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ABOUT COVER

Editorial Board Member of the *World Journal of Virology*, Celso Cunha holds a PhD in Biology (awarded 1992) and is currently Associate Professor of Habilitation in the Institute of Hygiene and Tropical Medicine at NOVA University (IHMT-NOVA; Lisbon, Portugal). In his professional career, his academic and administrative duties have included coordination of a Master's Program in Biomedical Sciences and membership on the scientific committees for two PhD Programs. In addition to his ongoing leadership of a research group focusing on host-pathogen interactions in hepatitis B and D infections at IHMT-NOVA, he also serves as Director of the Institute's Medical Microbiology Unit and as a member of the Institute's Counsel. Outside of the IHMT-NOVA, he serves on several evaluation panels for various international agencies related to his research interests. (L-Editor: Filipodia)

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The primary aim of *World Journal of Virology* (*WJV*, *World J Virol*) is to provide scholars and readers from various fields of virology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJV mainly publishes articles reporting research results obtained in the field of virology and covering a wide range of topics including arbovirus infections, viral bronchiolitis, central nervous system viral diseases, coinfection, DNA virus infections, viral encephalitis, viral eye infections, chronic fatigue syndrome, animal viral hepatitis, human viral hepatitis, viral meningitis, opportunistic infections, viral pneumonia, RNA virus infections, sexually transmitted diseases, viral skin diseases, slow virus diseases, tumor virus infections, viremia, and zoonoses.

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REVIEW

Emerging therapeutics in the management of COVID-19

Asim Kichloo, Michael Albosta, Akshay Kumar, Michael Aljadah, Mohamed Mohamed, Zain El-Amir, Farah Wani, Shakeel Jamal, Jagmeet Singh, Akif Kichloo

ORCID number: Asim Kichloo 0000-0003-4788-8572; Michael Albosta 0000-0003-4187-4911; Akshay Kumar 0000-0003-2718-0606; Michael Aljadah 0000-0003-1858-2670; Mohamed Mohamed 0000-0002-9173-5824; Zain El-Amir 0000-0001-7649-5634; Farah Wani 0000-0002-4683-6845; Shakeel Jamal 0000-0003-2359-8001; Jagmeet Singh 0000-0001-7179-1020; Akif Kichloo 0000-0002-4566-0294.

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Asim Kichloo, Department of Internal Medicine, Samaritan Medical Center, Watertown, NY 13601, United States

Michael Albosta, Mohamed Mohamed, Zain El-Amir, Shakeel Jamal, Department of Internal Medicine, Central Michigan University, Saginaw, MI 48602, United States

Akshay Kumar, Department of Cardiothoracic Surgery, University of Pittsburgh, Pittsburgh, PA 15260, United States

Michael Aljadah, Deparment of Internal Medicine, Medical College of Wisconsin, Milwaukee, WI 53226, United States

Farah Wani, Department of Family Medicine, Samaritan Medical Center, Watertown, NY 13601, United States

Jagmeet Singh, Department of Transplant Nephrology, Geisinger Commonwealth School of Medicine, Sayre, PA 18510, United States

Akif Kichloo, Department of Anesthesiology and Critical Care, Saraswathi Institue of Medical Sciences, Uttar Pradesh 245304, India

Corresponding author: Michael Albosta, MD, Doctor, Department of Internal Medicine, Central Michigan University, 1632 Stone Street, Saginaw, MI 48602, United States.
albos1ms@cmich.edu

Abstract

The severe acute respiratory syndrome coronavirus 2 (coronavirus disease 2019, COVID-19) pandemic has placed a tremendous burden on healthcare systems globally. Therapeutics for treatment of the virus are extremely inconsistent due to the lack of time evaluating drug efficacy in clinical trials. Currently, there is a deficiency of published literature that comprehensively discusses all therapeutics being considered for the treatment of COVID-19. A review of the literature was performed for articles related to therapeutics and clinical trials in the context of the current COVID-19 pandemic. We used PubMed, Google Scholar, and Clinicaltrials.gov to search for articles relative to the topic of interest. We used the following keywords: "COVID-19", "therapeutics", "clinical trials", "treatment", "FDA", "ICU", "mortality", and "management". In addition, searches through the references of retrieved articles was also performed. In this paper, we have elaborated on the therapeutic strategies that have been hypothesized or trialed to-date, the mechanism of action of each therapeutic, the clinical trials finished or in-

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process that support the use of each therapeutic, and the adverse effects associated with each therapeutic. Currently, there is no treatment that has been proven to provide significant benefit in reducing morbidity and mortality. There are many clinical trials for numerous different therapeutic agents currently underway. By looking back and measuring successful strategies from previous pandemics in addition to carrying out ongoing research, we provide ourselves with the greatest opportunity to find treatments that are beneficial.

Key Words: COVID-19; Therapeutics; Infectious disease; SARS-CoV-2; Pharmacology; Virology

Core Tip: As coronavirus disease 2019 continues to affect the global community, researchers are working diligently to determine the efficacy of therapeutic agents to fight this virus in clinical trials. Currently, there is a lack of published literature that comprehensively discusses all of the therapeutic agents under investigation. In this manuscript, we provide readers with a thorough and comprehensive evaluation of the current state of therapeutics including the proposed mechanisms of action, pharmacokinetics, recommended dosages, adverse effects, and efficacy data from clinical trials.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), a disease caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been labelled a pandemic by the World Health Organization after its emergence from Wuhan, Hubei Province, China in December 2019. It has since infected more than 60 million people worldwide. The presentation of the disease varies, however the most common symptoms include fever, cough, and dyspnea[1-3]. Other possible symptoms include rhinorrhea, sore throat, headache, gastrointestinal (GI) disturbances, and fatigue[3]. Because of the extensive morbidity and mortality related to COVID-19 infection, researchers and clinicians are racing to find effective therapeutics for the treatment of this disease. On March 28, 2020 the United States Federal Drug Administration (FDA) issued an emergency authorization for chloroquine (CQ) phosphate and hydroxychloroquine (HCQ) sulfate as a treatment for adults and adolescents weighing greater than 50 kg, who are hospitalized, and for whom a clinical trial is not available or feasible[4]. Furthermore, on May 1, 2020 an emergency authorization was issued for Remdesivir to be used for adults and children with severe disease, defined as SpO_2 less than 94% on room air, requiring supplemental oxygen, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO)[4]. Clinical trials for numerous therapeutics are on-going worldwide. In this review, we will familiarize readers with the current therapeutics being investigated for the treatment of COVID-19, including their mechanisms of action, rationale for use, adverse effects, and information from clinical trials in the currently published literature. A summary of the therapeutics can be found in monoclonal antibodies (Table 1), antivirals (Table 2), cell and RNA-based therapies (Table 3) and miscellaneous treatment (Table 4).

METHODS

A literature review was performed for articles related to therapeutics and clinical trials

Table 1 Monoclonal antibodies (a survey)

Drug	Current use/FDA approval	Proposed mechanism of action	Published trials
Sarilumab	FDA approved for use in rheumatoid arthritis	Monoclonal antibody, IL-6 receptor antagonist	(1) Sanofi and Regeneron [10]; (2) Benucci *et al*[11]; and (3) See Clinicaltrials.gov for ongoing trials
Siltuximab	FDA Approved for use in Multicentric Castleman's disease	Monoclonal antibody, IL-6 receptor antagonist	(1) Gritti *et al*[14]; and (2) See Clinicaltrials.gov for ongoing trials
Leronlimab	Not currently FDA approved, however under investigation for COVID-19 and HIV	Monoclonal antibody, CCR5 antagonist	(1) CytoDyn [17]; and (2) See Clinicaltrials.gov for ongoing trials
PD-1 inhibitors	FDA approved for the treatment of various malignancies	Inhibition of PD-1 pathway	No currently published trials
Gimsilumab	Not currently FDA approved. Clinical Trials are underway testing Gimsilumab as a treatment for ankylosing spondylitis as well as ARDS	Monoclonal antibody against GM-CSF	See Clinicaltrials.gov for ongoing trials

FDA: Federal Drug Administration; IL: Interleukin; COVID-19: Coronavirus disease 2019; HIV: Human immunodeficiency virus; PD-1: Programmed cell death protein 1; GM-CSF: Granulocyte macrophage colony stimulating factor; ARDS: Acute respiratory distress syndrome.

Table 2 Antivirals (a survey)

Drug	Current use/FDA approval	Proposed mechanism of action	Published trials
Arbidol	Approved in other countries for influenza treatment and prophylaxis, however not approved in the United States	Antiviral, inhibits viral-mediated fusion with target membrane, blocking viral entry into target cells	Zhang *et al*[38]
ASC09	Not currently FDA approved. Trials are underway testing ASC09 as a treatment for HIV and COVID-19	Antiviral, Protease inhibitor	See Clinicaltrials.gov for ongoing trials
Azvudine	Currently being tested in clinical trials for HIB and COVID-19	Antiviral, nucleoside reverse transcriptase inhibitor	See Clinicaltrials.gov for ongoing trials
Favipravir	Approved in other countries for the treatment of influenza, however not FDA approved in the United States	Antiviral, Inhibits RNA-dependent RNA polymerase	(1) Cai *et al*[52]; (2) Chen *et al*[33]; and (3) See Clinicaltrials.gov for ongoing trials
Baloxavir marboxil	Approved for treatment of uncomplicated influenza A and B in individuals age 12 and older who have been symptoms for no more than 48 h	Antiviral, cap-dependent endonuclease inhibitor	Lou *et al*[59]
Remdesivir	FDA Emergency Use Authorization for COVID-19	Antiviral, inhibitor of RNA-dependent RNA polymerase	(1) Wang *et al*[68]; (2) NIH (ACTT trial)[69]; (3) Beigel *et al*[71]; and (4) See Clinicaltrials.gov for ongoing trials

FDA: Federal Drug Administration; COVID-19: Coronavirus disease 2019; HIV: Human immunodeficiency virus; NIH: National Institutes of Health; ACTT: Adaptive COVID-19 Treatment Trial.

in the context of the current COVID-19 pandemic. We used PubMed, Google Scholar, and Clinicaltrials.gov to search for articles relative to the topic of interest. We used the following keywords: "COVID-19", "therapeutics", "clinical trials", "treatment", "FDA", "ICU", "mortality", and "management". In addition, searches through the references of retrieved articles was also performed. Three reviewers were responsible for performing article selection based on relevance to our topic. Inclusion criteria included both published and pre-published works that were available in English, and articles related to therapeutics and clinical trials for COVID-19 in all settings. We excluded abstracts, non-English articles, and those unrelated to therapeutics and COVID-19.

MONOCLONAL ANTIBODIES

Sarilumab

Chemical composition: Sarilumab (Kevzara) is a fully human monoclonal antibody that acts as an interleukin (IL)-6 receptor antagonist, which leads to blockage of the

Table 3 Cell and RNA-based therapies

Drug	Current use/FDA approval	Proposed mechanism of action	Published trials
Mesenchymal stem cells	FDA approved for graft versus host disease	Prevention of cytokine release as well as promotion of cellular repair/regeneration	(1) Leng *et al*[75]; and (2) See Clinicaltrials.gov for ongoing trials
MultiStem	Currently being studied for treatment of ischemic stroke, ulcerative colitis, acute myocardial infarction, and graft *vs* host disease	Immune system modulation, anti-inflammatory, pro-angiogenic	See Clinicaltrials.gov for ongoing trials
RNA based therapies	Have been utilized as anticancer and antiviral therapy. Have also been implemented in genetic diseases	Interfere with gene expression through RNA interference	See Clinicaltrials.gov for ongoing trials

FDA: Federal Drug Administration.

Table 4 Miscellaneous therapeutics

Drug	Current use/FDA approval	Proposed mechanism of action	Published trials
APN01	Known to have anti-hypertensive and anti-neoplastic properties	Cleaves angiotensin II to form angiotensin-1-7	See Clinicaltrials.gov for ongoing trials
Chloroquine/hydroxychloroquine	Anti-malarial, anti-viral, and anti-rheumatic effects. Previous studied in the 2004 SARS outbreak	Poorly understood. Likely mechanism includes accumulation of basic drug in lysosomes, altering pH and disrupting enzymes involved in post-translation protein modification	(1) Gautret *et al*[105]; (2) Tang *et al*[107]; (3) Borba *et al*[108]; (4) Horby *et al*[109]; and (5) Boulware *et al*[110]
Azithromycin	Macrolide antibiotic, classically using in the treatment of several bacterial infectious processes	Bacteriostatic properties due to binding of the 50 s ribosomal subunit, inhibiting bacterial protein synthesis. Against SARS-CoV-2, it is hypothesized that intracellular accumulation alters pH, leading to interference with viral activities	(1) All trials have been performed using Azithromycin as an adjunct to CQ/HCQ; and (2) No clinical trials evaluating the efficacy of azithromycin alone
Colchicine	Treatment for gout. Implicated in familial Mediterranean fever, primary biliary cirrhosis, psoriasis, sarcoidosis, scleroderma, amyloidosis, pericarditis, Sweet syndrome, and Behcet disease	Anti-inflammatory agent, binds to beta-tubulin in neutrophils leading to inhibition of assembly and polymerization of microtubules. This leads to decrease in several neutrophilic inflammatory processes	Gendelman *et al*[123]
Corticosteroids/methylprednisolone	Used in a variety of clinical instances as anti-inflammatory agents	Extensive anti-inflammatory and anti-fibrotic properties, thought to decrease inflammation	(1) Wu *et al*[135]; (2) Wang *et al*[133]; and (3) Horby *et al*[136]
Ivermectin	Used as an anti-parasitic agent, however has shown antiviral activity against numerous pathogens	May play a role in inhibiting viral nuclear import into the host cell *via* interactions with IMPalpha/B1	Caly *et al*[143]
Convalescent plasma	Has been used in previous pandemics, including SARS, MERS, Ebola, and H1N1 for the purpose of passive immunization	By sharing plasma of individuals who have previously been infected, passive immunization occurs	(1) Li *et al*[148]; (2) Shen *et al*[149]; and (3) Duan *et al*[150]
ECMO	Used to support cardiac and pulmonary function in critically ill patients	Assists the cardiorespiratory system functioning in patients with severe ARDS	Currently, no randomized clinical trials have evaluated the efficacy of using ECMO in the treatment of COVID-19

FDA: Federal Drug Administration; COVID-19: Coronavirus disease 2019; SARS: Severe acute respiratory syndrome; ECMO: Extracorporeal membrane oxygenation; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; ARDS: Acute respiratory distress syndrome; CQ: Chloroquine; HCQ: Hydroxychloroquine.

development of IL-6 mediated inflammation[5]. It is currently best known for its role in the treatment of rheumatoid arthritis. It is a covalent heterotetramer made up of two disulfide linked heavy chains linked to a kappa light chain[6].

Mechanism of action: The proposed mechanism of action of Sarilumab against COVID-19 is due to its ability to act as an IL-6 receptor antagonist[7]. It has been demonstrated that patients with severe COVID-19 infection are more likely to have elevated levels of several biomarkers, including IL-6[8]. The binding of SARS-CoV-2 to

the alveolar epithelial cells leads to the activation of the innate and adaptive immune systems, which leads to the production of several pro-inflammatory cytokines[7]. IL-6 promotes T-cell activation, B-cell differentiation, and induces the production of acute phase reactants from the liver[7]. In addition, elevated levels of IL-6 have been associated with cardiovascular diseases such as atherosclerosis, heart failure, angina, and hypertension[7].

Pharmacokinetics: Sarilumab is shown to be well absorbed in Rheumatoid Arthritis patients[6]. In one study of the pharmacokinetics of Sarilumab in 1770 patients with rheumatoid arthritis, 631 patients received 150 mg and 682 patients received 200 mg of Sarilumab every two weeks for up to one year[6]. On average, Tmax was observed between 2 to 4 d[6]. The volume of distribution at steady state is 7.3 L[6]. As with other monoclonal antibodies, it is believed to be degraded into peptides and amino acids[6]. It is not eliminated by either the hepatic or renal systems, but rather it is eliminated predominately through proteolytic pathways[6].

Adverse effects: There is limited clinical trial data available describing the adverse effects of Sarilumab for the treatment of COVID-19. A review of the use of Sarilumab in the treatment of rheumatoid arthritis along with other disease modifying antirheumatic drugs (DMARDs) found that neutropenia (9.8% to 14.2%), upper respiratory infections (6.4% to 7.1%), elevated alanine aminotransferase (ALT) (6.7% to 6.8%), and local injection site erythema (5.3%) were some of the more common side effects[9]. When Sarilumab was used as monotherapy, neutropenia (15.6%), nasopharyngitis (6%), and injection site erythema (6.2%) were among the most common side effects[9].

Dosage: Published clinical trial data for Sarilumab is lacking. Current unpublished data available has suggested using either 200 mg or 400 mg intravenous doses, depending on the study protocol[10,11].

Randomized clinical trials: There are no currently published clinical trials demonstrating efficacy for Sarilumab in the treatment of COVID-19. However, a large phase 2/3, randomized placebo-controlled study of Sarilumab in hospitalized patients with severe COVID-19 is ongoing[10]. In addition, a case series by Benucci *et al*[11] described the clinical course of eight patients hospitalized in Italy with COVID-19. Patients were given 400 mg of Sarilumab in addition to HCQ, azithromycin, darunavir, cobicistat, and enoxaparin at 24 h after hospitalization[11]. An additional 200 mg dose was given to patients after 48 and 96 h, respectively. In this series, 7 patients saw substantial improvements in their SpO_2/FiO_2 ratio and were discharged home after testing negative for COVID-19 within 14 d[11]. Only 1 of the patients, who was 83 years old, died after 13 d[11]. Further clinical trials are needed to evaluate the efficacy and safety of Sarilumab for the treatment of COVID-19.

Siltuximab

Chemical composition: Siltuximab (Sylvant) is a chimeric monoclonal antibody that acts *via* inhibition of IL-6, similar to Sarilumab[12]. It is known for its role in treating a variety of malignancies, including multicentric Castleman's disease, multiple myeloma, myelodysplastic syndrome, prostate cancer, ovarian cancer, and lung cancer[12].

Mechanism of action: The primary mechanism of Siltuximab is *via* binding to and/or neutralization of IL-6[12]. As discussed previously, IL-6 is a proinflammatory cytokine that has been shown to be elevated in patients suffering from severe COVID-19[8].

Pharmacokinetics: Siltuximab is primarily distributed within the intravascular space[13]. It is approved in the United States to be given at doses of 11 mg/kg over the course of a one hour infusion once every three weeks[13]. The steady state is reached by the sixth dose, accumulating at 1.7 times higher than the concentration achieved *via* a single dose[13]. The volume of distribution in a 75 kg man is approximately 4.5 L, and the half-life is approximately 20.6 d[13]. It is cleared *via* first order elimination at a rate of 0.23 L per day[13].

Profit and adverse effects: The safety and efficacy of Siltuximab in the treatment of COVID-19 has not yet been established. Further clinical trials are needed to determine adverse effects of this medication. The most common adverse effects of Siltuximab therapy when used for the purposes of treating Castleman's disease and Multiple Myeloma include weight gain, hyperuricemia, respiratory infections, rash, and

pruritus[12].

Dosage: In the only clinical trial currently reported from Italy, patients received the standard dose of Siltuximab, 1 1mg/kg IV infusion over the course of one hour[14]. In addition, a second dose was able to be given at the physician's discretion[14].

Randomized clinical trials: Currently, there is no published data regarding the usage of Siltuximab for the treatment of COVID-19. Currently, an unpublished study from Italy evaluated the use of Siltuximab in 21 patients admitted to the hospital with confirmed COVID-19[14]. All of the patients who were available for follow up had CRP levels normalized (median time to follow up = 8 d). Additionally, 7 patients experienced a reduced need for ventilation, 9 patients experienced clinical stabilization of their position, while 5 patients experienced worsening of their condition described as the need for intubation during the course of the study[14].

Leronlimab (PRO 140)

Chemical composition: Leronlimab is a humanized immunoglobulin (Ig) G4 monoclonal antibody that acts as a CCR5 antagonist[15]. It is currently in clinical trials for the treatment of human immunodeficiency virus (HIV)[15].

Mechanism of action: Leronlimab is a CCR5 receptor antagonist. CCR5 is a fusion co-receptor used by the HIV-1 virion to enter into human cells[16]. It is thought that the CCR5 receptor plays a role in immune cell trafficking to sites of inflammation, and for this reason there is a potential benefit for the use of this drug in the treatment of COVID-19[17].

Pharmacokinetics: A clinical trial by Jacobson *et al*[18] examined the use of Leronlimab in the treatment of HIV. Subjects were given either placebo, a 162 mg dose, or a 324 mg dose of Leronlimab weekly for three weeks[18]. The average peak concentration of the drug was 6.1 mg/L and 13.8 mg/L for the 162 mg group and the 324 mg group, respectively[18]. The average half-life was 3.4 and 3.7 d for each respective group[18]. There is little information available regarding the metabolism and elimination of Leronlimab.

Adverse effects: There are no documented adverse effects regarding the use of Leronlimab in the treatment of COVID-19. Jacobson *et al*[18] found in their study that the most frequent adverse effects of Leronlimab in the treatment of HIV included diarrhea (14%), headache (14%), lymphadenopathy (11%), and hypertension (9%).

Randomized clinical trials: Currently, no published randomized clinical trials have evaluated the use of Leronlimab in the treatment of COVID-19. However, in New York, 10 severely ill patients with COVID-19 have received treatment with Leronlimab[17]. After three days, eight of these patients showed significant improvement in levels of cytokines, including IL-6, as well as improvements in CD4/CD8 T-cell ratios[17]. Currently, patients are enrolling in Phase 2 and Phase 2b/3 trials for the use of Leronlimab in the treatment of severe COVID-19[17].

Programmed cell death inhibitors

Chemical composition: Antibodies that block programmed cell death (PD-1) are known as immune checkpoint inhibitors. Immune checkpoints refer to inhibitory pathways that are crucial for maintaining self-tolerance and controlling the physiologic immune responses in peripheral tissues to minimize tissue damage when responding to pathogenic infections. Many immune checkpoints are initiated by ligand-receptor binding, which allows for blockade by antibodies[19].

Mechanism of action: When PD-1 binds to its ligand (PD-L1), it has an immunosuppressive effect[19]. PD-1 and its ligands have traditionally been studied for antitumor treatment because of the ability of cancer to dysregulate the expression of these checkpoint proteins, which allows it to escape T-cell mediated cell death.

Pharmacokinetics: The pharmacokinetics of immune checkpoint inhibitors like PD-1 blocking antibodies are impacted by time-varying clearance and the target-mediated drug position[20]. Differences in patient-specific characteristics only account for some of the variability in the pharmacokinetics of immune checkpoint inhibitors. Immune checkpoint inhibitors appear to have little to no impact on liver and renal function[20]. They display limited diffusion outside of the vascular space[20]. They have a long half-life and are cleared through a receptor-mediated mechanism in both linear and

nonlinear phases[20]. Clearance may occur through nonspecific degradation in tissues and plasma[21].

Adverse effects: There is currently no clinical trial data available describing the adverse effects of PD-1 blocking antibodies in the treatment of COVID-19. Previously reported adverse effects vary including the following disturbances: gastrointestinal (bloody diarrhea, abdominal pain, and pyrexia), hepatic (jaundice, and asymptomatic liver enzyme elevation), endocrine (hypophysitis, hypo/hyperthyroiditis, primary adrenal insufficiency, and hypercalcemia), skin (rash and Stevens-Johnson syndrome), rheumatological (mild arthralgia, myalgia, and arthritis), neurological, renal, pulmonary (pneumonitis), cardiac (myocarditis, myositis), and many others[22]. It should be noted that the incidence of these adverse effects varies[22]. In one smaller study of 19 patients receiving PD-1 therapy, some patients experienced flares of pre-existing autoimmune disease with treatment[23]. Similar results were also seen in patients receiving anti-PD-1 therapy in a larger, multicenter trial[24].

Dosage: There is limited data regarding dosing for the use of PD-1 inhibitors in the treatment of COVID-19. In one clinical trial, 200 mg of the PD-1 inhibitor Camrelizumab was administered one time intravenously[25].

Randomized clinical trials: There are no currently published randomized clinical trials regarding the use of PD-1 inhibitors in patients with COVID-19. Researchers at Southeast University in China are currently studying the efficacy of Camrelizumab in patients with severe pneumonia associated with lymphocytopenia in COVID-19 patients in order to restore immunoactivity[25].

Gimsilumab

Chemical composition: Gimsilumab (KIN-1901) is a fully human monoclonal antibody that antagonizes granulocyte macrophage colony stimulating factor (GM-CSF)[26]. This monoclonal antibody is fully human and is directed at a proinflammatory cytokine that is thought to play a role in autoimmunity and inflammation[26].

Mechanism of action: GM-CSF is a hematopoietic growth factor that can stimulate the proliferation of granulocytes and macrophages which can contribute to increased inflammation and cytokine release in COVID-19 patients[3]. It is found in synovial fluid in patients who have spondylarthritis; therefore, this drug has been studied in the setting of ankylosing spondylitis because it may neutralize the cytokine activity and benefit patients[26].

Pharmacokinetics: Because this drug is still under investigation and is relatively new, little information is available about the pharmacokinetics of Gimsilumab.

Adverse effects: There is currently no clinical trial data available describing the adverse effects of Gimsilumab in the treatment of COVID-19. Since the drug is still under investigation and is relatively new, little information is available about the adverse effects of Gimsilumab.

Dosage: Dosage information for Gimsilumab as a treatment for COVID 19 is not currently available. One study looking at Gimsilumab in the treatment of ankylosing spondylitis administered single or repeat subcutaneous injections once weekly for four weeks[26]. A trial for the use of Gimsilumab in patients with COVID-19 is planning to administer high dose Gimsilumab on day one and low dose on day eight of treatment, unless the patient is discharged or no longer in need of supplemental oxygen or ventilator support for over 48 h on day 8[27]. Unfortunately, specific information regarding what constitutes "high dose" and "low dose" is not available.

Randomized clinical trials: Gimsilumab was originally being studied as a treatment for ankylosing spondylitis and thus far a Phase 1 study has been completed which demonstrated a favorable safety and tolerability profile with no serious adverse events. However, because of its possible applicability in COVID-19, trials will be focused on the prevention of acute respiratory distress syndrome (ARDS) and cytokine storm instead of Phase 2 trials on rheumatic diseases[26]. It has been found that the percentage of GM-CSF expressing white blood cells are higher in the blood of intensive care unit (ICU)-admitted COVID-19 patients when compared to healthy controls, as well as non-ICU patients[28]. One study is examining the efficacy and safety of Gimsilumab in people with lung injury or ARDS secondary to COVID-19 infection in a randomized double blinded trial[27]. This study will have a 2-wk treatment period

and a 22-wk follow-up period[27].

ANTIVIRALS

Arbidol (umifenovir)

Chemical composition: Arbidol{ethyl-6-bromo-4-[(dimethylamino)methyl]-5-hydroxy-1-methyl-2-[(phenylthio)methyl]-indole-3-carboxylate} is a broad-spectrum antiviral compound[29]. Although it has been used for the treatment and prevention of influenza in Russia and China for decades, it has not been approved for this purpose by the Food and Drug Administration in the United States[29].

Mechanism of action: The anti-viral mechanism involves inhibition of virus-mediated fusion with the target membrane and a resulting block of virus entry into target cells[30]. It inhibits viral glycoprotein conformational changes during membrane fusion by interacting with the phospholipid membrane and protein motifs enriched in aromatic residues[30].

Pharmacokinetics: Regarding metabolism, 33 metabolites of Arbidol have been identified in human plasma, urine, and feces[31]. The drug is rapidly absorbed when administered orally, with a t_{max} of 1.38 h[31]. The main biotransformation pathways of Arbidol are sulfoxidation, glucuronidation, sulfate conjugation, and dimethylamine N-demethylation[31]. The primary urine metabolites are glucuronide and sulfate conjugates[31]. The liver and intestines are primarily responsible for the metabolism of Arbidol in humans, with CYP3A4 being a major isoform and other P450 enzymes and flavin-containing monooxygenases playing less significant roles in metabolism[31]. It has a long elimination half-life, which is reported to be 25 h, and high plasma exposure[31].

Adverse effects: One study reported that 43.7% of patients had digestive upset, such as mild diarrhea and nausea, with Arbidol treatment[32]. In this study, however, no patients stopped treatment with Arbidol due to adverse effects[32]. Another study showed an increase in serum uric acid in 2.5% of patients taking Arbidol[33].

Dosage: Current recommendations for Arbidol dosing are as follows: 200mg orally 3 times a day for no more than 10 d in adults[34]. In clinical trials, 200 mg orally 3 times a day for 7-10 d or longer is currently being used and investigated[33,35-37].

Randomized clinical trials: Zhang *et al*[38] conducted a retrospective case-control study to evaluate the efficacy of Arbidol as a post-exposure prophylactic medication on family members and health care workers who were exposed to patients confirmed to have SARS-CoV-2 infection by real-time reverse transcription polymerase chain reaction (RT-PCR) and chest computed tomography (CT) scan. Logistic regression based on the data of the family members and health care workers with Arbidol or Oseltamivir prophylaxis showed that Arbidol post-exposure prophylaxis was protective against the development of COVID-19 [hazard ratio 0.025, 95% confidence interval (CI) 0.003-0.209, P = 0.0006 for family members and hazard ratio 0.056, 95%CI = 0.005-0.662, P = 0.0221 for health care workers][38]. They suggested Arbidol could reduce the infection risk of the novel coronavirus in hospital and family settings[38]. Though the study had a number of limitations and warrants further research, most healthcare facilities in China have already adopted the usage of Arbidol as a standard protocol for post-exposure prophylaxis of COVID-19 transmission among its healthcare workers.

ASC09

Chemical composition: ASC09, which is also referred to as TMC-310911, is not currently FDA approved for the treatment of COVID-19[39]. It is similar in structure to darunavir and is an investigational drug currently under study for use in HIV-1 infections as well as for treatment for COVID-19[40,41].

Mechanism of action: ASC09 is an HIV protease inhibitor[39]. Regarding HIV-1, the drug binds to the protease enzyme in order to inhibit the cleavage of Gag-Pol polyproteins and Gag polyproteins. This inhibition prevents the formation of mature virus particles capable of infection[40].

Pharmacokinetics: The drug is metabolized mainly by CYP enzymes[42]. The terminal

elimination half-life of ASC09 ranged from 1.25 to 3.751 h in one study. Multiple oral doses that ranged from 150 mg twice daily to 900 mg twice daily were also studied and showed that the terminal elimination ranged from 12.23 to 16.48 h[42].

Adverse effects: In one Phase IIa study the authors looked at the adverse effects of ASC09 in HIV patients[43]. The study found that the most common adverse events were fatigue and nausea, which occurred in at least 10% of the 33 participants[43]. Gastrointestinal-related adverse effects occurred in approximately 27% of participants[43]. No deaths or serious adverse events were reported. No adverse events resulted in patient discontinuation of the study. There were rises in liver enzymes in two patients, although the presence of cytomegalovirus hepatitis in one patient may have accounted for this abnormality[43].

Dosage: Dosage information for ASC09 as a treatment for COVID 19 is not currently available. One clinical trial planned to give ASC09/ritonavir in 300 mg/100 mg tablets twice daily for 14 d[44].

Randomized clinical trials: To date, there are no completed clinical trials evaluating the efficacy of ASC09 in the treatment of COVID-19. One current clinical trial is set to evaluate the efficiency and safety of ASC09/ritonavir and lopinavir/ritonavir for COVID-19 infections[44]. The study is a randomized, open-label trial and is estimated to have 160 participants[44]. Further clinical trials are needed to determine whether ASC09 is an efficacious therapeutic option.

Azvudine

Chemical composition: Azvudine, also known as FNC, is a cytidine analogue. It is a substrate for deoxycytidine kinase and is phosphorylated to deoxycytidine[45]. Azvudine is used in the treatment of HIV-1 infected patients and has been introduced in large part due to the emergence of resistance against previously created nucleoside analogues, namely 3TC[45].

Mechanism of action: Azvudine is a nucleoside reverse transcriptase inhibitor (NRTI) that has activity against HIV-1, HIV-2, hepatitis B, and hepatitis C[45]. The drug is activated after phosphorylation into an NRTI-triphosphate derivative[45]. As an NRTI-triphosphate derivative, the drug competes with deoxynucleoside triphosphates for incorporation into the viral strand by the enzyme reverse transcriptase[45]. The NRTI derivative lacks a 3'-OH group, so the incorporation into the viral strand prevents elongation[45].

Pharmacokinetics: Azvudine is currently being tested in clinical trials for HIV treatment and COVID-19; therefore, there is currently no published information about the pharmacokinetics of Azvudine. Regarding HIV, computer modeling has been used to predict the binding of Azvudine to reverse transcriptase[45].

Adverse effects: There is currently no clinical trial data available describing the adverse effects of Azvudine in the treatment of COVID-19.

Dosage: Dosage information for Azvudine as a treatment for COVID-19 is not currently available.

Randomized clinical trials: To date, there are no completed clinical trials evaluating the efficacy of Azvudine in the treatment of COVID-19. One clinical trial is studying the efficacy of Azvudine in the treatment of COVID-19-related pneumonia[46]. The study is a randomized, double blinded, double dummy, parallel controlled study[46]. Further clinical trials are needed to determine whether Azvudine is an efficacious therapeutic option.

Favilavir/Favipiravir/T-705/Avigan

Chemical composition: Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is a pyrazine-carboxamide derivative and a pyrazine analogue that was initially approved for use against influenza[47]. Its activity is primarily against RNA viruses. It has known activity against influenza and has been promising in the treatment of avian influenza. It has been studied for the treatment of Ebola virus, Lassa virus, and COVID-19[47].

Mechanism of action: Favipiravir is converted to its active form, favipiravir-ribofuranosyl-5'-triphosphate, which then inhibits viral RNA-dependent RNA polymerase. Inhibition of the viral polymerase halts transcription and replication of

the viral genome[47]. It is thought that it is incorporated into an RNA strand, preventing elongation and proliferation of the viral genome[48]. Favipiravir is also reported to prevent the entry and exit of the virus in host cells[49].

Pharmacokinetics: Favipiravir is reported to have a bioavailability of 97.6%[50]. Its volume of distribution is approximately 15-20 L, and 54% of the drug appears to be plasma protein bound with 65% of this fraction being bound to albumin and 6.5% bound to a1-acid glycoprotein[50]. The drug is predominantly excreted renally, and its elimination half-life is reported to be between 2 to 5.5 h[50].

Adverse effects: The safety profile of Favipiravir in the treatment of COVID-19 is yet to be established. It is known to cause QT prolongation[50]. Additionally, it is recommended that cardiac and hepatic monitoring take place during treatment[50]. Favipiravir is a known teratogen and should be avoided in women who may become or are confirmed to be pregnant[48].

Dosage: Currently, the safety and efficacy of Favipiravir for the treatment of COVID-19 is being evaluated in a number of clinical trials. As such, there is not currently a proven recommended dosage. Open-label studies in China have used 1600 mg twice daily on the first day of treatment and 600 mg twice daily used for the following 7-10 or 14 d, respectively[33,51,52]. There is a need for continued clinical trials to determine an efficacious dose.

Randomized clinical trials: Limited clinical trial data regarding the efficacy of Favipiravir for COVID-19 infections is available. A small, open label, prospective, randomized multicenter study in China evaluated the use of Favipiravir *vs* Arbidol for patients with COVID-19[33]. It was found that the use of Favipiravir was associated with a greater degree of clinical recovery, defined as greater than 72 h of temperature less than 36.6 degrees C, respiratory rate less than 24/min, oxygen saturation greater than 98% on room air, and either mild or no cough when compared to Arbidol[33]. Clinical recovery rates were greater in both moderate (71% *vs* 56%) and severe cases of COVID-19 (6% *vs* 0%)[33]. In an additional open-label, nonrandomized trial of patients in China with non-severe COVID-19 infection, it was found that the use of Favipiravir was associated with decreased median time to viral clearance when compared to a control group receiving lopinavir/ritonavir treatment (4 d *vs* 11 d)[52]. The patients also noted improvements on chest CT scan on day 14[52]. Additional clinical trials are currently underway[53-55].

Xofluvaa (Baloxavir marboxil)

Structure/mechanism of action: Baloxavir marboxil is a cap-dependent endonuclease protein inhibitor that acts on influenza A and B viruses[56,57]. This inhibits mRNA synthesis, thus blocking viral replication[56,57]. It is currently FDA approved for the treatment of acute uncomplicated influenza A and B infections in individuals aged 12 and older who have been symptomatic for no more than 48 h.

Pharmacokinetics: Baloxavir marboxil is metabolized to an active form, Baloxavir acid[56]. The median time to peak plasma concentration is 4 h, and the mean half-life is 79.1 h[56]. It is 93 to 94% protein bound, and is primarily excreted in the feces (80%) with smaller amounts being excreted renally (15%)[56]. It is metabolized by the UGT1A3 and CYP3A4 pathways[56].

Adverse effects: Currently, only one randomized clinical trial has assessed the use of Baloxavir marboxil in the treatment of COVID-19. Because of this, there is limited data available regarding side effects of Baloxavir marboxil in COVID-19 patients. In studies evaluating the drug for the treatment of patients with influenza, the most common adverse effects were diarrhea (3.2%), bronchitis (2.6%), nasopharyngitis (1.5%), nausea (1.5%), and sinusitis (1.1%), although it was thought that these adverse events were not likely due to the trial regimen[58].

Dosage: The dosing protocol used in one of the only documented randomized control trials was 80 mg of Baloxavir marboxil once daily on day 1 and day 4[59]. Additionally, if patients still test positive for COVID-19 on day 7, an additional 80 mg dose can be given[59]. The total amount of doses given is not to exceed three 80 mg doses[59].

Randomized clinical trials: Randomized clinical trial data for the use of Baloxavir marboxil in COVID-19 is limited. One exploratory, single center, open-label, randomized control trial in China compared the addition of Baloxavir marboxil to

Favipiravir and control along with the current standard antiviral treatment regimen in patients with confirmed COVID-19 infection[59]. The current standard regimen included either Lopinavir/Ritonavir in combination with inhaled interferon-alpha or Darunavir/Cobicistat and Arbidol in combination with inhaled interferon alpha[59]. 29 patients were included in the study, and they were assigned to either receive Baloxavir marboxil, Favipiravir, or control, in addition to standard antiviral therapy. Twenty-four patients in the trial tested negative for COVID-19 within 14 d of starting the trial. The percentage of patients turning virus negative was 70%, 77%, and 100% in the Baloxavir group, Favipiravir group, and control group respectively[59]. Additionally, the daily viral load of the patients in each group was measured throughout the course of the trial, and the addition of Baloxavir or Favipiravir did not appear to improve the time to achieve half viral clearance when compared to control[59]. Based on this study, there is no evidence that Baloxavir marboxil is an effective treatment against COVID-19 patients. Additional studies may be necessary to confirm these findings.

Remdesivir

Chemical composition: Remdesivir (GS-5734) is a phosphoramidate prodrug of a 1'-cyano-substituted nucleotide analogue[60]. Its triphosphate form (RDV-TP) resembles adenosine triphosphate (ATP) and is used as a substrate of several viral RNA-dependent RNA polymerase enzymes or complexes. It is a broad-spectrum antiviral medication, with activity against RNA viruses such as Ebola, Marburg, MERS-CoV, SARS-CoV, respiratory syncytial virus (RSV), Nipah virus (NiV), and Hendra virus. It has shown prophylactic and therapeutic efficacy in nonclinical models of these coronaviruses[61-63].

Mechanism of action: Replication of SARS-CoV-2 depends on the viral RNA-dependent RNA polymerase, which is the target of the nucleotide analogue Remdesivir. The SARS-CoV-2 RNA-dependent RNA polymerase is composed of the non-structural proteins nsp8 and nsp12[64]. Enzyme kinetics indicated that this RNA-dependent RNA polymerase efficiently incorporates the active triphosphate form of Remdesivir into RNA[64]. Additionally, the mechanism of Remdesivir's anti-MERS-CoV activity is likely through premature termination of viral RNA transcription as shown in biochemical assays using recombinant EBOV, NiV, and RSV polymerase[64]. This drug has shown potent inhibitory activity against Remdesivir with intact proof reading and with low level of resistance to target mutations[64].

Pharmacokinetics: Remdesivir has a short plasma half-life of 0.39 h[65]. When given to cynomolgus monkeys, a 10 mg/kg dose rapidly distributed to the testes, epididymis, eyes, and brain within 4 h[65]. Levels measured in the brain were much lower than other tissues due to poor blood-brain barrier penetration, however levels in the brain were detectable at 168 h after the dose was given. It is primarily eliminated renally (74%), with a smaller amount of fecal excretion (18%)[66].

Adverse effects: During a study by Grein *et al*[67], the most common adverse events noted during use of Remdesivir in patients with COVID-19 included rash, diarrhea, hypotension, abnormal liver function and renal impairment. Serious adverse events such as acute kidney injury, septic shock, and multi-organ failure were noted in 23% of patients[67]. During the study, 60% of participants suffered at least one adverse event and 8% discontinued treatment prematurely[67].

Dosage: Current dosage recommendation of Remdesivir in COVID-19 is a bolus dose of 200 mg IV diluted in normal saline (0.9%) or 5% dextrose to be given over 60 min on day 1, followed by 100 mg IV to be given diluted over 60 min for the next 9 d[66].

Randomized clinical trials: Wang *et al*[68] enrolled and randomly assigned 237 patients to a treatment group (158 to Remdesivir and 79 to placebo). Remdesivir use was not associated with a difference in time to clinical improvement [hazard ratio 1.23 (95%CI 0.87–1.75)][68]. Although not statistically significant, patients receiving Remdesivir had a faster time to clinical improvement than those receiving placebo among patients with symptom duration of 10 d or less [hazard ratio 1.52 (0.95-2.43)][68]. Adverse events were reported in 102 (66%) of 155 Remdesivir recipients *vs* 50 (64%) of 78 placebo recipients[68]. Remdesivir was stopped early because of adverse events in 18 (12%) patients *vs* four (5%) patients who stopped placebo early[68].

On April 29, 2020, the National Institute of Allergy and Infectious Diseases (NIAID) announced interim results of a randomized controlled trial named ACTT (Adaptive COVID-19 Treatment Trial) involving 1063 patients conducted at 68 sites (47 in United

States and 21 in Europe and Asia)[69]. Preliminary results indicate that the median time to recovery was 11 d for patients treated with Remdesivir compared to 15 d for those who received placebo, thereby suggesting that patients who received Remdesivir had a 31% faster time to recovery than those receiving placebo ($P < 0.001$)[69]. However, the survival benefit with Remdesivir was not statistically significant compared to the control, as the Remdesivir group had a mortality rate of 8.0% compared to 11.6% for the placebo group ($P = 0.059$)[69].

The SIMPLE trial is an open-label, randomized, phase 3 clinical trial comparing the clinical improvement of 5-d (short-course) *vs* 10-d (long-course) treatment duration of Remdesivir ($n = 397$) in hospitalized patients with severe (evidence of pneumonia and reduced oxygen levels, not requiring mechanical ventilation) COVID-19, in addition to the standard of care in 15 countries[70]. Secondary objectives included rates of adverse events and additional measures of clinical response in both treatment groups. The study showed that the 10-d course had similar outcomes compared to the 5-d course [odds ratio (OR) 0.75, 95%CI 0.51-1.12] assessed on day 14, without any new safety signals[70]. An exploratory analysis of this study suggested a larger benefit if Remdesivir was initiated within 10 d of symptom onset[70]. Pooled data from both study arms found that at day 14, 62% *vs* 49% of participants were discharged from the hospital, if Remdesivir was started within 10 d *vs* after 10 d of symptoms, respectively[70].

Beigel *et al*[71] conducted a double-blind, randomized, placebo-controlled trial using IV Remdesivir in adults hospitalized with COVID-19 with evidence of lower respiratory tract involvement. Patients were randomly assigned to receive either Remdesivir (200 mg loading dose on day 1, followed by 100 mg daily for up to 9 additional days) or placebo for up to 10 d[71]. The primary outcome was the time to recovery, defined by either discharge from the hospital or hospitalization for infection-control purposes[71]. Preliminary results from the 1059 patients (538 assigned to Remdesivir and 521 to placebo) indicated that those who received Remdesivir had a median recovery time of 11 d (95%CI, 9.0 to 12.0) as compared with 15 d (95%CI, 13.0 to 19.0) in those who received placebo (rate ratio for recovery, 1.32; 95%CI, 1.12 to 1.55; $P < 0.001$)[71]. The Kaplan-Meier estimates of mortality by 14 d were 7.1% with Remdesivir and 11.9% with placebo (hazard ratio for death, 0.70; 95%CI, 0.47 to 1.04)[71]. Serious adverse events were reported for 21.1% in the Remdesivir group and 27% in the placebo group. Thus, Remdesivir was superior to placebo in shortening the time to recovery in adults hospitalized with COVID-19 and evidence of lower respiratory tract infection[71].

CELL AND RNA-BASED THERAPIES

Mesenchymal stem cells

Structural composition: Mesenchymal stem cells (MSCs) are considered to be a highly proliferative, minimally invasive, potential treatment of COVID-19[72]. MSCs are stem cells that are isolated from various body tissues, including dental pulp, menstruation blood, bone marrow, adipose tissues, buccal fat pad, and the fetal liver[72]. MSCs are multipotent stem cells and can be expanded easily to a clinical volume[73]. Clinical trials have not shown adverse reactions to allogeneic MSCs[73].

Mechanism of action: It is believed that COVID-19 triggers immune system over-activation that is responsible for damaging infected tissue. The immune system produces large amounts of pro-inflammatory factors, inducing a cytokine storm that may induce the overproduction of immune cells[74]. It is thought that MSCs prevent the cytokine storm by preventing the release of cytokines by the immune system and promoting repair through the reparative properties of stem cells[72]. Once the MSCs are given through an intravenous injection, the MSC population is trapped in the lung, which may help in the recovery of the lung's microenvironment, protect alveolar epithelial cells, cure lung dysfunction, and intercept pulmonary fibrosis in COVID-19-related pneumonia[75].

Adverse effects: There is currently no clinical trial data available describing the adverse effects of MSCs in the treatment of COVID-19.

Dosage: Dosage information for MSCs as a treatment for COVID-19 is not currently available.

Randomized clinical trials: Several clinical trials have been registered to investigate

the use of MSCs in the treatment of COVID-19. One study by Leng *et al*[75] evaluated the effects of MSC transplantation in 7 patients with COVID-19 pneumonia. At 48 h post MSC transplant, all 7 patients showed improvement of clinical symptoms[75]. MSCs have shown promise in the treatment of ARDS, inflammation, pneumonia, and sepsis, all of which contribute significantly to mortality in COVID-19 patients[76]. The safety and efficacy of intravenous MSC therapy has not been shown, and there is some concern about this mode of delivery because of the high levels of procoagulant tissue factor present in MSC infusions[76]. This could of course be dangerous for patients with COVID-19, who are already thought to be in a hypercoagulable state[76].

MultiStem

Structural composition: MultiStem is an allogeneic cell therapy made of multipotent adherent bone marrow cells[77]. MultiStem cells have been studied for the treatment of ischemic stroke, ulcerative colitis, acute myocardial infarction, and graft *vs* host disease prophylaxis in allogenic hematopoietic stem cell transplant[78].

Mechanism of action: MultiStem cells appear to be therapeutic due in part to their pro-angiogenic effects and their ability to modulate the immune system[79]. MultiStem cells lack major histocompatibility complex (MHC) II, which means that they do not create a proliferative response when cultured alongside allogeneic T-cells and ultimately that they reduce T cell proliferation when the T cells are stimulated with irradiated, allogeneic stimulator cells[80]. MultiStem is also immunosuppressive due to the presence of soluble factors[78].

Adverse effects: There is currently no clinical trial data available describing the adverse effects of MultiStem in the treatment of COVID-19. One study looked at the administration of MultiStem alongside hematopoietic stem cell transplants[81]. They found that overall, there was good tolerance to the therapy with no associated infusion toxicity, increased infection incidence, or graft failure[81].

Dosage: There is currently no clinical trial data available describing the appropriate dosage for MultiStem in the treatment of COVID-19.

Randomized clinical trials: There is currently no clinical trial data available describing the efficacy and safety of MultiStem in the treatment of COVID-19. One clinical trial is hoping to look at the efficacy of MultiStem in the treatment of COVID-19 induced ARDS. The study is a multicenter, open-label, single active treatment arm study followed by a double-blind, randomized, placebo-controlled phase. The goals of this study are to evaluate the safety and efficacy of MultiStem for people with moderate to severe ARDS[82].

RNA based therapies

Structural composition: Small interfering RNAs, also known as short interfering RNAs, silencing RNAs, or siRNAs, are non-coding, double stranded RNA molecules that are typically 20-25 base pairs in length.

Mechanism of action: SiRNA molecules are capable of regulating gene expression through RNA interference[83]. RNA interference allows for post-transcriptional gene silencing and degradation of target mRNAs[84]. SARS-CoV-2 has a protease sequence (specifically in protease 3CL) known as nsp5 that appears to be highly conserved, making this sequence a potential target of siRNA therapeutics[85]. Other targets that have been considered are the viral helicase and the viral RNA-dependent RNA polymerase[85]. It is believed that siRNAs can target these highly conserved sequences of SARS-CoV-2 and suppress the viral impact in the lungs, ultimately allowing for treatment of COVID-19 infection[86].

Pharmacokinetics: Information regarding the pharmacokinetics of RNA based therapy in the treatment for COVID-19 is not currently available.

Adverse effects: There is currently no clinical trial data available describing the adverse effects of RNA based therapy in the treatment of COVID-19. Previous work has shown that though beneficial, siRNA can induce unwanted side effects[85]. The side effects associated with siRNA therapy may be due to a phenomenon called off-targeting, which is when siRNAs interfere with transcripts besides the target RNA[87]. The first evidence of this effect was reported in 2003 by Jackson *et al*[89] Strategies have been proposed to minimize off-targeting, most of which deal with planning siRNA experiments and designing appropriate siRNA for therapeutics[87].

Dosage: Dosing information for RNA based therapies as a treatment for COVID-19 is not currently available.

Randomized clinical trials: To date, there are no published clinical trials evaluating the efficacy of RNA based therapy in the treatment of COVID-19. Researchers have previously tested the efficacy of *in vitro* utilization of amidoamine nanocarriers for siRNA onto lung epithelial cells, which may be useful in targeting SARS-COV-2 because the primary site of infection is the ciliated cells of the human lung[86,89].

MISCELLANEOUS TREATMENT

APN01: recombinant human angiotensin-converting enzyme

Structural composition: APN01 is a soluble, glycosylated recombinant form of the human angiotensin-converting enzyme 2 (rhACE2). It has both antihypertensive and antineoplastic properties[90].

Mechanism of action: The drug is thought to cleave angiotensin II to form angiotensin-1-7. Angiotensin-1-7 is thought to have a variety of functions, including counteracting the cardiovascular actions of angiotensin II and inhibiting cyclooxygenase-2. It is believed that when pro-inflammatory prostaglandins are made, the angiotensin-1-7 G-protein-coupled receptor Mas is activated, potentially diminishing tumor cell proliferation[90]. Previous research showed that ACE2 is a key receptor for SARS-CoV-2, and that APN01 could block early stages of SARS-CoV-2 infections, suggesting that treatment with APN01 may be useful in the treatment of COVID-19[91]. One publication showed that SARS-COV-2 replicates in human blood vessels and kidneys, and this replication may be blocked by APN01[91]. By binding to the spike protein, rhACE2 can reduce binding to ACE2 at the cell membrane, leading to decreased internalization of SARS-COV-2 and reduced viral load[91].

Pharmacokinetics: Information regarding the pharmacokinetics of APN01 in the treatment for COVID-19 is not currently available.

Adverse effects: Information regarding the adverse effects of APN01 in the treatment for COVID-19 is currently not available.

Dosage: Dosage information for APN01 as a treatment for COVID-19 is not currently available.

Randomized clinical trials: APN01 is currently in clinical trials for its efficacy in treating COVID-19. One study aims to enroll 200 participants in a randomized, double-blinded study[92]. In vitro and *in vivo* studies are needed to truly understand the effects of APN01 in COVID-19 infections.

CQ/HCQ

Structural composition: CQ and HCQ belong to a class of drugs known as 4-Aminoquinolines[93]. Both have a flat aromatic core and are weakly basic[94]. CQ has been historically used as an antimalarial and anti-amoebic agent, while HCQ has been used as an antirheumatic agent, as well as more recently as a therapy for the Zika virus and the 2004 SARS outbreaks[95]. HCQ is a more tolerable and safer derivative of CQ and has potent activity against SARS CoV-2 *in vitro*[96]. HCQ differs from CQ due to the presence an N-Hydroxyethyl side chain in place of the N-diethyl group, which makes it more soluble[95].

Mechanism of action: Numerous mechanisms have been postulated as to how CQ/HCQ achieves its anti-malarial, ani-viral, and anti-rheumatic effects, however the overall picture remains poorly understood. The most common theory is that as they are both weak bases, they tend to accumulate in lysosomes, increasing the pH and disrupting several enzymes that ultimately leads to the inhibition of the post-translational modification of newly synthesized proteins[97]. This disruption of the protein degradation pathway interferes with antigen processing and can prevent MHC class II mediated antigen presentation resulting in its anti-rheumatic effects[97]. Another theory is that they may interfere with endosome mediated viral entry secondary to alkalization of the entry endosomes[97]. This change in endosomal pH also allows CQ/HCQ to downregulate toll-like receptors (TLRs) along with TLR-mediated signal transduction[95]. They also are known to decrease the production of cytokines such as

IL-1, IL-6, and tumor necrosis factor by mononuclear cells[98].

Pharmacokinetics: CQ/HCQ are absorbed in the upper gastrointestinal tract with the fraction absorbed being approximately 74%[93]. They are approximately 50% plasma protein bound, with an extremely long half-life (> 40 d)[93,99]. It is believed that the extended half-life is likely due to a large volume of distribution[93]. They are primarily metabolized by the liver, specifically the CYP3A4 and CYP2C3 enzymes, and are renally cleared[93]. CQ/HCQ bind strongly to melanin, and can deposit in high concentrations in the eyes and skin[100].

Adverse effects: CQ/HCQ are both well tolerated medications. The most common side effects include gastrointestinal effects such as nausea, vomiting, dyspepsia and cramps[101]. It may also cause headaches, tinnitus, itching, and rashes[102]. The dose limiting side effect is macular retinopathy, however this is more common in CQ as opposed to HCQ[103] Both medications have QT prolongation effects which may lead to cardiac arrest, especially when combined with Azithromycin, which has been done for the treatment of COVID-19[104].

Dosage: HCQ dosage consists of 6 d of therapy, with a 400 mg dose given every 12 h for the first day, and 200 mg given every 12 h for the subsequent 5 d[105]. HCQ was given emergency use authorization by the FDA in March 2020, however, was subsequently revoked in June 2020 due to safety concerns. It is currently not recommended unless a patient is enrolled in a clinical trial[106].

Randomized clinical trials: HCQ had been implemented in the treatment of COVID-19 prior to any meaningful randomized clinical trials. This was done on the basis of *in vitro* data as well as an open label non-randomized clinical trial published in March 2020 by Gautret *et al*[105]. This trial had enrolled 20 patients to receive HCQ 200 mg three times a day for a total of 10 d[105]. The control group (16 patients) were patients who had refused treatment or had contraindications to HCQ. The primary endpoint was virologic clearance at day 6 post-inclusion. Results showed that at day 6 post-inclusion, 70% of patients receiving HCQ had achieved virologic clearance as compared to 12.5% in the control group[105]. The limitations of this study include small sample size as well as lack of randomization and blinding.

The first true randomized control trial to evaluate efficacy of HCQ for treatment COVID-19 was conducted by Tang *et al*[107] This was a multi-center, open label, randomized control trial comparing viral clearance at 28 d in 150 patients, randomized to a HCQ arm and a standard of care arm with intention to treat analysis. Patients who had received HCQ received a dose of 600 mg twice daily for three days followed by 400 mg twice daily for 2-3 wk[107]. The results showed no discernable difference in viral clearance between the HCQ group and standard of care group (85.4% and 81.3% respectively)[107].

A randomized control trial to assess safety and efficacy of higher doses of CQ was conducted by Borba *et al*[108] in March 2020. This was a parallel, double masked, randomized clinical trial involving 81 patients. The participants were divided into two groups, with 41 patients receiving high dose CQ and 40 receiving low dose CQ. High dose CQ was considered 600 mg twice daily for 10 d while low dose was considered 450 mg twice daily for the first day and then once daily for the subsequent 4 d[108]. Primary outcome was a decrease in mortality by 50% in the high dose group as opposed to the low dose group[108]. Results demonstrated a higher mortality at day 13 in the high dose group compared to the low dose group (39% and 15% respectively)[108]. Due to safety concerns secondary to increased adverse effects in the high dose group the trial was discontinued prematurely[108].

The RECOVERY trial was a UK-based randomized clinical trial to test numerous drugs for COVID-19, including HCQ[109]. A total of 1542 patients were randomized to HCQ as compared with 3132 randomized to standard of care alone. The primary endpoint was 28-d mortality, which showed no significant difference between the two groups (25.7% HCQ *vs* 23.5% standard care) [95%CI 0.98-1.26; $P = 0.1$][109]. On June 5th 2020, the chief investigators of the study had released the afore-mentioned preliminary results as well as a statement conveying the lack of any meaningful mortality benefits in patients with COVID-19 and that the investigators have stopped enrolling participants in the HCQ arm of the RECOVERY trial[109]. The results of the HCQ arm of RECOVERY trial have not been published.

HCQ has also been evaluated as a possible post-exposure prophylaxis agent for COVID-19. This was done in the context of a randomized, double-blinded, placebo-controlled trial[110]. The study population included adults who had high-risk household

or occupational exposure (distance less than 6 ft for more than 10 min without a mask) or moderate risk (the same distance with a face mask but no eye shield) to someone with laboratory confirmed COVID-19[110]. Within 4 d of exposure, patients were randomly assigned to receive either placebo or HCQ. The primary outcome was the incidence of laboratory-confirmed COVID-19 or illness compatible with COVID-19 within 14 d[110]. There were 821 asymptomatic participants, and incidence of COVID-19 in the high-risk exposure population did not differ significantly[110].

Azithromycin

Structural Composition: Azithromycin is an azalide which is a subclass of macrolide antibiotics derived from the prototype Erythromycin[111]. Azithromycin has a nitrogen added to the 14-membered ring of erythromycin, creating a new 15 membered compound[111]. The addition of nitrogen creates a dibasic molecule which results in improved antimicrobial activities, pharmacokinetics and fewer side effects as compared with Erythromycin[112].

Mechanism of action: Azithromycin is a bacteriostatic antibiotic which prevents bacterial growth *via* inhibition of bacterial protein synthesis by binding to the 50 s ribosomal subunit[111]. Due to its dibasic nature Azithromycin is taken up by white blood cells (WBC) and fibroblasts resulting in a neutrophilic intracellular:extracellular ratio of 226:1 after 24 h of incubation[113]. This WBC uptake is believed to be the reason for Azithromycin's effective intracellular and extracellular activity, as well as increased drug levels localized to the site of infection[113]. Regarding the mechanism of action against SARS-CoV-2, one in-vitro study suggested that due to its intracellular accumulation, Azithromycin increases the pH of intracellular organelles[114]. This alteration in pH would interfere with intracellular viral activities, a mechanism very similar to CQ/HCQ[114]. Another potential mechanism of action is the anti-inflammatory activity shown by macrolides, thus alleviating the proinflammatory state of COVID-19[106].

Pharmacokinetics: Azithromycin is rapidly absorbed after oral dosing, with excellent tissue penetration and a long half-life of roughly 68 h[115]. It has a large volume of distribution of approximately 31 L/kg. The primary route of elimination is *via* biliary excretion[116]. Approximately 6% of azithromycin is excreted unchanged in urine[116].

Adverse effects: Azithromycin is generally safe and well tolerated. The most commonly reported side effects are gastrointestinal and include nausea, diarrhea and abdominal pain[117]. Rash, transaminitis, and hepatomegaly have also been seen with azithromycin[117]. Rarely it may cause QT interval prolongation and should be used with caution when administering to patients concomitantly with other QT-prolonging drugs such as CQ/HCQ[117].

Dosage: Since the outbreak of COVID-19, many clinicians are using Azithromycin off-label, usually concomitantly with HCQ. Due to lack of supporting evidence regarding its efficacy, it is no longer used in the treatment of COVID-19. It was given as a 500 mg dose on day 1, followed by 250 mg for the next four days for a total of a 5-d treatment course[105]. Currently it is only recommended that Azithromycin be administered in the context of clinical trials[106].

Randomized clinical trials: All clinical trials conducted utilizing Azithromycin for the treatment of COVID-19 have been performed in the context of Azithromycin being administered as an adjunct to CQ/HCQ. The are no well-controlled, prospective, randomized clinical trials evaluating the efficacy of azithromycin for the treatment of COVID-19.

Colchicine

Structural composition: Colchicine is a tricyclic, lipid soluble alkaloid. Its chemical formula is N-(5,6,7,9, tetrahydro-1,2,3,10, tetramethoxy-9 oxobenzo[a] hep-tain-7-yl) acetamide. Colchicine has long been used for the treatment of gout, but has since been known to be used in the treatment of several disorders including familial Mediterranean fever, primary biliary cirrhosis, psoriasis, sarcoidosis, scleroderma, amyloidosis, pericarditis, Sweet syndrome, and Behcet's disease[118].

Mechanism of action: There are several proposed mechanisms regarding the potential efficacy of Colchicine as a therapeutic for COVID-19. First, Colchicine is believed to exert its anti-inflammatory effects through binding to beta-tubulin in neutrophils[119]. This allows for the inhibition of assembly and polymerization of microtubules,

interfering with several cellular functions. This includes production of chemokines and decreasing neutrophil degranulation, chemotaxis, and phagocytosis[119]. Additionally, Colchicine has been shown to be an inhibitor of the NLRP3 inflammasome, which plays a major role in the pathophysiology of ARDS[120]. Several SARS-CoV-2 proteins have been hypothesized to activate the NLP3 inflammasome, thus leading to the development of ARDS[120]. Inactivation of the neutrophilic function and inhibition of the NLP3 inflammasome lead to the anti-inflammatory and cytokine suppression effects that may contribute to Colchicine's potential mechanism against COVID-19.

Pharmacokinetics: After administration, Colchicine is absorbed in the jejunum and ileum[121]. The peak plasma concentration after oral administration occurs between roughly 30 and 90 min [119,121]. The half-life is thought to be between 9.3 and 10.6 h, and bioavailability has been shown to range from 24% to 88%[119,122]. Colchicine is thought to be widely taken up by tissues, and its protein binding is between 10% and 31%[119,121]. It is thought to be metabolized by the cytochrome P450 CYP3A4 (20%) and it is excreted primarily *via* the feces[118,119]. It is also thought that 10%-20% of the available metabolites are excreted *via* the urine[118].

Adverse effects: There is currently no clinical trial data available describing the adverse effects of colchicine in the treatment of COVID-19. Colchicine is thought to be generally safe and well tolerated, although the therapeutic window is narrow[118]. Current data suggests that side effects of Colchicine in non COVID-19 use includes GI side effects such as vomiting, diarrhea, cramping, and abdominal pain[118,119]. Additionally, leukopenia and neuromuscular complications are rare side effects that have been reported[118].

Dosage: Dosage information for Colchicine as a treatment for COVID 19 is not currently available.

Randomized clinical trials: To date, there are no published clinical trials evaluating the efficacy of Colchicine in the treatment of COVID-19. A retrospective study by Gendelman *et al*[123] examined the protective role of Colchicine against COVID-19 by determining the rate of baseline usage of the drug in patients with RT-PCR confirmed COVID-19 infection and those who tested negative. The total sample included 14520 subjects, of which 1317 tested positive. There was no significant difference found in Colchicine usage between those who tested positive (0.53%) and those who tested negative (0.48%)[123]. This retrospective study did not suggest a protective effect of Colchicine against COVID-19. Further clinical trials are needed to determine whether Colchicine is an efficacious therapeutic option.

Corticosteroids/methylprednisolone

Structural composition: Methylprednisolone is a glucocorticoid that is a prednisolone derivative. It is more potent than prednisone[124]. Methylprednisolone exists in both a succinate formulation (Solu-Medrol), and an acetate suspension (Depo-Medrol).

Mechanism of action: It is speculated that the anti-inflammatory and antifibrotic properties of corticosteroids may prevent an extensive cytokine response, which would result in a faster resolution of pneumonia and systemic inflammation[125,126]. Methylprednisolone is a corticosteroid; therefore, it binds to the glucocorticoid receptor and inhibits proinflammatory signals and promotes anti-inflammatory signals[127]. The binding of the drug to the glucocorticoid receptor alters gene expression, leading to downstream effects over the course of hours to days[127]. These effects may be anti-inflammatory when administered at lower doses, or immunosuppressive when administered at higher doses[127].

Pharmacokinetics: The bioavailability of oral methylprednisolone acetate is 89.9%, and the average volume of distribution is approximately 1.38 L/kg[128,129]. It is 76.8% protein bound in plasma[129]. It is thought that the drug is metabolized mostly by 20-ketosteroid reductases and 11beta-hydroxysteroid dehydrogenases[129]. The half-life is 2.3 h[128,129]. Its clearance rate is 336mL/h/kg on average[129]. One study in animals showed 25-31% of the drug was eliminated in urine while 44-52% was eliminated *via* fecal route[130].

Adverse effects: Information regarding adverse events related specifically to COVID-19 infections is not available. One study of methylprednisolone use in Kawasaki disease showed that adverse effects included sinus bradycardia, hyperglycemia, and hypertension[131]. Hypothalamic-pituitary-adrenal (HPA) suppression is also possible

with methylprednisolone administration[132].

Dosage: One retrospective study used 1-2 mg per kg daily IV for 5-7 d[133]. A randomized control study administered 40 mg IV every 12 h for 5 d[134].

Randomized clinical trials: One multicenter, open label randomized controlled study completed in China compared the use of methylprednisolone paired with standard care in patients that had progressed to acute respiratory failure. The results of this study have not yet been published[134]. Additional studies regarding the efficacy of methylprednisolone have been completed. One retrospective, observational single-center study collected data from 201 confirmed COVID-19 infected patients who had pneumonia that progressed to ARDS[135]. In these patients, methylprednisolone appeared to reduce the risk of death[135]. In another retrospective, observational single-center study, data was collected from 46 patients who had severe, confirmed COVID-19 pneumonia that progressed to acute respiratory failure. The study found that methylprednisolone use was associated with shortened disease and improved clinical symptoms (including fever and hypoxia)[133].

As part of the RECOVERY trial, dexamethasone has been evaluated as a potential treatment for COVID-19[137]. This was a controlled, open-label, adaptive trial in which a total of 2114 patients were allocated to receive Dexamethasone (6 mg once daily for up to 10 d) and 4321 were allocated to usual standard of care[136]. The primary outcome was all-cause mortality at 28-d post randomization[136]. Preliminary results showed a decrease in mortality in the Dexamethasone arm as opposed to the standard of care arm (22.9% *vs* 25.7% mortality respectively)[136]. It was also noted that there were variations in the proportional and absolute mortality rate reductions based on the level of ventilatory support that patients initially required[136]. Dexamethasone decreased mortality by one-third in patients receiving mechanical ventilation (29.3% *vs* 41.4% mortality) and by one-fifth in those who were receiving non-invasive oxygenation (23.3% *vs* 26.2% mortality)[136].

Ivermectin

Structural composition: Ivermectin is a semisynthetic derivative of avermectin B1, consisting of an 90:10 mixture of 22,23-dihydro-avermectin B1a and 22,23-dihydro-avermectin B1b[137,138]. Avermectins are a group a pentacyclic sixteen-membered lactones derived from the soil bacterium Streptomyces avermitilis[139]. It is a broad spectrum antiparasitic, however it has shown antiviral activity against a number of pathogens including dengue virus, yellow fever virus, and HIV1 virus, among others[140-142].

Mechanism of action: Although the potential mechanism of action of Ivermectin against SARS-CoV-2 is unknown, the novel coronavirus SARS-CoV-2 is very similar to the better studied SARS-CoV. They are both single-stranded positive sense RNA viruses, and SARS-CoV is thought to utilize IMPalpha/B1, a heterodimer responsible for integrase protein nuclear import[143]. It is thought that Ivermectin may play a role in inhibiting viral nuclear import into the host cell through its interactions with IMPalpha/B1[143].

Pharmacokinetics: Ivermectin is only approved for oral administration in humans. Following oral administration, plasma concentrations are similar to the dose received[138]. It is widely distributed in the human body, bound strongly to plasma proteins at 93.2%[137]. After administration, the compound has been found to be present in adipose tissue, skin, fascia, and nodule[137]. It is found at highest concentrations in adipose tissue[137]. The drug is metabolized by the cytochrome P450 system, and is excreted almost exclusively in feces over a 12 d period, with only 1% of the dose given being excreted in the urine[137,138].

Adverse effects: The adverse effects of Ivermectin as a therapy for COVID-19 are unknown, however it is thought to be very well tolerated in the treatment of parasitic infections. Side effects noted from clinical trials include fatigue (0.9%), abdominal pain (0.9%), anorexia (0.9%), constipation (0.9%), diarrhea (1.8%), nausea (1.8%), dizziness (2.8%), vertigo (0.9%), tremor (0.9%) and Mazzotti reaction, including arthralgias, lymph node enlargement and tenderness, edema, and urticarial rash[138].

Dosage: No human trials have been published regarding the usage of Ivermectin as a treatment modality for COVID-19. For the treatment of strongyloidiasis, Ivermectin is given as a single oral dose providing 200 mcg per kilogram of body weight[138]. For Onchocerciasis, a single dose providing 150 mcg per kilogram of bodyweight is

given[138].

Randomized clinical trials: To date, there have been no studies published regarding the efficacy of Ivermectin as a potential treatment for COVID-19 in humans. However, Caly *et al*[143] tested the anti-viral effects of Ivermectin against COVID-19 infected Vero/hSLAM cells in vitro, and found that at 24 h, the amount of viral RNA was reduced by 93% in the infected cells. At 48 h, there was greater than 5000 fold decrease in the amount of viral RNA seen in COVID-19 infected cells[143]. There was no toxicity observed in any of the samples during the study[143]. While this is promising, clinical trials are needed to determine the safety and efficacy of Ivermectin as a potential treatment for COVID-19.

Convalescent plasma

Mechanism of action: Convalescent plasma is the collection of plasma from an individual who has previously been infected with COVID-19 and developed antibodies. Administration of the plasma from patients with resolved infections leads to passive immunization, and reception of these antibodies in those who are currently sick may lead to reduced symptom burden and mortality. The use of convalescent plasma during pandemics is not a new trend, as it was used during the SARS, MERS, Ebola, and H1N1 pandemics as well[144-147]. Theoretically, the use of convalescent plasma should be given to infected patients early in the course of illness before the immune system has had the time to develop antibodies on its own.

Adverse events: Adverse effects have varied. Li *et al*[148] reported adverse events in 2 of 52 patients receiving convalescent plasma, which included transfusion reactions such as febrile non-hemolytic and severe transfusion associated dyspnea. Shen *et al*[149] reported no adverse events in 5 critically ill patients receiving convalescent plasma. Finally, a study by Duan *et al*[150] found no serious adverse events in 10 ICU patients receiving plasma, however one patient did develop an evanescent facial red spot.

Dosage: Dosing of convalescent plasma for the treatment of COVID-19 has varied among the current available studies. Dosage of convalescent plasma given in a clinical trial by Li *et al*[148] was 4-13 mL/kg of recipient body weight, transfused at 10 mL for 15 min, followed by 100 mL per hour. Shen *et al*[149] gave 200-250 mL twice on the same day as collection from the donor. Lastly, Duan *et al*[150] gave patients one dose of 200 mL of convalescent plasma.

Randomized clinical trials: Li *et al*[148] performed an open-label, multicenter, randomized clinical trial at 7 hospitals in Wuhan, China. The trial enrolled 103 patients hospitalized with COVID-19. Fifty-two patients received convalescent plasma in addition to standard treatment (based on Chinese national treatment guidelines and hospital practice guidelines for COVID-19) and 51 patients received standard treatment alone[148]. Patients received plasma at a dose of 4-13 mL/kg of recipient body weight[148]. The primary outcome was time to clinical improvement within a 28 d period, defined as either discharge or a reduction of 2 points on a 6 point COVID-19 severity scale[148]. For all patients, the authors found no significant difference between the convalescent plasma group and control (51.9% *vs* 43.1%; $P = 0.26$)[148]. Furthermore, there was no significant difference in 28 d mortality between the two groups (15.7% *vs* 24.0%; $P = 0.30$)[148]. Adverse events were reported in two patients receiving convalescent plasma treatment, both of which were transfusion related reactions[148]. This trial did not demonstrate significant time to improvement in patients receiving convalescent plasma for the treatment of COVID-19.

Shen *et al*[149] describe a case series of 5 critically ill patients in Shenzhen, China with COVID-19 and ARDS receiving convalescent plasma in addition to antiviral agents. The patients received two transfusions of 200-250 mL of convalescent plasma in one day. In this trial, viral load declined and was negative in all five patients within 12 d of treatment[149]. Furthermore, all patients saw reductions in temperature within 3 d, improvements in chest imaging, and improvements in PaO2/FiO2 ratio. Four of five patients receiving mechanical ventilation no longer required respiratory support within 9 d of receiving convalescent plasma[149]. No adverse events were reported. Although this study was a small case series, and there were no controls, it shows promise for the use of convalescent plasma in the treatment of COVID-19.

Lastly, Duan *et al*[150] performed a pilot study including 10 patients with severe COVID-19. Patients received a single 200 mL dose of convalescent plasma in addition to various antiviral therapies and intravenous methylprednisolone. Within 3 d, all 10 patients had significant improvement in their symptoms including fever, cough,

shortness of breath, and chest pain[150]. Additionally, 2 of 3 patients receiving mechanical ventilation were weaned to high flow nasal cannula, and one patient was able to discontinue high flow nasal cannula[150]. All patients showed improvement of pulmonary lesions on CT after transfusion. Lastly, neutralizing antibody titers increased in 5 patients, and viral RNA decreased to undetectable levels in 3 patients after 2 d, three patients after 3 d, and one patient after 6 d[150]. This trial, again, was performed without controls. It does however show promise for the use of convalescent plasma in patients with severe COVID-19.

ECMO

ECMO is often used as a last resort in patients with critical pulmonary or cardiovascular compromise. ECMO has various configurations that can be altered based on the needs of the patient. The potential use of ECMO has been a hot topic in recent discussions. Positive ECMO experiences in critically ill patients infected with Middle Eastern respiratory syndrome (MERS) has encouraged some to use ECMO in those infected with COVID-19[151]. Theoretically, it is possible for ECMO to be used in COVID-19 infected patients to support cardiac and pulmonary function; however, the efficacy and validity in the clinical setting remains unanswered.

Mechanism of action: Most often, COVID-19 patients who may benefit from ECMO are those with ARDS refractory to standard treatment. Patients in this clinical stage have impaired gas exchange due to alveolar inflammation and edema. Therefore, patients require oxygenation assistance. There are several configurations of ECMO. Veno-venous ECMO (V-V ECMO drains blood from a large peripheral vein, oxygenates it *via* a synthetic lung and returns it to the circulation *via* a large peripheral vein. Newly oxygenated blood then flows through the normal circulatory pathways to provide oxygen to the rest of the body. V-V ECMO settings require that the patient's heart is functioning appropriately to ensure adequate blood distribution[152]. Suggested criteria for V-V ECMO use are PaO2/FiO2 < 100 mmHg, PCO2 > 60 mmHg, and/or arterial pH < 7.2[153].

When a patient's cardiovascular function is compromised, such as with COVID-19-induced myocarditis, veno-arterial ECMO (V-A ECMO) is the preferred ECMO configuration. In V-A ECMO, venous blood is drained, oxygenated *via* a synthetic lung and finally returned to the patient's circulatory system *via* a large peripheral artery that drains towards the aorta. The ECMO-induced increase in aortic blood flow improves peripheral perfusion[154]. V-V/V-A ECMO may be modified in difficult circumstances, such as those with superimposed sepsis or multi-organ dysfunction, in order to add extra lumen and convert the ECMO system from a double lumen to a triple lumen ECMO system. The additional lumen may help optimize settings per patient requirements[155].

Randomized clinical trials: To date, there are no published clinical trials evaluating the efficacy of ECMO in the treatment of COVID-19. ECMO has been used in patients who have been confirmed to have COVID-19 and in those suspected to have COVID-19 but whose status has not been confirmed. There has been some published work on the efficacy of ECMO despite the lack of clinical trials. One preliminary study form China showed a high mortality rate for COVID-related ARDS patients, reporting a mortality of 50% in a cohort of 28 patients[156]. A pooled analysis of the data from China, which included the data for 562 COVID-19 patients, studied the effects of ECMO and non-ECMO treatment in the 46% of patients who developed ARDS. Those who did not get treated with ECMO had a mortality rate of 70.9%, while those treated with ECMO had a mortality of 94.1%[157]. While some data exists that may speak to the efficacy and validity of using ECMO in those infected with COVID-19, there is still a need for randomized clinical trials to understand the effects of this therapy.

CONCLUSION

The COVID-19 pandemic has placed a tremendous burden on our healthcare systems, as well as on researchers and clinicians who are racing to find therapeutics that may be beneficial in combatting this morbid disease. Currently, there is no single treatment that has been proven to provide significant benefit in reducing morbidity and mortality. There are many clinical trials for numerous different therapeutic agents currently underway. By looking back and measuring successful strategies from previous pandemics in addition to carrying out ongoing research, we provide ourselves with the greatest opportunity to find treatments that are beneficial. It is

reasonable that we continue to work together as a global community to explore different treatment modalities.

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Editorial Board Member, Sharof M Tugizov, DSc, PhD, Professor, Department of Medicine, University of California, Division of Infectious Diseases, San Francisco, CA 94143, United States. sharof.tugizov@ucsf.edu

AIMS AND SCOPE

The primary aim of *World Journal of Virology* (*WJV*, *World J Virol*) is to provide scholars and readers from various fields of virology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJV mainly publishes articles reporting research results obtained in the field of virology and covering a wide range of topics including arbovirus infections, viral bronchiolitis, central nervous system viral diseases, coinfection, DNA virus infections, viral encephalitis, viral eye infections, chronic fatigue syndrome, animal viral hepatitis, human viral hepatitis, viral meningitis, opportunistic infections, viral pneumonia, RNA virus infections, sexually transmitted diseases, viral skin diseases, slow virus diseases, tumor virus infections, viremia, and zoonoses.

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EDITORIAL

New Year's greeting and overview of *World Journal of Virology* in 2021

Dong-Mei Wang, En-Qiang Chen, Mahmoud El-Bendary

ORCID number: Dong-Mei Wang 0000-0002-6348-321X; En-Qiang Chen 0000-0002-8523-1689; Mahmoud El-Bendary 0000-0002-3751-5927.

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Dong-Mei Wang, Science Editor Development Department, Baishideng Publishing Group Inc, Pleasanton, CA 94566, United States

En-Qiang Chen, Center of Infectious Diseases, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China

Mahmoud El-Bendary, Department of Tropical Medicine and Hepatology, Faculty of Medicine, Mansoura University, Mansoura 35111, Egypt

Corresponding author: Dong-Mei Wang, BSc, Assistant Editor, Science Editor Development Department, Baishideng Publishing Group Inc, 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, United States. d.m.wang@wjgnet.com

Abstract

The 2020 year-end wrap-up session of Baishideng Publishing Group was held on December 31, 2020. All staff attended this session. We shared our key results area and made a business plan regarding the journal management. *World Journal of Virology (WJV)* is now abstracted and indexed in PubMed and PubMed Central. It received 23 manuscripts and published 9 papers which included 6 articles reporting coronavirus 19 in 2020. On the other hand, we made major strategies for *WJV*'s development in 2021. At present, *WJV* only has 28 Editorial Board members and cannot receive many manuscripts. We must redouble our efforts to invite more highly influential scientists to join our Editorial Board member and write high-quality manuscripts.

Key Words: *World Journal of Virology*; Publication; Editorial board; Virology

Core Tip: At the 2020 year-end wrap-up session of Baishideng Publishing Group, we made a report on the work of *World Journal of Virology* (*WJV*) in 2020 and made a business plan regarding the development of *WJV*.

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INTRODUCTION

World Journal of Virology (*WJV*) is a high-quality, online, open-access, single-blind peer-reviewed journal published by the Baishideng Publishing Group (BPG). *WJV* accepts both solicited and unsolicited manuscripts. Articles published in *WJV* are high-quality, basic and clinical, influential research articles by established academic authors as well as new researchers. The paramount objective of *WJV* is to showcase and promote distinguished research in the field of virology, to help advance development of this field. The types of articles published in *WJV* include Editorial, Opinion Review, Frontier, Review, Minireview, Basic Study, Clinical Research, Systematic Review, Meta-analysis, Evidence-based Medicine, Field of Vision, Clinical Guidelines, Letter to the Editor, and Case Report[1].

REVIEW OF OUR WORK IN 2020

Submissions and publishing articles

In 2020, we received 23 submissions from around the world. The Science Editor Development Department made the first decisions for 18 manuscripts, and edited and made the second decisions for 11 manuscripts, and rejected 2 manuscripts (8.7%, 2/23). The Production Department published 9 articles (39.1%, 9/23) (Figure 1).

Among these 9 articles, 6 were published on the theme of the coronavirus 19 (COVID-19), 4 were invited manuscripts (44.4%, 4/9), 5 were unsolicited manuscripts (55.6%, 5/9), and 1 original article (11.1%, 1/9), 7 review articles (77.8%, 7/9), 1 other type article (11.1%, 1/9) (Figure 2A). The articles came from 6 countries and regions, including 3 articles from Chinese authors (33.3%, 3/9), 2 American authors (22.2%, 2/9), 1 Italian author (11.1%, 1/9), 1 Irish author (11.1%, 1/9), 1 South African author (11.1%, 1/9), and 1 author from Saudi Arabia (11.1%, 1/9) (Figure 2B).

Invited manuscripts

In 2020, *WJV* accepted a total of 97 manuscript titles, including 74 for review articles (76.3%, 74/97), 21 for original articles (21.7%, 21/97), and 2 for editorials (2.1%, 2/97) (Figure 3). Among them, 9 (9.3%) manuscripts have been submitted online, and 4 (4.1%) manuscripts were submitted after the deadline. Eighty-four (86.6%) manuscripts have not been submitted yet.

Review statistics

In 2020, *WJV* invited peer reviewers and editorial board members to review manuscripts 664 times. Of these invitations, 100 were accepted (15.1%, 100/664), 78 were rejected (11.70%, 78/664), 486 did not receive a response (73.2%, 486/664). Of the 100 accepted invitations, 31 peer review comments were submitted on time (31.0%, 31/100), 67 failed to submit peer review comments on time (67.0%, 67/100), and 2 have not yet submitted peer review comments.

Editorial Board members

In 2020, the *WJV* Editorial Board consisted of 28 members[2]. In addition, 4 scientists applied for the Editorial Board and are awaiting evaluation. *WJV* Editorial Board members are from 17 countries and regions, including 6 from the United States (21.4%), 4 from Italy (14.3%), 3 from Ireland (10.7%), 2 from Egypt (7.1%), and 13 from various countries and regions (46.4%) (Figure 4). Seventeen Editorial Board members (60.7%) have completed a peer review on a manuscript, and nine members (32.1%) have not yet completed a peer review on a manuscript.

CONCLUSION

The BPG appreciates the continuous support and submissions from authors and the dedicated efforts and expertise by our invited reviewers, many who are also serving on the Editorial Board.

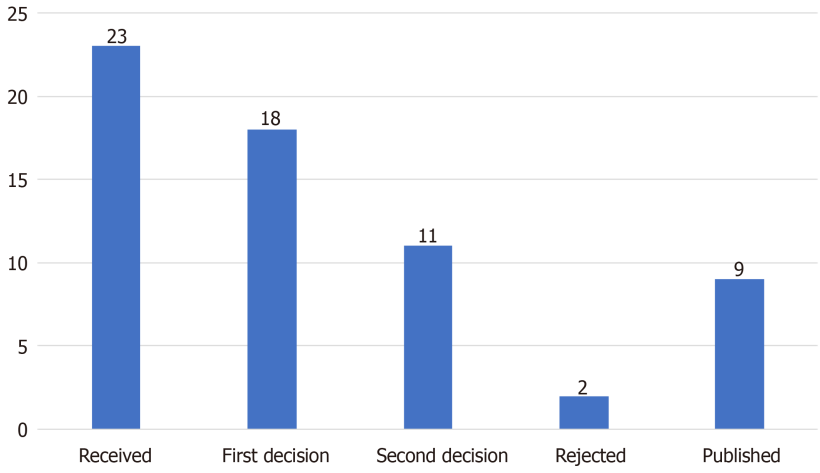

Figure 1 *World Journal of Virology* 2020 manuscript processing. The numbers of manuscripts processed from submission through publication.

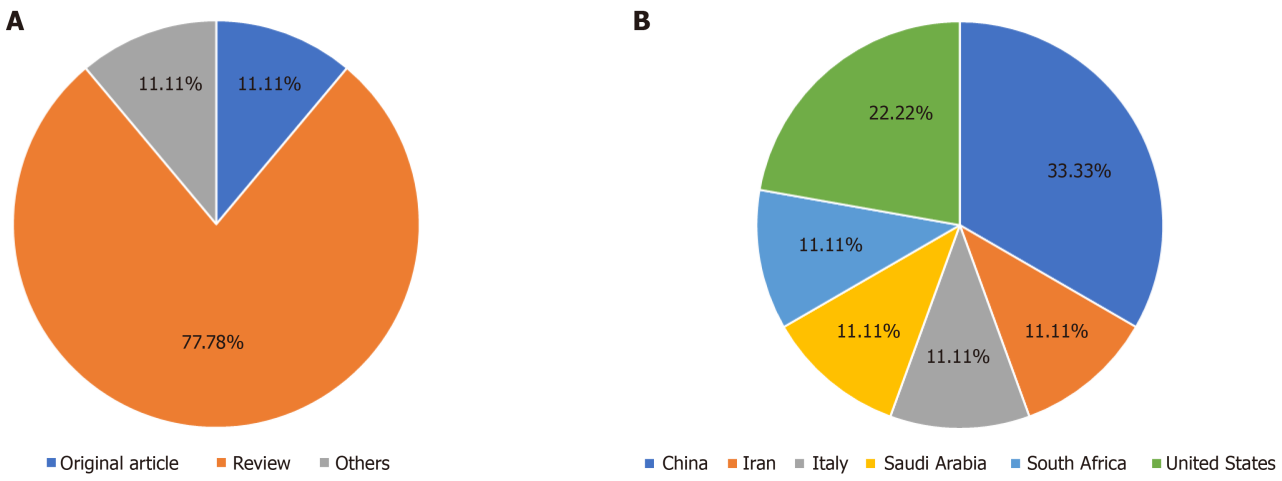

Figure 2 Bibliographic data for articles published by the *World Journal of Virology* in 2020. A: Article types; B: Authors' countries.

Despite the challenges caused by the worldwide COVID-19 pandemic, scientists made great progress in the field of virology during 2020. We would appreciate the consideration of *WJV* for virology submissions. In 2021, we will continue to apply current publishing standards and take stronger steps to grow the Editorial Board and attract more high-quality submissions.

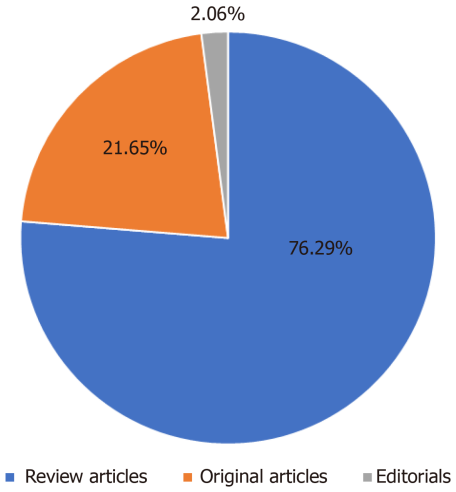

Figure 3 Titles of invited manuscripts submitted for the various types of articles for consideration of publication in 2021 by the *World Journal of Virology*.

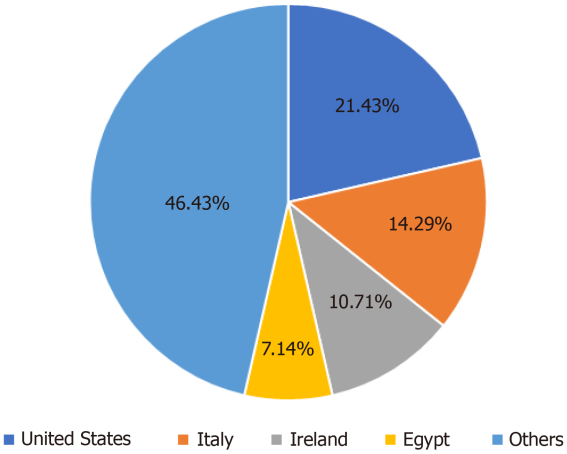

Figure 4 *World Journal of Virology* Editorial Board members are from 17 countries or regions.

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REVIEW

Bottom-up analysis of emergent properties of N-acetylcysteine as an adjuvant therapy for COVID-19

Asimina Dominari, Donald Hathaway III, Abdulhusein Kapasi, Trissa Paul, Sarabjot Singh Makkar, Valeria Castaneda, Sirisha Gara, Bishnu Mohan Singh, Kuchalambal Agadi, Maliha Butt, Varadha Retnakumar, Spandana Chittajallu, Rahima Taugir, Muhammad Khawar Sana, Manish KC, Sarah Razzack, Niala Moallem, Alina Alvarez, Michael Talalaev

ORCID number: Asimina Dominari 0000-0002-4023-9767; Donald Hathaway III 0000-0002-1613-6362; Abdulhusein Kapasi 0000-0001-5913-6912; Trissa Paul 0000-0002-2884-5756; Sarabjot Singh Makkar 0000-0003-0008-4876; Valeria Castaneda 0000-0003-0673-8690; Sirisha Gara 0000-0002-5903-8420; Bishnu Mohan Singh 0000-0002-5711-9948; Kuchalambal Agadi 0000-0001-8025-1261; Maliha Butt 0000-0002-5563-062X; Varadha Retnakumar 0000-0001-9018-8235; Spandana Chittajallu 0000-0002-3985-0809; Rahima Taugir 0000-0001-5769-6203; Muhammad Khawar Sana 0000-0003-1952-8203; Manish KC 0000-0003-1693-6068; Sarah Razzack 0000-0002-8405-5505; Niala Moallem 0000-0003-4913-7684; Alina Alvarez 0000-0002-3814-1904; Michael Talalaev 0000-0002-5343-5038.

Author contributions: Dominari A and Hathaway III D defined the topic and designed the study, Dominari A coordinated the writing of the manuscript, Alvarez A and Talalaev M provided critical reviews, all authors contributed to the literature search, all authors wrote the original manuscript, all authors assisted in reviewing and editing the manuscript, all authors consented for publication of the finalized manuscript.

Asimina Dominari, Donald Hathaway III, Abdulhusein Kapasi, Trissa Paul, Sarabjot Singh Makkar, Valeria Castaneda, Sirisha Gara, Bishnu Mohan Singh, Kuchalambal Agadi, Maliha Butt, Varadha Retnakumar, Spandana Chittajallu, Rahima Taugir, Muhammad Khawar Sana, Manish KC, Sarah Razzack, Niala Moallem, Alina Alvarez, Michael Talalaev, Division of Research and Academic Affairs, Larkin Health System, South Miami, FL 33143, United States

Corresponding author: Donald Hathaway III, MD, Division of Research and Academic Affairs, Larkin Health System, 7031 SW 62nd Avenue, South Miami, FL 33143, United States. donald.hathaway@larkinhospital.com

Abstract

N-acetylcysteine (NAC) is an abundantly available antioxidant with a wide range of antidotal properties currently best studied for its use in treating acetaminophen overdose. It has a robustly established safety profile with easily tolerated side effects and presents the Food and Drug Administration's approval for use in treating acetaminophen overdose patients. It has been proven efficacious in off-label uses, such as in respiratory diseases, heart disease, cancer, human immunodeficiency virus infection, and seasonal influenza. Clinical trials have recently shown that NAC's capacity to replenish glutathione stores may significantly improve coronavirus disease 2019 (COVID-19) outcomes, especially in high risk individuals. Interestingly, individuals with glucose 6-phosphate dehydrogenase deficiency have been shown to experience even greater benefit. The same study has concluded that NAC's ability to mitigate the impact of the cytokine storm and prevent elevation of liver enzymes, C-reactive protein, and ferritin is associated with higher success rates weaning from the ventilator and return to normal function in COVID-19 patients. Considering the background knowledge of biochemistry, current uses of NAC in clinical practice, and newly acquired evidence on its potential efficacy against COVID-19, it is worthwhile to investigate further whether this agent can be used as a treatment or adjuvant for COVID-19.

Key Words: N-acetylcysteine; Antioxidant; COVID-19; SARS-CoV-2; Treatment

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Core Tip: N-acetylcysteine is a long known antioxidant that is currently best studied for its use as an antidote for acetaminophen overdose. Its off-label use in various diseases, such as chronic respiratory disease, heart disease, cancer, human immunodeficiency virus infection, and seasonal influenza, has shown promising results, as have recent clinical trials investigating the potential benefits of N-acetylcysteine in patients with coronavirus disease 2019.

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INTRODUCTION

N-acetylcysteine (NAC) is a glutathione precursor derived from L-cysteine, long known for its antioxidant properties. NAC has a variety of clinical benefits, seen in cough, dry eyes, and influenza. It is also commonly used as an antidote for acetaminophen overdose and as a means to reduce nitrate tolerance. This medication has been recommended by the World Health Organization as an antidote in poisoning since the 1960s. NAC is also a common ingredient found in certain cosmetics and vitamin supplements[1].

NAC has been proposed as a potential prophylactic or adjuvant for coronavirus disease-19 (COVID-19) therapy, a cost-effective alternative for mild to severe cases. NAC is routinely used in the prevention and adjuvant treatment in conditions with thick and tenacious mucus production, such as pneumonia, cystic fibrosis, chronic bronchitis, and postoperative pulmonary complications. It has unbound sulfhydryl groups that break disulfide bonds of the glycoprotein matrix within the mucus, which helps dissolve the mucus, making NAC a potent mucolytic. NAC is not only responsible for managing the redox state by replenishing the thiol stores, but it is also a cysteine precursor, making it a durable antioxidant[2].

The number of Americans who have perished from COVID-19 is nearly double that of World War I and almost two to three times that of Nagasaki's atomic bombing. Therefore, it is vital to use the best therapeutic approaches possible to help contain COVID-19. There are currently numerous studies being carried out to test the efficacy of NAC in COVID-19 patients. A clinical trial called 'Efficacy and Safety of Nebulized Heparin-NAC in COVID-19 Patients by Evaluation of Pulmonary Function Improvement' investigates whether this method can decrease ventilator use in COVID-19 patients. Another clinical trial called "A study of NAC in Patients With COVID-19 Infection" is testing the number of patients being taken off the ventilator, the number of patients released from the Intensive Care Unit, and the number of patients discharged from the hospital after treatment with NAC (for a complete list of current clinical trials on the use of NAC in COVID-19, please refer to the "Ongoing Clinical Trials" section). NAC could also be immensely beneficial as prophylaxis in front-line workers, but its benefits are yet to be studied. Further testing is necessary for assessing potential medical gain and validation of this therapeutic approach[2,3].

STRUCTURE

NAC is known by many different names, such as acetylcysteine, NAC, or R-mercaptate. The organic compounds class is known as N-acyl-alpha-amino acids[4]. Cysteine is converted to NAC *via* acetylation. Cysteine, among a few other amino acids, is a small molecule, and its structure is NH2-CH (CH2-SH) COOH[5]. Cysteine contains sulfanyl (-SH) in its side chain, which are helpful in the movement of living cells and ions by forming channels. The formation of disulfide bonds between cysteine

are known to unravel different proteins. Cysteine is made of many occupied and unoccupied orbitals such as O-2p, C-2p, S-4s+3d orbitals, N-no ($n > 3$), O-np ($n \geq 3$) and sulfur-ns+md ($n > 4$, $m > 3$), S-3sp, O-2sp[6-8]. Its structure can explain the function and clinical significance of NAC. According to the dynamic rotational isomeric state formalism, there is a frequent timed transition of a molecule from one isomeric state to another isomeric state. The transition rate can be calculated from the molecular dynamics simulations of Gly-Gly-X-Gly-Gly peptides, where X is one of the amino acids. This has been recorded in the lab experiments by the fluorescence tag, by Ramachandran[9].

Molecular dynamics, explained by the dynamic rotational isomeric state formalism, illustrate the torsional transition from Psi to Pi and vice versa. According to the study, these torsional rotations of amino acids are influenced by temperature, molecular weight, and pressure. They studied different amino acids and found that rate constants for different amino acids are reflective of the flexibility of the side chain. These transitions are determined by the carboxyl and amino end of the amino acids. Unlike other amino acids, Cys, Trp, Tyr, and Met don't have specified constants since they are known as "efficient quenchers"; they accept the free electrons into their outermost orbit and become stabilized. This process also gives NAC its antioxidant effects. NAC is a protein, and like other proteins, it is a dynamic molecule. The cysteine component of NAC contributes to this[6,9].

The chemical structure is C5H9NO3S. The IUPAC name for NAC is (2R)-2-acetamido-3-sulfanylpropanoic acid. Its molecular weight is 163.2 g/mol. It is an N-acetyl-L-Amino acid from the N-acetylated derivative of the natural amino acid L-cysteine[6]. NAC is composed of cysteine and an acetyl group attached to the amino group of cysteine[10,11]. It is a white crystalline powder with a slightly acidic odor and a sour taste. It has a specific optical rotation of +5 degrees at 20 °C, and it is stable in ordinary light and temperatures up to 120 °C. NAC is non-hygroscopic, meaning it oxidizes in moist air[12]. NAC exerts its antioxidant effects in multiple ways. It is a precursor of reduced glutathione (GSH) and cysteine *via* a deacetylation reaction. GSH, in turn, has both direct and indirect antioxidant effects. NAC acts as a direct antioxidant on NO_2 and Homeobox. NAC also acts as an antioxidant by breaking the thiolated proteins, a form of organosulfur compound (R-SH). By this action, it releases free thiols as well as reduced proteins like mercapto-albumin[2].

SOURCES

The human body can naturally produce cysteine in small amounts. This production requires adequate amounts of folate, iron, and vitamins B6 and B12. These nutrients can be found in beans, lentils, spinach, bananas, salmon, and tuna. Protein-rich foods are also a good source of cysteine. The top high-cysteine-containing foods include pork, beef, chicken, fish, lentils, oatmeal, low-fat yogurt, sunflower seeds, and cheese[13]. High dietary nitrogen sources are found in both animal sources, fruits, and vegetables. Meat sources include poultry, fish, shellfish, beef cuts such as tenderloin and top sirloin, and pork. The principal dietary sources of acetyl-coenzyme A are egg yolk, liver, kidney, broccoli, and milk. Substantial concentrations of pantothenic acid are also found in chicken, beef, potatoes, and whole-grain[14].

Plant foods rich in nitrogen sources are tofu and soy-based proteins, beans (lentils, black beans, kidney beans), and sesame seeds. According to the Centers for Disease Control and Prevention, leafy green vegetables, such as spinach, lettuce, and beetroot, are the richest nitrate source that can be included in the diet[15].

ANALYSIS AND EXTRACTION

Total NAC from human plasma can be obtained through liquid chromatography-tandem mass spectrometry[16]. Recognition by mass spectrometry can be done through positive electrospray ionization and various reaction surveillance modes. NAC transition pairs and isotope-labeled internal standards are obtained. Trichloroacetic acid has been shown to improve extraction recovery yields. The blank matrix can be used to reduce the effect of endogenous NAC[17].

Lewis *et al*[18] discussed the use of the high-performance liquid chromatography method for NAC in human plasma and urine using a dinitrophenyl derivative of NAC with a Carbon 18-bonded reverse-phase column A mobile methanol phase citrate solution, used to reach a retention time of congruent to 13 min at a flow rate of 1

mL/min. For the NAC assay in urine, there is a slight modification. The assays' sensitivity limits were determined as 60 ng/mL for the plasma and 200 μg/mL for the urine.

NAC's oxidation process yields disulfides and artifacts, making it difficult to perform an assay in biological systems. Also, biological systems have thiols like cysteine and glutathione that have physical and chemical properties like that of NAC. Hence, it is always important to receive NAC in its reduced form quickly. This is possible *via* chemical derivatization of NAC using several electrophilic agents, leading to the formation of secure adducts. These adducts are more easily separated by chromatography than the main compound and display properties like fluorescence, which helps recognize and quantify them. Reagents which are required for derivatization and assay of NAC include: N-(1-Pyrene) maleimide; N-(7-Dimethylamino-4-methylcoumarinyl) maleimide; 4-(Aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole; Ammonium 7-fluoro-2,1,3-benzoxadiazole; 2,4-Dinitro-l-fluorobenzene; Monobromobimane; and o-Phthalaldehyde. The derivatization is done in basic pH since most of the reagents interact with the thiolate anion of NAC. However, oxidation of NAC increases quickly with basic pH such that the derivatizing agents must interact quickly with the remaining NAC in the sample before thiol is extracted. As thiols are present in the biological samples, it is important to add sufficient reagents to permit quantifiable recovery of the NAC adduct from such biological specimens. The assay protocol for NAC should include the capacity to ascertain the redox condition of the thiol. Acid precipitation and reduction allow for oxidized NAC formation in disulfide forms, and NAC intermingled with disulfides and proteins. This can be done by dividing the soluble and protein components of the specimen by acid precipitation, followed by reducing these constituents with reducing agents like dithiothreitol. Finally, extra NAC derivatives are obtained from the oxidized specimens[19-22].

STORAGE

A study conducted by Siddiqui *et al*[23], 2016, NAC was reported to be the most fragile cell reinforcement agent among endogenous thiol mixes. It was found to be more stable in an aqueous arrangement. It was exposed to dependability reads for 24 h with a 4 h span, and the outcomes were as far as rate debasement. The outcomes recommend that there was a corruption of 0.89% and 0.48% in the solution put away at room temperature and in refrigerated conditions, individually[23]. Unopened vials of acetylcysteine sodium solutions ought to be stored at 15-30 °C. Following the exposure to air, the orally taken solutions should be stored at 2-8 °C to hinder oxidation and should be utilized within 96 h[6]. Acetylcysteine arrangement doesn't contain any antimicrobial operator; therefore, care must be taken to limit the sterile arrangement's pollution. Once opened, the vial should be put away in the fridge, and the opened vial ought to be disposed of after 96 h.

In the long haul (2 mo) steadiness study conducted by He *et al*[24] in mice using analytical methods, N acetylcysteine amide and N acetylcysteine spiked in plasma at -20 °C, with a recovery extending from 103.5% to 111.5% for N- acetylcysteine amide and from 99.7% to 105.4% for NAC, demonstrating that keeping the agent at -20 °C is an option when plasma can't be examined right away. In fluid arrangements (10 mmol/L NH4HCO3, pH: 7.4), recuperation paces of 91.8% to 102.1% were acquired for NAC amide and 4 °C or -20 °C for NAC at room temperature, demonstrating that watery/stock arrangements are steady for long-term studies. This proves that NAC amide was likewise stable in physiological saline at RT and 4 °C (91.0%-116.1%), while less stability was seen in 5% glucose at high fixation at RT (86.6%), recommending that NAC amide ought to be ideally put away at 4 °C when 5% glucose is utilized in future clinical settings[24].

BIOLOGICAL MECHANISMS AND HEALTH BENEFITS

NAC plays several roles in medicine, and different mechanisms of action have been postulated for the various roles. When used for acetaminophen poisoning, it acts by restoring hepatic concentrations of GSH, an antioxidant that metabolizes acetaminophen into nontoxic soluble intermediates. When there is acetaminophen overdose, reduced glutathione stores in the liver are depleted, resulting in the accumulation of the toxic intermediate N-acetyl-p-benzoquinone imine. NAC helps

replenish glutathione stores by being metabolized into L-cysteine, which is a glutathione precursor. It is suggested that the thiol group contained in NAC can also directly inactivate the toxic metabolite[25].

NAC is also used as a mucolytic through the lytic effect of its free sulfhydryl group on the disulfide bonds in mucus, which helps lower the viscosity of mucus. It is found to have positive neuropsychotropic effects through its metabolite L-cysteine, which also serves as a precursor of cysteine, a substrate for the cystine-glutamate antiporter on astrocytes. Increased cystine levels increase glutamate release into the extracellular space. Thus, NAC has been suggested as an adjuvant in the treatment of Parkinson's disease, Alzheimer's disease, neuropathic pain, and stroke[26].

The role of NAC in viral infections has been investigated since the early 1990s. In 1993, Roederer *et al*[27] investigated the role of thiol replenishment therapy in the treatment of human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS). They showed that NAC can inhibit inflammatory stimulation *in vivo*, including that caused by HIV replication[27]. On the other hand, Geiler *et al*[28] explained that NAC can inhibit H5N1 replication and H5N1-induced production of pro-inflammatory molecules. The mechanism behind these findings is mostly explained by NAC's effect on reactive oxygen species (ROS). ROS is produced *via* multiple pathways during viral infections, including mitochondrial reactions, degradation of lipids and proteins, and, importantly, from respiratory burst reactions in phagocytes. Several viruses such as HIV-1, Respiratory Syncytial Viral, H5N1 have been shown to increase oxidative stress in the host by dysregulating the oxidative stress pathways and causing an escalation of ROS synthesis. While high levels of ROS help in the phagocytosis and apoptosis of infectious organisms, low levels promote viral replication and mutations resulting in the development of resistant strains. ROS also causes significant host cell damage and lysis[29]. NAC scavenges ROS directly through direct interaction with target proteins containing a cysteine residue or thiol group such as Raf-1, MEK, and ERK *via* a thiol-disulfide exchange reaction, and indirectly by increasing synthesis of GSH. This potent antioxidant catalyzes the reduction of hydrogen peroxide to water and oxygen and the reduction of peroxide radicals to alcohols and oxygen. NAC also protects cells from apoptosis by chemically forming inactive adducts or complexes with several 18b-glycyrrhetinic acid derivatives, which induce apoptosis by activation of caspase-8 and caspase-9 and downregulation of anti-apoptotic proteins like c-FLIP, XIAP, and Mcl-1[30].

NAC has various anti-inflammatory actions, including the inhibitory effect on inflammatory cytokines such as CXCL8, CXCL10, CCL5, that are responsible for neutrophil recruitment, Th1 response, and NK and CD8 cell trafficking, as well as on interleukin-6 (IL-6), which is responsible for stimulation of acute-phase responses, hematopoiesis, and immune reactions. It also regulates proinflammatory kinases, such as nuclear factor kappa B (NF-kB) and p38 through activation of GSH and direct antioxidant effect of its free thiol group. NF-kB is a redox-sensitive transcription factor that regulates the expression of pro-inflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor-alpha, as well as genes associated with apoptosis, such as p53, and is activated by increased ROS levels. NAC, a glutathione precursor, inhibits NF-kB by S-glutathionylation of the p65 subunit of NF-kB, resulting in blockage of TNF-alpha activation and nuclear translocation of NF-kB-p65. The latter results in reduced synthesis of inflammatory cytokines[31].

NAC has also been reported to promote lymphocyte proliferation, which is inversely affected by oxidative stress and low GSH levels. T cell exhaustion, which refers to low levels of CD4+ and CD8+ levels, commonly occurs in chronic viral infections and is considered to be caused by inflammatory cytokines, TNF-alpha, IL-6, IL-10. NAC's antioxidant effect helps to improve the redox balance, which helps protect and promote lymphocyte proliferation[32].

Another mechanism of its anti-inflammatory effect is the inhibition of the NLR family pyrin domain containing 3 (NLRP3) inflammasome pathway. NLRP3 inflammasome is a well-known trigger of the cleavage and activation of caspase-1, leading to maturation and secretion of interleukin-1β and interleukin-18. Overactivation of this inflammasome is critical in the pathogenesis of several disorders, such as Crohn disease, atherosclerosis, gout, type 2 diabetes mellitus, and chronic infections. Data from both severe acute respiratory syndrome coronavirus (SARS-CoV)-1 and SARS-CoV-2 patients show evidence of increased NLRP3 inflammasome activity. NAC blocks NLRP3 inflammasome activation by interfering with the priming step required to induce NLRP3 expression. It is also shown to work in a dose-dependent manner to reduce mRNA expression of NLRP3 inflammasome and caspase-1, a large pro-inflammatory enzyme that causes the production of interleukin-1β and interleukin-18, as well as the recruitment of neutrophils[33].

As it has come to be known, NAC has been used in practice for several decades now. It has served as a mucolytic agent, contributing to the breakdown of mucus in the respiratory tract and keeping the tract moist to decrease irritation. By reacting with hydroxyl radicals, superoxide, hydrogen peroxide, and peroxynitrite radicals, NAC helps reduce the disulfide bonds in proteins[34]. Since it is a cysteine pro-drug and a GSH precursor, it can also help scavenge free radicals such as those mentioned above. NAC has anti-inflammatory activity already mentioned in the previous section, and it accomplishes this *via* the inhibition of nuclear factor-kappa light chain enhancer of activated B cell (NF-kB). An example of a disease with oxidative stress implicated in its pathogenesis and progression is chronic obstructive pulmonary disease (COPD). The oxidative species are from the inhalation of cigarette smoke and those formed within the body by inflammatory cells. This leads to an increase in oxidant stress in the lung. NAC's antioxidant property plays a crucial role in COPD patients to reduce their symptoms, acute exacerbations, and the decline in lung function[35].

The known health benefits of NAC are mainly exerted at the cellular level. A study conducted by Kinscherf *et al*[35] in 1994, using healthy human subjects, showed that people with intracellular glutathione levels of 20-30 nmol/mg had higher numbers of CD4+ T cell numbers than people who had higher or lower glutathione levels. Once the patients in the 4-wk observation period moved from the optimal to the suboptimal range, which meant from 20-30 nmol/mg to 10-20 nmol/mg, they ended up with a 30% decrease in CD4+ T cells. This 30% decrease was prevented by using NAC as a treatment. They found that NAC causes a relative increase of CD4+ T cell numbers even though the glutathione levels decrease but not by increasing the glutathione levels either. They discovered that NAC, which determines glutathione levels, has a strong influence not only on cysteine and glutathione levels but also on T cells in the human body[36].

SAFETY PROFILE AND ADVERSE EFFECTS

NAC is administered in the intravenous, oral, and nebulized forms. It is used as adjuvant therapy in respiratory conditions and can be administered in a nebulized form or be directly instilled. The inhaled form can be given by nebulization through a face mask, mouthpiece, or tracheostomy. Alternatively, inhalation through a tent or croupette is also available[37]. Acetylcysteine solutions of 10% and 20% are used in adult, geriatric and pediatric patients receiving the inhaled dosage employing face mask, mouthpiece, or tracheostomy. The 20% solution is diluted with sodium chloride or sterile water for inhalation. The 10% solution can be used undiluted[37].

When administered orally at a dose of 1200 mg/d for six months, De Flora *et al*[37] found that NAC reduced symptoms of influenza in patients over the age of 65 years with chronic degenerative diseases. The NAC recipients suffered from influenza less and only had fewer influenza-like episodes with fewer days confined to bed. Though NAC played no role in viral seroconversion, symptomatic infection episodes were considerably less[37].

The effectiveness and tolerability profile of high-dose NAC was studied in a trial, where NAC at a dose of 1200 mg/d, 600 mg/d, or placebo was given once daily for 10 d to patients with COPD exacerbations Evidence showed that a significant proportion of patients had normalization of C-reactive protein (CRP) levels which was obtained with both NAC 600 and 1200 mg/d compared to placebo. The same study demonstrated NAC's therapeutic superiority in decreasing the IL-8 Levels with a dose of 1200 mg/d rather than 600 mg/d. Both treatment regimens' effects were equally effective in terms of lung function and other clinical outcomes, including the intensity and frequency of cough and Korsakoff sounds. Adverse events were reported only in one patient amongst the 1200 mg/d NAC groups, whereas; two events were seen in the placebo group[38].

Therefore, oral NAC (600 mg/d) could function as a preventive measure in those who are repeatedly exposed to possible SARS-CoV-2 carriers like health workers and those who cannot work at home. Healthcare workers worldwide have become infected while caring for hospitalized patients; therefore, 600 to 1200 mg daily NAC could potentially help to flatten the exponential curve in several countries[39].

In severe cases of COVID-19, ventilator use is common, with roughly 3.2% of all cases requiring mechanical ventilation at some point during the illness. The use of NAC as a prophylactic intervention for mechanical ventilation complications, such as ventilator-associated pneumonia (VAP), has been studied in a randomized controlled trial involving nasogastric administration of 1200 mg NAC daily. It was found that

patients treated with NAC had fewer incidences of VAP and a shorter hospital stay. Also, the complete recovery from VAP was more frequently observed in the NAC group[40].

NAC can also be of benefit in the treatment of patients with acute respiratory distress syndrome. A clinical trial conducted in the United States and Canada found that intravenous NAC (70 mg/kg body weight), when given every 8 h for ten days, effectively reduced glutathione in RBCs, thereby decreasing lung injury. Additionally, it helped increase the cardiac index[41]. Administration of NAC (50 mg/kg body weight in 250 mL of 5% dextrose for 6 d) was found to protect the lung tissue in acute respiratory distress syndrome patients. The effectiveness of NAC was quantified by measuring the expired ethane and malondialdehyde along with glutathione disulfide and GSH in the epithelial lining fluid[42]. In another study, intensive care unit (ICU) patients who received NAC at a dose of 150 mg/kg body weight on the first day, followed by 50 mg/kg for a total of 3 d, appeared to have a better clinical outcome when compared to the placebo group[43].

The use of NAC has been established in a clinical study in which isosorbide dinitrate, given its vasodilator properties, was given to six male participants for a period of 48 h. NAC was administered at 24 h in a dose of 2 g intravenously, followed by 5 mg/kg/h. The plasma concentration of angiotensin II increased for the duration of the first 24 h of isosorbide dinitrate administration, but the levels decreased by 28 ng/L to 14 ng/L ($P < 0.05$) just 2 h after NAC was started[44]. This effect could postulate that NAC's protective effects counteract the harmful effects of angiotensin II in SARS-CoV-2. NAC has an exceptional safety history in clinical trials. The side effects of oral NAC include stomatitis, nausea, vomiting, gastroesophageal reflux. If an anaphylactoid reaction occurs with intravenous NAC, then oral NAC may be used instead[45,46]. Bronchoconstriction and extended coughing, and worsening of asthma were the side effects of nebulized NAC[47,48].

The harmful effects of NAC are mainly dependent on its route of administration. A clinical study investigated the pharmacological profile of a six-month administration of oral NAC in 26 volunteers. The main adverse effects seen were mostly gastrointestinal symptoms; intestinal gas, diarrhea, nausea, and fatigue, with the maximum nontoxic dose being 800 mg/m^2/d[49]. Another trial studied the effects of oral administration of NAC at high doses of up to 8000 mg/d in HIV patients, and no adverse effects were reported[50]. Severe anaphylactoid reactions like hypotension, bronchospasm, and angioedema were noted to occur with initial loading infusions of NAC, which resulted in temporary increased plasma concentrations of NAC. These symptoms were promptly resolved after discontinuation of the drug[51]. Nevertheless, severe systemic reactions are rare. NAC does not require dosage adjustments in renal or hepatic impairment[52]. The risk of sound-alike error can be observed with acetylcysteine, which may be confused with acetylcholine, and mucomyst, which may be confused with Mucinex.

All patients (adult and pediatric) should receive an aerosolized bronchodilator 10-15 min before NAC administration. In adults, 3 to 5 mL of the 20% solution or 6 to 10 mL of the 10% solution is given through nebulization up to 3 or 4 times/d. The standard dosing range for the 20% solution is 1 to 10 mL and 2 to 20 mL for the 10% solution every 2 to 6 h. For inhalation of the 10% or 20% solution in the form of a heavy mist *via* a tent or croupette, the dose must be individualized and may require up to 300 mL solution/treatment. Children and adolescents are usually given the adult dosage, but in infants, 1 to 2 mL of 20% solution or 2 to 4 mL of 10% solution is used. NAC can also be given through direct administration into the tracheostomy in adults. 1 to 2 mL of the 10% or 20% solution is introduced every 1 to 4 h. When administered through a percutaneous intratracheal catheter, 1 to 2 mL of the 20% or 2 to 4 mL of the 10% solution should be instilled every 1 to 4 h *via* a syringe attached to the catheter. In children and adolescents, 1 to 2 mL of 10% to 20% solution can be instilled every 1 to 4 h as needed *via* the endotracheal tube. The dosage remains the same for percutaneous endotracheal instillation[53].

Different adverse events have been reported with NAC, and they range from nausea to death. Although NAC's severe reactions look like anaphylaxis, they are non-immunological and hence classified as anaphylactoid reactions. Other adverse events that have been reported infrequently in studies of NAC include dizziness, fever, vertigo, localized skin rash, dyspnea, tachycardia, hypertension, cardiac arrest[54]. Oral NAC has been rarely associated with serious adverse events. However, repeated high doses may cause nausea, vomiting, diarrhea, and rarely headache, rash, hypotension, and respiratory distress[55].

Urticaria and hepatotoxicity have also been reported. High-dose Intravenous NAC has been associated with anaphylactoid reactions like flushing, rash/pruritus,

angioedema, bronchospasm, nausea/vomiting, hypotension, tachycardia, and respiratory distress[56]. There are also case reports that describe ECG abnormalities, status epilepticus, and a serum sickness-like illness[57-59].

NAC is contraindicated in persons with previous severe anaphylactoid reactions or hypersensitivity reactions associated with its use. Should be cautiously used in pregnant women as it crosses the placental barrier, those with a family history of drug allergy, and patients with asthma or bronchospasm. It should not be used in acute paraquat poisoning. Nebulised NAC should be used cautiously in patients with respiratory insufficiency, an inadequate cough mechanism, or gag reflex depression. At the same time, oral NAC can exacerbate vomiting for which precautions should be taken to use in patients with esophageal varices and peptic ulcers. Acetylcysteine effervescent tablets should also be cautiously used in patients with sodium-restricted diets like hypertension, heart failure, and renal disease[60].

CLINICAL APPLICATIONS

NAC has been used for more than 30 years and is best known for its use in acetaminophen overdose. It can be used in several other diseases like chronic bronchitis, HIV, influenza, heart disease, and several other poisonings. It can be used in acetaminophen overdose and respiratory diseases like pneumonia, tracheobronchitis, cystic fibrosis, tracheostomy patients, postoperative pulmonary complications, and posttraumatic chest conditions. Its off-label uses are acute hepatic failure and prevention of contrast-induced nephropathy[45].

Acetaminophen overdose

The treatment for acetaminophen overdose is NAC. It is proved that NAC's early administration within 8 to 24 h prevents mortality[45]. Interestingly, it has recently been suggested that a shorter 12-h regimen of NAC be used in these patients, instead of the conventional regimen of 20-21 h in duration. The rationale behind this recommendation is the ability to preserve resources in the current shortage conditions while ensuring effective treatment of the most common cause of excessive medicine ingestion[61].

Respiratory diseases

A study by Cotgreave *et al*[61] observed the levels of NAC in the bronchoalveolar lavage of six healthy volunteers following administration of 600 mg of NAC orally for four weeks. Although the levels of NAC, cysteine, and glutathione in the bronchoalveolar lavage fluid did not increase, the levels of protein-bound NAC and both free and total plasma glutathione were shown to rise significantly[62]. On the other hand, a study by Rodenstein *et al*[62] demonstrated that NAC given orally to people with respiratory disorders led to a similar NAC level in the plasma and lung tissue. NAC has been used as a mucolytic agent in chronic bronchitis. Although initial studies like the one by Millar *et al*[63] showed no significant effect in patients with chronic bronchitis, a study by Parr *et al*[64] showed that there is a substantial decrease in the number of incapacitated days in the individuals suffering from chronic bronchitis.

Additionally, Rasmussen *et al*[65] conducted a double-blind, placebo-controlled, six-month comparison study, which showed that the NAC treatment group had a lower number of sick-leave days and exacerbation days. Jackson *et al*[66] conducted a multicenter, double-blind, placebo-controlled study that found that the difficulty in expectoration and cough severity improved and was more evident in patients using NAC. Behr *et al*[67] studied the effect of NAC administration for 12 wk on 18 patients suffering from fibrosing alveolitis, a disease known for the uncontrolled activation of the oxidative stress response, as well as for the reduced levels of GSH in the lower respiratory tract. This treatment led to improved pulmonary function tests and an increase in total and reduced glutathione[68]. NAC has shown some preventive effect of microembolism in a rat model having acute respiratory distress syndrome by decreasing alveolar edema, fibrin deposition, and plasma viscosity.

Cancer

NAC has been proven to have some beneficial effects on cancer and its management. Though evidence is still preliminary, a few studies have shown its efficacy when combined with chemotherapeutic agents. De Flora *et al*[69] have studied NAC's effect on GSH metabolism and the biotransformation of carcinogenic compounds. *In vitro* and *in vivo* studies have shown that NAC counteracted the mutagenicity of direct-acting

compounds and, at high concentrations, inhibited procarcinogens' mutagenicity[70]. This study has also combined NAC with doxorubicin and found that, under certain experimental conditions, it can be highly effective by working synergistically with doxorubicin to reduce tumor formation and prevent metastases. Pre-treatment with NAC increased the non-protein content of P388 Leukemia cells nearly threefold, without negatively affecting the chemotherapeutic activity of doxorubicin against this tumor.

Heart disease

NAC is also useful in heart disease. It affects the levels of homocysteine and possibly even the levels of lipoprotein A. Moreover, it protects against ischemic and reperfusion damage and increases the efficacy of nitroglycerine. Gavish and Breslow *et al*[71] proved that NAC administration to patients with increased lipoprotein levels had reduced plasma lipoprotein levels by 70%. Wiklund *et al*[72] postulated that NAC administration reduces plasma homocysteine levels by 45% but did not show any effect on lipoprotein levels. Bostom *et al*[73] reported that even in dialysis patients who have high homocysteine levels and are refractory to vitamin B supplementation, oral NAC supplementation resulted in a 16% decrease in non-fasting pre-hemodialysis total plasma homocysteine[74]. In combination with nitroglycerin and streptokinase, NAC decreased the oxidative stress and preserved left ventricular function in patients with evolving acute myocardial infarction[75]. In combination with nitroglycerin, NAC should be used with caution because of the adverse effects[76].

Cigarette smoking

Oral supplementation with NAC is necessary for smokers and people exposed to second-hand smoke, as NAC has been proven to decrease smoking-induced mucus cell hyperplasia, epithelial hypertrophy, and the time required for the secretory cells to return to normal[77].

HIV

HIV-positive individuals have low cysteine and GSH levels. Supplementation of NAC in these individuals has been studied, and the results are still unclear. Wu *et al*[76] observed that NAC administration had increased the ability of cells to form T-cell colonies in people with AIDS[78]. Herzenberg *et al*[77] noted that the oral administration of NAC in HIV-infected individuals improves GSH levels and aids in the improvement of survival rates in this population[79]. Sandilands *et al*[80] suggested that NAC administration to HIV-infected individuals prevented the progression to AIDS. Though further evidence is needed to determine NAC's efficacy in HIV-positive individuals, based on the available evidence, NAC supplementation can be considered an essential component of anti-HIV treatment in individuals with low GSH levels[81].

Other uses

NAC usage in individuals with influenza and influenza-like episodes decreased the symptoms but did not prevent the disease. NAC is also used in myoclonic epilepsy, where it has been shown to reduce the myoclonus. Finally, NAC is of benefit in Sjogren syndrome, where it is considered to help improve ocular soreness, irritability, halitosis, and daytime thirst[82].

PREVIOUS HUMAN EXPERIENCE

NAC is a powerful drug used for a variety of treatments, including pulmonary and liver diseases. Different *in vitro* and *in vivo* studies were performed to demonstrate NAC's efficacy as an antioxidant in COPD. Data has shown that oxidative stress acts as an essential pathogenetic factor in altering the lungs of patients with COPD. Open-label and double-blinded clinical studies with patients with and without COPD were used to conclude that the ability of NAC to protect the lungs against toxic agents is through its antioxidant properties. Results show that in patients with COPD, a dose of 600 mg daily accounted for the reduced risk of exacerbations and viscosity of expectorations. After two months of treating patients with NAC, the viscosity improved by 80%, the severity of the cough improved by 71%, and the difficulty of expectoration by 74%. However, a different double-blind, double-dummy, controlled study with 120 patients suggested that 1200 mg was the correct dosage to see improvements in COPD patients[83].

Another study with acute coronary syndrome patients was designed to determine

the effectiveness of rapid intravenous hydration with sodium bicarbonate plus NAC to prevent contrast-induced nephropathy in patients undergoing percutaneous coronary intervention. The study focused on 120 patients that were consequently divided among group A and group B. The first group received an initial intravenous bolus of 5 mL/kg/h of alkaline saline solution with 154 mEq/L of sodium bicarbonate in 5% glucose and H_2O plus 2400 mg of NAC in the same solution. The next day, patients received two doses of 600 mg NAC. In contrast, Group B was treated with perfusion of isotonic saline (0.9%) at a rate of 1 mL/kg/h for 12 h after percutaneous coronary intervention plus two doses of 600 mg NAC orally the next day. After collecting samples and stating that the development of acute contrast-induced nephropathy refers to an increase in serum creatinine concentration of 0.5 mg/dL or more, data analysis was performed. Data indicated that rapid hydration with saline bicarbonate and high doses of NAC before contrast injection helps prevent renal dysfunction, and the rate of contrast-induced nephropathy decreases drastically[84].

The alleviation of polychlorinated biphenyls (PCBs) 52-induced hepatotoxicity with NAC was tested by performing an *in vitro* study in human and rat cells. Human L-02 cells supplemented with 15% fetal bovine serum and 100 U/mL penicillin-streptomycin, in addition to rat Brl-3A cells cultured with 3% fetal bovine serum and 100 U/mL penicillin-streptomycin, were utilized for the investigation. It is known that PCBs may induce human hepatotoxicity since they are a type of persistent chlorinated pollutant. In this study, cells were treated with 40 μmol/L of PCB52 for 48 h after NAC/saline pre-treatment. Exposure to PCB52 Leads to excessive production of ROS-releasing inflammatory mediators, which play an essential role in hepatotoxicity. Consequently, data was analyzed with different laboratory techniques to gather ROS levels. Results show that NAC pretreatment drastically reduced ROS levels in both rat and human cells. NAC ameliorated PCB52 reduction of cell viability, implying that the alleviation of PCB52-induced hepatotoxicity could result from the elimination of ROS[85].

CURRENT CORONAVIRUS DISEASE 2019 MANAGEMENT AND POTENTIAL IMPLICATIONS OF N-ACETYLCYSTEINE AS A SUPPLEMENTARY AGENT

The therapeutic options for COVID-19 have constantly been evolving. Many studies have shown that certain dietary elements and vitamin supplements could be promising[86] and, according to the World Health Organization's International Clinical Trials Registry Platform, there are about 3369 studies on management of COVID-19. Currently, COVID-19 management is based on the severity of the disease, patient age, and history of comorbidities[87] (Table 1). The following drugs are used as a possible therapy though still lacking evidence of efficacy. Chloroquine acts by blocking the cell fusion of the virus and also increases endosomal pH[88]. It is an autoimmune and antimalarial drug used alone or together with remdesivir and has the highest efficacy in controlling coronavirus infection[89]. The use of chloroquine or hydroxychloroquine in combination with azithromycin has been evaluated in several retrospective observational, and uncontrolled studies[90,91]. In patients on first treatment with antiviral drugs like lopinavir or ritonavir, the viral road decreased and helped with the recovery[92]. Rosuvastatin is capable of binding and inhibiting the main protease enzyme of COVID-19. Statins act by reducing chemokine release, levels of adhesion molecules, and by modulating T-cell activity. The use of statins has been postulated to affect mortality in COVID-19[93]. Monoclonal antibodies like tocilizumab act against IL-6 receptors and prevent the development of cytokine storm and severe inflammation[94]. Anakinra is another antibody utilized in the treatment of critically ill patients. By blocking the IL-1 receptor, Anakinra reduces cytokine release triggered by the virus[95]. Treatment with vitamin C enhances the internal production of vasopressors and reduces the need for norepinephrine treatment[96].

The worldwide spread of COVID-19 continues with no effective treatment in the medical armamentarium and with the first Food and Drug Administration's approved vaccines only rolling out since December 2020. It would thus be of benefit to once again look into our current understanding of the pathogenic mechanisms of SARS-CoV-2 infection. More specifically, the significant variability among the responses of different patients to COVID-19 and the importance of excessive inflammatory reaction and redox decompensation observed in critical cases of COVID-19 are both worth highlighting[97].

Table 1 Principles of coronavirus disease 2019 management according to disease severity and presence of comorbidities

Severity	No comorbidities present	Comorbidities present
Mild	Conservative at home	Steroids,or/and plasma therapy
Moderate	Conservative at home	Steroids, or/and plasma therapy
Severe	Hospitalized: Treatment focused on the complication	Intravenous fluid, oxygen, corticosteroids

Angiotensin-converting enzyme (ACE) and ACE2 proteases are present on the surface of many cell types and have the same substrates angiotensin I and angiotensin II, but the opposite activities. ACE increases levels of angiotensin II, thereby mediating vasoconstriction, apoptosis, as well as the induction of oxidative stress and inflammatory reaction. ACE2 is responsible for a decrease in angiotensin II levels and for induction of ang (1-7) peptide. As a result, ACE2 counteracts the pro-inflammatory effects of ACE[97]. By binding ACE2 at its entry into human cells, SARS-CoV-2 decreases ACE2 availability and promotes ACE activity. The latter sets the background for induction of oxidative stress, as angiotensin II stimulates the NADPH oxidase pathway for production of ROS and peroxynitrite anions[98]. The imbalance between ACE and ACE2 can become even more evident in patients with an endogenous tendency towards higher levels of ACE. It is known that ACE/ACE2 ratios can differ among people and ACE-predominant individuals can be susceptible to excessive inflammation[97].

The main defense mechanism against free radical damage is through natural scavenging systems, such as the system of reduced GSH. GSH donates an electron to an unstable molecule, such as ROS, and then becomes reactive and can rapidly bind to another reactive glutathione molecule, forming a glutathione disulfide. This is feasible under normal circumstances because of the abundant concentration of GSH in cells. GSH insufficiency arising either in the context of COVID-19 or as baseline low levels due to other conditions have been postulated to have an association with the overwhelming oxidative stress leading to COVID-19 complications. On one hand, SARS-CoV-2 infection in itself induces the synthesis of free radicals, thereby consuming GSH supplies. Given that intracellular levels of GSH tend to remain relatively stable and are regulated by various environmental stimuli, such as NF-κB, hypoxia, ROS, and reactive nitrogen species, it is no surprise that in a COVID-19 patient, less GSH may be available for other cellular functions. On the other hand, low GSH levels have additionally been identified in a series of pathologic conditions that are currently considered as risk factors for severe COVID-19: older age, male sex, diabetes mellitus, hypertension, obesity, and even certain medications[97].

The extensive study of the above biochemical mechanisms and the failure of antiviral and anti-inflammatory agents to show positive results have led several researchers to explore the effects of NAC as an adjuvant treatment in patients with COVID-19.

In July 2020, a study by Ibrahim *et al*[36] found that having glucose 6-phosphate dehydrogenase (G6PD) deficiency facilitates SARS-CoV-2 infection due to glutathione depletion. NAC can be administered to help replenish glutathione stores. They found that patients with severe COVID-19 benefited from the intravenous (IV) administration of NAC. NAC blocks the hemolysis that G6PD deficiency patients are predisposed to. It also blocks the elevation of liver enzymes, CRP, and ferritin. Blocking these enzymes allowed the G6PD deficient patients to be taken off the ventilator and the veno-venous extracorporeal membrane oxygenator and led to a full recovery. Additionally, NAC was administered to another 9 ventilator-dependent COVID-19 patients who did not have G6PD deficiency. They found that NAC promoted the clinical improvement and reduced CRP levels in all patients and ferritin in 9/10 patients. In COVID-19 patients, there are high serum levels of pro-inflammatory cytokines being reported. IL-6 has also been shown to play an essential role in the cytokine storm that is associated with COVID-19. IL-6 and CRP are one of them, and NAC has been found to reduce the IL-6 dependent CRP elevation during the H1N1 influenza pneumonia. Morbidity and mortality of the human coronavirus, causing lower respiratory tract infections, originates from the host's immune response, which includes the cytokine storm perpetuated by IL-6.

De Alencar *et al*[99] conducted a double-blind, randomized, placebo-controlled trial of NAC for the treatment of severe COVID-19 respiratory disease. The rationale behind this study was the potential for improvement in COVID-19 outcomes through mitigation of oxidative stress. In this trial, 135 patients with severe COVID-19,

saturation < 94%, tachypnea of > 24 breaths/min were included, and received 300 mg/kg NAC or placebo. 23.9% of patients on placebo and 20.6% of patients of NAC received mechanical ventilation (P = 0.675), while the need for ICU admission was 42.3% in the placebo group and 47.1% in the NAC group. The mortality rate and hospital stay were the same for both groups. The study concluded that NAC can be safely tolerated but does not seem to be of benefit to severely ill patients with COVID-19.

Alamdari *et al*[97] studied the effects of methylene blue-vitamin C-NAC (MCN, 1 mg/kg methylene blue, 1500 mg/kg vitamin C, 1500 mg/kg NAC) administration as last resort therapy in five critically ill COVID-19 patients with elevated levels of nitrite, nitrate, and methemoglobin among others. Four out of five patients recovered and were discharged from the ICU, but one patient died from sepsis shortly after initiation[100]. The results of this study demonstrate that treatment with MCN is both safe and feasible. Oxidative stress is shown to play a major role in COVID-19 and the need for earlier initiation of NAC therapy, before critical disease develops, is expressed.

A different application of NAC in COVID-19 has been presented by Melisa *et al*[101]. A patient with critical COVID-19 developed a superinfection with Pseudomonas aeruginosa and Staphylococcus aureus and progressed to respiratory failure with persistent hypercapnia. In addition to standard of care, consisting of antiviral and antibiotic agents, respiratory, and nutritional support, the patient underwent bronchoalveolar lavage with a 10-15 g NAC nebulized inhalation solution. The patient gradually recovered showing that NAC can have a dual role in COVID-19: Mucus dissolving expectorant and antioxidant effects. However, what is lacking right now is the presence of large-scale studies in order to confirm the individual outcomes.

ONGOING CLINICAL TRIALS

The clinical use of NAC in COVID-19 is still under investigation. There are few ongoing trials, but no results have been posted as of the time of this writing. The trials are as follows.

A pilot double-blinded randomized placebo-controlled multicenter clinical trial was posted in July 2020 with an estimated 1180 participants at King Saud University: The study attempts to evaluate NAC therapy's efficacy in the management of adult hospitalized patients with COVID-19, focusing on the regulation of inflammatory response. The current estimated completion date for this trial is on August 30, 2021[99].

Study of NAC in patients with COVID-19: This study has started recruiting patients. The expected time frame is from May 1, 2020 - May 2021. This study has two arms A and B. Arm A has mechanically-ventilated patients and patients managed in the critical care unit. In contrast, arm B has non-mechanically-ventilated, noncritical care patients. Patients in both arms in the experimental group and the intervention group will be treated with NAC administered intravenously at a dose of 6 g/d, along with supportive care and medications specific for COVID-19. The latter will be determined by the physician on an individual basis[100].

Patients in the experimental group will receive treatment for a maximum of three weeks or until the fulfillment of one of the criteria mentioned in the corresponding table. The treatment group will utilize NAC and peripheral blood for both mechanically-ventilated and non-mechanically-ventilated patients. In the NAC treatment group, treatment may be held for ≤ 48 h, if clinically indicated. Patients can resume treatment if the drug was discontinued for no more than 48 h. The peripheral blood used in the treatment group uses a total of 16mL of whole blood collected in CPT tubes at baseline, the first day of Cycle 2 (or as close as feasible, when still coordinating sample collection across patients in a critical-care unit), and at the end of the study[100].

Efficacy of NAC in preventing COVID 19 from progressing to severe disease: This study is a randomized clinical trial and was first started on September 23, 2020, and will run through May 31, 2021, with a sample size of 200 participants[101].

A randomized double-blinded placebo-controlled study to evaluate the safety, efficacy, tolerability, and pharmacokinetics of OP-101 (Dendrimer N-acetyl-cysteine) in severe COVID-19: The anticipated primary completion date is within a week as of this writing, on October 10, 2020, and will be one of the earliest phase 2 trials with anticipated results. The primary outcome in this trial is "treatment-

emergent adverse events", and secondary outcomes include time to improvement based on the World Health Organization 7-point ordinal scale, time to improvement in oxygenation, time to resolution of fever, number of days of resting respiratory rate, and the time to discharge from the clinic or to the point of the National Early Warning Score, which consists of physiological parameters: respiration rate (per minute), SpO2 Scale 1 (%), SpO2 Scale 2 (%), use of air or oxygen, systolic blood pressure (mm Hg), pulse (per minute), consciousness, temperature (°C). Furthermore, this study is unique in assessing the baseline percent change in cytokines, including IL-6, CRP, and ferritin[102] (Tables 2 and 3).

CONCLUSION

NAC is a long-known antioxidant whose main clinical application is in the treatment of acetaminophen overdose. Its mucolytic and anti-inflammatory properties make it useful in chronic bronchitis, and its ability to reduce homocysteine levels is of benefit to people with heart disease. Moreover, it helps mitigate the impact of environmental toxins and malignancy by preventing reactive oxygen species overproduction. NAC use has also shown promising results in the treatment of various viral infections. By increasing glutathione levels, it impedes viral replication and decreases viral load. Several studies have illustrated the antiviral activity of NAC against influenza A strains H3N2 and H5N1. Recently, several studies have attempted to explore the effects of NAC in severe COVID-19 patients and the results vary. Although it seems that the ability of NAC to reduce the formation of pro-inflammatory cytokines and mitigate the impact of cytokine storms could lead to better outcomes in COVID-19 patients, there is currently not enough evidence to support this. Our hopes are that ongoing clinical trials and future studies will be able to confirm both the positive outcomes and safety profile of in COVID-19.

Table 2 Details of clinical trial

	Arm	Intervention/Treatment
NCT04455243	Experimental: Intervention group	Drug N-acetylcysteine is given as 150 mg/kg q 12 h PO or IV every 12 h for 14 d diluted in 200 mL diluent (D5 % NS)
	Placebo comparator: Control group	Matching drug placebo is administered in the same schedule and volume as N-acetylcysteine
NCT04374461	Experimental: Arm A. (1) Transfer out of the critical care unit; (2) Extubation; (3) Toxicity; and (4) Death	Drug NAC. Others: Peripheral blood dosages are given in both groups as mentioned above
	Experimental: Arm B. (1) Discharge from the hospital; (2) Admission to a critical care unit; (3) Intubation; (4) Toxicity; and (5) Death	Drug NAC. Others: Peripheral blood dosage details as mentioned above
NCT04419025	Active Comparator: NAC Patients receiving N-acetylcysteine	Drug: N-acetylcysteine. In-patient: (1) Oral formulation 600 mg capsules of NAC q4 h until discharge; and (2) 1200 mg PO BID × 1-wk post-discharge Outpatient :2400 mg PO × 1 then 1200 mg PO BID × 2 wk
	No Intervention: Control patients not receiving N-acetylcysteine	
NCT04458298	Experimental: Cohort A: OP-101 2 mg/kg. Participants will receive a single intravenous (IV) infusion of OP-101 2 milligram per kilogram (mg/kg) on Day 1	Drug: OP-101 will be administered as an IV infusion
	Experimental: Cohort B: OP-101 4 mg/kg. Participants will receive a single IV infusion of OP-101 4 mg/kg on Day 1	Drug: OP-101 will be administered as an IV infusion
	Experimental: Cohort C: OP-101 8 mg/kg Participants will receive a single IV infusion of OP-101 8 mg/kg on Day 1	Drug: OP-101 will be administered as an IV infusion
	Placebo Comparator: Cohort D: Placebo Participants will receive a single IV infusion of matching placebo on Day 1	Drug: Placebo. Matching placebo infusion will be administered intravenously

PO: Peros; NAC: N-acetylcysteine; NAC: N-acetylcysteine; BID: Bisindie; PO: Peros.

Table 3 Summary of ongoing clinical trials of N-acetyl cysteine and corona virus disease 2019

Nct	Drug or other interventions	Diseases	Location (State, Country)	Status (Recruiting or completed)	Results (Yes or not available)	Phase
NCT04455243	N-acetyl cysteine *vs* placebo	COVID 19	Riyadh, Saudi Arabia	Not yet recruiting	Pending	3
NCT04374461	N-acetyl cysteine *vs* peripheral blood	COVID 19	New York, United States	Trial began May 2020	Pending, expected May 2022	2
NCT04419025	N-acetyl cysteine	COVID 19 SARS COV 2, SARS associated Coronavirus disease, Oxidative stress	Massachusetts, United States	Trial began September 2020	Pending, expected May 2021	4
NCT04458298	OP-101 (Dendrimer N-Acetylcysteine) Placebo	COVID 19	California, United States	Trial began July 2020	Pending, expected February 2021	2

COVID 19: Corona virus disease 2019; SARS COV 2: Severe acute respiratory syndrome coronavirus 2.

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MINIREVIEWS

Are nucleotide inhibitors, already used for treating hepatitis C virus infection, a potential option for the treatment of COVID-19 compared with standard of care? A literature review

Anna Maria Spera

ORCID number: Anna Maria Spera 0000-0003-1292-3040.

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Anna Maria Spera, Department of Infectious Diseases, University of Study of Salerno, Salerno 84131, Italy

Corresponding author: Anna Maria Spera, MD, Doctor, Department of Infectious Diseases, University of Study of Salerno, Largo Ippocrate, Salerno 84131, Italy.
annamariaspera@hotmail.it

Abstract

Coronavirus disease 2019 (COVID-19) is global pandemic with various clinical presentations, ranging from cold to sometimes unrecoverable acute respiratory distress syndrome. Although urgently needed, currently there are no specific treatments for COVID-19. Repurposing existing pharmaceuticals to treat COVID-19 is crucial to control the pandemic. *In silico* and *in vitro* studies suggest that a nucleotide inhibitor called Sofosbuvir, has also antiviral activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), apart from suppressing other positive-strand ribonucleic Acid viruses with conserved polymerase (hepatitis C virus). The aim of this study was to assess if Sofosbuvir improves clinical outcomes in patients with moderate or severe COVID-19. A comprehensive overview of scientific literature has been made. Terms searched in PubMed were: COVID-19, SARS-CoV-2, nucleotide inhibitors, pandemic, Sofosbuvir. Results clinical trials conducted among adults with moderate or severe COVID-19 were analyzed. Patients were divided in treatment and control arms, receiving Sofosbuvir plus standard care and standard care alone respectively. The addition of Sofosbuvir to standard care significantly reduced the duration of hospital stay compared with standard care alone in clinical trials examined. If efficacy of these repurposed, cheap and easily available drug against SARS-CoV-2 is further demonstrated, it could be essential to refine the treatment of COVID-19.

Key Words: COVID-19; SARS-CoV-2; Pandemic; Nucleotide inhibitors; Sofosbuvir; Coronavirus

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Core Tip: Coronavirus disease 2019 represents a terrible, still unsolved, global problem affecting not only the healthcare system but also the economic and social one. All countries are facing and fighting against this pandemic but there is still no specific treatment for its eradication. Recently some nucleotide inhibitors, already approved and employed for the treatment of hepatitis c virus infection, have been repurposed for treatment of severe acute respiratory syndrome coronavirus 2 infection, because of some common features among coronaviruses and hepatitis c virus. Herein briefly I focused on the effects of this compound on coronavirus disease 2019, based on its pharmacokinetic properties and on results of several completed clinical trials.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infection caused by a coronavirus (CoV), an enveloped positive-sense ribonucleic acid (RNA) virus with a crown-like appearance due to spike-like projections on its surface[1]. The identification of 27 cases of pneumonia of unknown etiology on 31 December 2019 in Wuhan City, China, revealed a new virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COVID-19, as named by the World Health Organization (WHO)[2]. According to Zhou *et al*[3], SARS-CoV-2 can affect the respiratory, gastrointestinal, hepatic and central nervous system tracts of several organisms, such as humans, cattle, bats, rodents, birds and other wild animals. Given that COVID-19 has been preempted by two different events in the past (2002 and 2012) caused by crossover of animal betacoronaviruses to humans that resulted in severe disease, until the outbreak of severe acute respiratory syndromes, these zoonotic viruses were not considered highly pathogenetic to humans but only responsible for mild infections in immunocompetent people[4]. Such zoonotic spillover determines pathogen transmission from a vertebrate animal to a human. Furthermore, there is evidence of human-to-human virus transmission: Humans may change from hosts into new stable infection reservoirs[5]. Moreover, some people can act as superspreaders; overall, patients can be infectious not only during their symptomatic phase but also during their clinical recovery; as the viral loads found in the nasal cavity are higher than those of the throat, there is no difference in viral burden among symptomatic and asymptomatic patients, as Zou *et al*[6] recently clarified. According to Cheng *et al*[7], the receptor used by SARS-CoV-2 to enter the respiratory mucosa is angiotensin receptor 2 (ACE2), which is highly expressed in the Asian population; this finding may represent an interesting target for future therapeutic options, as reported. The clinical presentation of COVID-19 varies among individuals, ranging from an asymptomatic status to severe respiratory distress and multiorgan failure. SARS-CoV-2 also has neuroinvasive potential, as hypothesized by Li *et al*[8], entering the central nervous system, invading the olfactory nerve and bulb or the sensory fibers of the vagus nerve innervating the respiratory tract and thus causing hyposmia and dysgeusia. The disease can progress in a week to interstitial pneumonia, and in the worst cases, patients develop silent "happy" hypoxemia (respiratory failure without subjective perception of dyspnea) with evidence of hypocapnia by compensatory hyperventilation. Complications typically developed by elderly people and patients affected by underlying comorbidities include acute lung injury, acute respiratory distress syndrome, shock and acute kidney impairment[5]. Recovery begins in the 2nd or 3rd week, and the median duration of hospital stay for recovered patients is almost 10 d. Differential diagnosis of COVID-19 includes all types of respiratory viral infections, atypical organisms such as mycoplasma and chlamydia and bacterial infections[5].

CURRENT TREATMENT ONGOING

Clinical management of COVID-19 is based only on life support, treatment of symptoms and prevention of respiratory failure, as there are currently no registered drugs for treating this disease. Nevertheless, clinical trials based on antiviral, immunomodulatory and anti-inflammatory drugs are ongoing, moving from the SARS-CoV and MERS-CoV experience as well as *in vitro* observations. No conclusive evidence is available regarding the use of steroids; according to Russel *et al*[9] and Zhou *et al*[10], it is necessary to evaluate use on a case-by-case basis, considering both risks and benefits[9,10]. Lin *et al*[11] recommend the use of anticoagulation therapy at the early stage of the disease, particularly when the D-dimer value is 4 times higher than normal, as the infection and related factors can overactivate the coagulation cascade, possibly resulting in ischemic events and disseminated intravascular coagulation. The use of antiviral agents is controversial. In fact, although Chu *et al*[12], Lim *et al*[13] and Yao *et al*[14] demonstrated the efficacy of lopinavir/ritonavir (400/100 mg twice daily) against COVID-19, clinical evidence of its efficacy remains under debate. Al-Tawfiq *et al*[15] described the successful use of remdesivir, a nucleotide analog able to incorporate into the nascent viral RNA chain, causing its premature termination, but it is not yet recommended by the WHO[16]. Chloroquine and hydroxychloroquine, two drugs used for malaria and amoebiasis, demonstrate activity against SARS-CoV-2 *in vitro* and in animal models[17]. According to this study, the mechanism of action of these drugs seems to be an increase in endosomal pH, which prevents fusion between the virus and the host cell and also interferes with the ACE2 receptor targeted by the virus. Moreover, these drugs appear to have immunomodulatory activity. In addition to common side effects (nausea, vomiting, diarrhea, abdominal pain, extrapyramidal disorders), arrhythmogenic cardiotoxicity has been reported, and QT interval monitoring is mandatory with their use. When hypoxia or acute respiratory distress syndrome arises, oxygen therapy is required, basically administered through a nasal cannula, face mask or noninvasive CPAP. If an adequate arterial O_2 level is not reached ($SatO_2$ < 93%), invasive mechanical ventilation *via* intubation is necessary. Advanced techniques such as prone positioning should be considered[18], as should extracorporeal membrane oxygenation. The national multicenter clinical trial in Italy based on the use of tocilizumab, a monoclonal antibody against IL-6R, was prematurely interrupted[19] because no improvement in patients was shown. However, other possible therapeutic options represented by specific anti-inflammatory molecules and multiple monoclonal antibodies/immunostimulants are under investigation. Some options include anti-IL-17, interferon and mesenchymal stromal cells able to reduce inflammation and stimulate regeneration of tissues[20], amplification of anti-2019nCoV specific T lymphocytes[21], the use of anti-Th1-mediated inflammatory cascades such as canakinumab (anti IL-1B)[22] and roflumilast (inhibitor of enzyme phosphodiesterase-4 already used to control neutrophilic inflammation in patients with COPD)[23]. Gurwitz *et al*[24] suggested that sartanics (angiotensin receptor 1 blockers) may be considered for their ability to inhibit binding between the spike S protein of the virus and ACE2, though other studies hypothesized that sartanics may predispose patients toward COVID-19 by targeting ACE receptors in pulmonary tissue. Another interesting option is based on the use of molecules able to target structural genes encoding the S, envelope or membrane protein along with small interfering RNAs[25]. Moreover, some broad-spectrum antiviral agents (*e.g.*, dsRNA-activated caspase oligomerizers) can cause selective apoptosis of host cells containing the virus, which should be exploited in fighting COVID-19; however, combination with other therapies (such as thiopurine compounds, naphthalene and protease inhibitors, zinc or mercury) is necessary because antivirals alone cannot block the virus from entering the cell or disrupt viral nucleic acid[25]. COVID-19-related bradykinin-dependent local lung angioedema can be treated with bradykinin receptor B1 and B2 antagonists and anti-inflammatory agents or neutralizing strategies for anti-S antibody-induced effects[26]. In addition, the use of passive immunotherapy with plasma derived from convalescent patients is still debated[27]. Vaccination may constitute a solution, but vaccine development is ongoing. All drugs currently employed or suggested for the treatment of COVID-19 are summarized in Table 1.

The aim of this review is to evaluate the possible role of nucleotide analogs in the treatment of this dangerous pandemic, given that no drugs currently available for the treatment of SARS-CoV-2 infections seem to be effective.

Table 1 Current ongoing treatment for coronavirus disease 2019

	Rationale of use	Notes
Steroids	Prevent and treat acute lung injury and respiratory distress due to host inflammatory response secondary to SARS-CoV-2 infection	May determine Hyper-glicemia, arterial hypertension
Anticoagulation therapy	Prevent and/or treat the over-activation of the coagulation cascade, responsible for ischaemic events and disseminated intravascular coagulation	May determines Hemorrhagic risk
Antiviral agents	Protease inhibitors (lopinavir), nucleotide analogue (remdesivir)	May determine Drug/drug interactions, allergic reactions, acquired resistance
Chloroquine/hydroxychloroquine	Increasing in endosomal pH, avoiding the fusion between the virus and the host cell, but also the interference with the ACE2 cell receptor targeted by the virus. immunomodulatory activity	May determine common side effects (nausea, vomiting, diarrhea, abdominal pain, extrapyramidal disorders), and arrhythmogenic cardiotoxicity (thus monitor QT interval)
Oxygen therapy	Treatment of hypoxia basically administered through a nasal cannula, face mask or noninvasive CPAP. If an adequate arterial O2 level is not reached (SatO2 < 93%), invasive mechanical ventilation *via* intubation is necessary. Advanced technique such as prone positioning should be considered as well as extracorporeal membrane oxygenation	
Antinflammatory molecules – multiple monoclonal antibodies/immunostimulants (anti IL-17, interferon and mesenchymal stromal cells)	Able to reduce inflammation and stimulate regeneration of tissues as well, the amplification of anti-2019nCoV specific T lymphocytes, the employment of anti-Th1-mediated inflammatory cascade such as canakinumab (anti IL-1B) and roflumilast (inhibitor of enzyme phosphodiesterase-4 already used to control neutrophilic inflammation in patients with COPD)	
Sartanics (angiotensin receptor 1 blockers)	Could be considered for their ability to inhibit the link between the spike S protein of the virus and ACE2	According to other studies could predispose to COVID targeting ACE receptors on pulmonary tissue
Some broad spectrum antiviral agents (dsRNA-activated caspase oligomerizer)	Cause selective apoptosis of host cells containing virus, this skill could be exploited in fighting COVID-19	
Bradykinin receptors B1 and B2 antagonists	COVID related bradykinin-dependent local lung angioedema	
Plasma	Passive immunotherapy	

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; ACE2: Angiotensin receptor 2; RNA: Ribonucleic acid.

NUCLEOTIDE ANALOGS IN THE TREATMENT OF CORONAVIRUS DISEASE 2019: WHERE ARE WE NOW?

A novel therapeutic approach for COVID-19 is based on the use of nucleotide analogs. One such analog is Sofosbuvir, a powerful anti-hepatitis C virus direct-acting agent that targets HCV polymerase NS5B, approved by national and international agencies. It has a demonstrated ability to suppress other positive-strand RNA viruses, such as members of Flaviviridae and Togaviridae, in addition to Coronaviridae[28].

Although not currently listed as a potential option for SARS-CoV-2 therapy, sofosbuvir may represent a key step in the control of the COVID-19 pandemic, as stated by Jácome *et al*[29]. Nevertheless, the winning strategy may instead be based on a multitargeted approach of different drugs targeting many viral proteins[29]. Sofosbuvir binds to the active site of HCV and is thus incorporated in the nascent strand, preventing the addition of the next nucleotide[29]. The replication mechanisms of coronaviruses, flaviviruses and togaviruses require an RNA-dependent RNA polymerase that is targeted by sofosbuvir, ribavirin and AZT[28]. This has been demonstrated by Elfiky *et al*[30] in a recent in silico study based on homology modeling: the docking scores that emerged from the study suggested the possible use of these antiviral drugs in the treatment of disease caused by SARS-CoV-2.

The RdRp enzyme of coronaviruses tightly embody biologically activated triphosphate forms of "four nucleotide/nucleoside analog" antiviral drugs (sofosbuvir, tenofovir alafenamide, alovudine and AZT), without further

incorporation thereafter, as clearly reported by Chien *et al*[31]. Therefore, all these compounds may be considered permanent terminators for SARS-CoV-2 RdRp[31,32] and are of curative significance for COVID-19, though the authors did not suggest the best RdRp inhibitor.

Sofosbuvir: An antiviral drug

The antiviral effect of sofosbuvir and its potent, fast action[33], even against liver cirrhosis, is well known, even in the setting of a lack of response to other medications, such as interferon and ribavirin[34]. Pivotal trials of this pangenotipic DAA[35] (Fission, Positron, Fusion and Photon 1)[36-38] report its high rate of success, significant efficacy, low rate of side effects and tolerability. Moreover, this antiviral compound does not interfere with the cytochrome P450 system or other major drug-metabolizing enzymes and has low drug-drug interactions. With a good pharmacokinetic profile, sofosbuvir can be prescribed as a single oral daily dose. The antiviral activity of the active form of sofosbuvir is related to the intracellular production of its active triphosphate metabolite by intracellular nucleoside diphosphate kinase (NDK), an enzyme encoded by National Military Establishment that is present in all cells, including the alveolar epithelial type II cells targeted/infected by SARS-CoV-2. The main role of NDK is to maintain an equilibrium between the concentrations of several nucleoside/nucleotide triphosphates, which are thus the source of RNA and deoxyribonucleic acid precursors such as CTP, UTP and GPT[39]. The presence of NDK-A and NDK-B in airway epithelial membranes has been suggested by Muimo *et al*[40] using isoform-specific antibodies, whereby local COVID-19-mediated lung inflammation enhanced sofosbuvir endothelial permeability and improved epithelial uptake during SARS-COV-2 infection. The extremely high intracellular stability of sofosbuvir and its triphosphate metabolite is a main feature of this antiviral drug and explains its significant and persistent HCV effect in inhibiting HCV-NS5B polymerase[41]. Moreover, intracellular levels of its triphosphate metabolite in alveolar epithelial type II cells may inhibit SARS-CoV-2 RdRp (in accordance with its EC50).

Notably, it must be emphasized that use of the currently employed nucleoside analog remdesivir has recently been reduced. In fact, the living WHO guidelines on drugs for COVID-19[16] released on September 4 and then updated in November 2020 strongly suggest no remdesivir use for patients with COVID-19 at any severity; this was based on results of a systematic review and network meta-analysis including data for 4 randomized trials with 7333 adult patients hospitalized for COVID-19. No effect on mortality, need for mechanical ventilation, or time to clinical improvement was found among COVID-19 patients treated with remdesivir. The conclusion is that remdesivir does not improve important patient outcomes. Jockusch *et al*[42] reported in Nature that RNA terminated by sofosbuvir is more resistant to SARS-CoV-2 proofreading than RNA terminated by remdesivir.

Several randomized and nonrandomized clinical trials have been performed comparing DAA-based regimens and standard of care (SOC) in hospitalized COVID-19 patients[43]. Trials eligible for inclusion were identified by reviewing clinicaltrials government, WHO International Clinical Trials Registry Platform and Cochrane Central Register of Controlled Trials. Three[44-46] of eight studies reviewed were considered by Simmons *et al*[43] because they met the inclusion criteria (completed trials about the comparison of predetermined DAA-based regimens and SOC for the treatment of COVID-19). The primary outcomes highlighted were clinical recovery in 14 d and all-cause mortality from enrollment to the end of the follow-up; the findings along with secondary outcomes are summarized in Table 2. An individual patient data meta-analysis was produced, and treatment effects were reported as risk ratios and mean differences for binary and continuous outcomes, respectively. Cox proportional hazards models were used to estimate the cause-specific hazard ratios for recovery, and the Fine and Gray competing risk model was employed to account for death as a competing risk. A sensitivity analysis for the primary outcomes involved excluding nonrandomized trials because of the potential risk of bias. A second analysis for primary binary outcomes was performed, including the worst outcomes not yet considered in the Intention to treat analysis of all the studies included in the meta-analysis.

The effects of nonrandomized treatment assignment were studied in a final sensitivity analysis in which the effect of sofosbuvir/daclatasvir on clinical recovery and death was estimated using the inverse probability weighting estimator adjusted for age, sex and comorbidities (hypertension, chronic pulmonary illness, diabetes mellitus). Data were analyzed using STATA vers 14.2 and Rstudio vers 3.5.3.

Three Iranian studies of the eight available were conducted among 176 hospitalized patients, with equal reported baseline characteristics among the intervention and

Table 2 Primary and secondary outcomes of studies included in simmons' meta-analysis

			Intervention arm (92 patients)	Control arm (84 patients)
Outcomes	Primary	Clinical recovery in 14 d	86 (93%)	57 (68%)
		All cause mortality	5 (5%)	17 (20%)
	Secondary	Duration of hospitalization	6 (IQR: 5-7)	8 (IQR: 6-11)
		ICU admission/imv needed	9 (10%)	24 (29%)

ICU: Intensive care unit.

control groups. Two of the three studies were randomized[44,45] and included patients affected by severe disease. A combination of DAA + SOC at the time of trial (hydroxychloroquine + lopinavir/ritonavir) was administered to the intervention arm of each trial; the control groups received only SOC (hydroxychloroquine plus lopinavir/ritonavir, hydroxychloroquine plus lopinavir/ritonavir and ribavirin, hydroxychloroquine plus lopinavir/ritonavir plus or without ribavirin), as reported in Table 3. Ninety-three percent of patients in the intervention arm and 68% in the control arms achieved clinical recovery after 14 d of randomization. Five percent in the intervention arms and 20% in the control arms died during the trial: a higher frequency of comorbidities, though not significant, was detected in the control arm. Significant differences in secondary outcomes (duration of hospitalization and intensive care unit admission or intermittent mandatory ventilation requirement) in favor of the DAA treatment-based group were found. Although limited by the small number of studies included and lack of full blinding and uniform reported primary outcomes, the cited meta-analysis revealed significant differences in clinical recovery and all-cause mortality in favor of sofosbuvir/daclatasvir regimens for the treatment of COVID-19. In conclusion, considering that managing a placebo-controlled trial during a pandemic is difficult, it is important to underline that the Iranian authors of those clinical trials took up a tough challenge, raising awareness of the whole scientific community about the use of sofosbuvir for the treatment of COVID-19 and encouraging larger randomized trials to establish the potential utility of nucleotide inhibitors for this disease. Moreover, given that sofosbuvir has been used for treating early stages of COVID-19, further studies are needed to evaluate whether this nucleotide analog may even be used to prevent SARS-CoV-2 contagion suddenly after the first exposure to this specific antigen.

CONCLUSION

The addition of Sofosbuvir to standard care significantly reduced the duration of hospital stay compared with standard care alone in clinical trials examined. If efficacy of these repurposed, cheap and easily available drug against SARS-CoV-2 is further demonstrated, it could be essential to refine the treatment of COVID-19.

Table 3 Therapeutic schedule of clinical trials considered in meta-analysis

	Disease stage	Treatment arm	Control arm	Duration
Eslami	Severe	35 patients: SOC (lopinavir/ritonavir + hydroxychloroquine) + Sof/dac started 24-48 h later (after PCR and TC confirmation of COVID-19)	27 patients: SOC (lopinavir/ritonavir + hydroxychloroquine) + ribavirin	14 d
Kasgari	Moderate	24 patients: Sof/dac + ribavirin	24 patients: Lopinavir/ritonavir + hydroxychloroquine ± Ribavirin	6 d?
Sadeghi	severe	33 patients: Sof/dac + lopinavir/ritonavir	33 patients: Lopinavir/ritonavir	14 d

SOC: Standard of care; COVID-19: Coronavirus disease 2019.

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MINIREVIEWS

Trends in the management of infectious disease under SARS-CoV-2 era: From pathophysiological comparison of COVID-19 and influenza

Masafumi Seki

ORCID number: Masafumi Seki 0000-0001-5414-8148.

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Masafumi Seki, Division of Infectious Diseases and Infection Control, Tohoku Medical and Pharmaceutical University, Miyagi 983-8536, Japan

Corresponding author: Masafumi Seki, MD, Professor, Division of Infectious Diseases and Infection Control, Tohoku Medical and Pharmaceutical University, Fukumuro 1-12-1, Miyagino-ku, Miyagi 983-8536, Japan. m-seki@tohoku-mpu.ac.jp

Abstract

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has become a historic pandemic, and dealing with it is one of the most important aspects of infectious disease treatment today. SARS-CoV-2 has been found to have characteristic and powerful infectivity (ability to propagate) and lethality (severity). With influenza, primary influenza pneumonia from the virus itself is known to exist in addition to secondary bacterial pneumonia. With COVID-19, on the other hand, it is important to provide diagnosis and treatment while keeping acute respiratory distress syndrome and pulmonary edema (alveolar flood) from a similar cytokine storm, as well as severe angiopathy, in mind. The importance of complying with hand hygiene and masks in infection control remains the same as in previous general infection control measures and responses to influenza virus infections and others, but in the future, vaccination will likely be the key to infection control in the community.

Key Words: COVID-19; SARS-CoV-2; Influenza; Angiotensin-converting enzyme 2; Vaccine; Alveolar flood

Core Tip: We are focusing the differences and similarity of coronavirus disease 2019 (COVID-19) and Influenza, and review the characteristic pathophysiology and basic concepts of treatment and prevention for COVID-19. Primary influenza pneumonia is known to exist in addition to secondary bacterial pneumonia, however, pulmonary edema (alveolar flood) from a similar cytokine storm, as well as severe angiopathy should be considered in COVID-19.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), which continues to spread around the world, has become the main focus of infectious disease treatment since the 2020 season. We have previously experienced acute epidemic viral infections, with influenza being typical, but a pandemic of this size has not been seen since the "Spanish flu" of 1918[1]. These two virus look similar, but we have found the critical differences between influenza and COVID-19. In this review, the epidemiological and clinical characteristics of COVID-19, compared with influenza, in addition to the trend of treatment and prevention, including anti-viral agents and vaccines, could be described.

EPIDEMIOLOGY

The virulence of viral infections is defined mainly by infectivity (ability to propagate) and lethality (severity). Coronavirus infections experienced in recent years include severe acute respiratory syndrome (SARS), which spread globally from Guangdong Province in China in 2002, and Middle East respiratory syndrome, which spread in the Middle East in 2012. Although both SARS and Middle East respiratory syndrome showed a moderate to high level of lethality, their ability to propagate was not as strong, and they came mostly to an end while being fairly limited in duration and geography[2].

Compared with these two serious coronavirus infections, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has somewhat stronger infectivity, but not as high a level of lethality/severity. Initially, COVID-19 was viewed as a viral infection similar to seasonal influenza, but it soon became empirically clear that COVID-19 was much more serious.

While its infectivity on average is about the same as that for influenza, it has been found that under the condition of the "three Cs"-crowded places, close-contact settings, and confined and enclosed spaces-a "cluster" is generated with nearly all people present becoming infected at a speed comparable to that of the measles[3]. This situation may be affected by the fact that there is a subtle mechanism somewhat like the human immunodeficiency viruses in which propagation occurs while evading attack by the human immune system, during which time, people are asymptomatic for about a week after being infected.

Although the overall fatality rate in Japan is very low, it is still much higher than that for influenza, and there have been many reports of increased severity in the aged; therefore, we cannot let our guard down. While COVID-19 is relatively mild in most young people, chest computed tomography scans have shown pneumonia presenting with characteristic bilateral ground-glass opacity, even in nearly asymptomatic patients. On the other hand, among older people, especially those with preexisting conditions, COVID-19 can become serious and potentially fatal at a very high rate[2]. Consequently, age is one of the most important factors in determining the prognosis of patients infected with SARS-CoV-2 (Figure 1). Therefore, COVID-19 may be viewed as a formidable infectious disease with two distinct manifestations.

CLINICAL CONDITION

Clinically, influenza and COVID-19 are both respiratory illnesses caused by viral infections, but show different symptoms and signs depending on the characteristics of influenza virus and SARS-CoV-2 (Table 1). Compared with influenza, COVID-19 seems to spread more easily and cause a more severe condition. Influenza and COVID-19 share many common signs and symptoms, but the loss of smell and taste is considered specific to COVID-19; therefore, diagnostic testing may be critical to

Table 1 Clinical differences between influenza and coronavirus disease 2019

Characteristics	Clinical differences
Signs and symptoms	Influenza: Mild to severe illness, including common signs and symptoms. COVID-19: More serious illnesses in some people. Change or loss of taste or smell may be included
Incubation period	Flu: 1-4 d after infection. COVID-19: 5 d, but symptoms can appear as early as 2 d or as late as 14 d after infection
Duration of the symptoms	Flu: 3-7 d. COVID-19: 2-3 wk
Asymptomatic patients	Flu: 10%. COVID-19: A few 60%

COVID-19: Coronavirus disease 2019; Flu: Influenza.

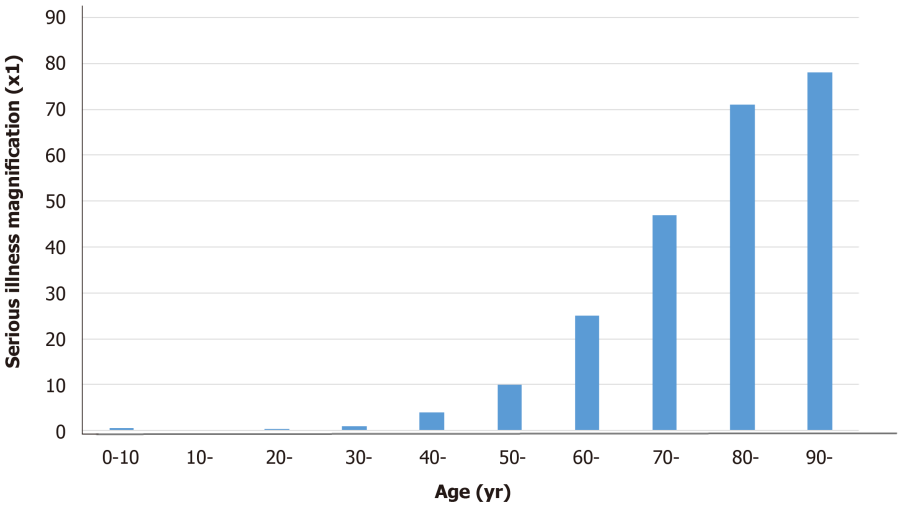

Figure 1 Magnification of serious illness by age in patients with coronavirus disease 2019. Numbers are calculated as the magnification rate in patients aged 30 years as ´1, and are significantly increased in older generations.

confirm a diagnosis.

In addition, it is well known that complications from pneumonia in influenza, particularly a high rate of secondary bacterial pneumonia, are a common mechanism in terms of disease severity[4]. However, pulmonary edema-like primary viral pneumonia, which does not occur at a high rate in influenza, has been found in nearly all cases of COVID-19[2,5].

Therefore, with influenza, strong inflammation is induced under relatively rare conditions in which an excessive immune response called a "cytokine storm" may occur, even with infection by the influenza virus alone. It has been shown that pulmonary edema (alveolar flooding) may occur from pneumonia and the breakdown of alveolar epithelial and pulmonary vascular endothelial cells[6]. By contrast, SARS-CoV-2 uses the angiotensin-converting enzyme 2 distributed in the human vascular endothelium as a receptor, and thus has a strong affinity for vascular endothelial cells in particular, which facilitates vascular permeability and makes angiopathy more likely[7]. In fact, pulmonary edema is thought to be significantly more likely to occur than general pneumonia, and typical findings of chest radiographs in patients with COVID-19 are very similar to cases of victims who drowned in freshwater (Figure 2). An abundance of extravascular fluid is found because of changes in osmotic pressure and the junction between alveolar spaces and plasma membranes.

Therefore, it has been suggested that COVID-19 is essentially pulmonary edema and pulmonary microthrombosis due to cytokine disease from viral infection and subsequent vascular destruction. These conditions progress particularly rapidly in older people with comorbidities, ultimately leading to multi-organ failure[8,9].

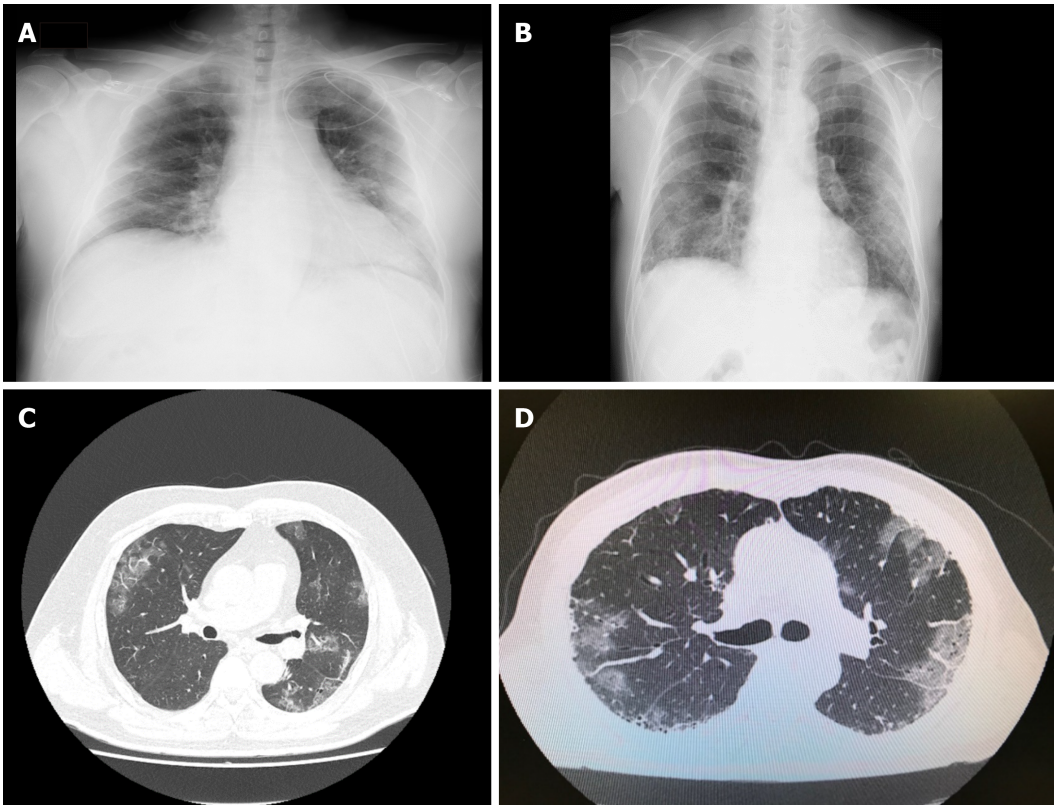

Figure 2 Coronavirus disease 2019 are very similar to cases of victims who drowned in freshwater. A and C: Representative chest X-rays; B and D: Computed tomography scans of a patient with coronavirus disease 2019 (COVID-19); A and B: A patient with COVID-19; C and D: A drowning victim. The drowning victim had a stroke while in the bath. Both the patient with COVID-19 and the drowning victim have similar characteristic ground-glass opacity lesions close to the pleura in both lung fields.

TREATMENT

For the treatment of influenza, a large number of anti-influenza drugs have been developed that target the virus itself. In recent years, the appearance of baloxavir (trade name: Xofluza) has attracted much attention. Infectious disease treatment is based on elimination of the causative agent, and this has been a very effective anti-influenza strategy[10].

Many drugs are currently being developed for the treatment of COVID-19[11]. As of this writing, remdesivir is the first drug to be used that acts against the virus itself, and has shown good efficacy. Therefore, remdesivir has been approved in insurance systems in Japan, and is currently used to treat many severe cases of COVID-19 that require oxygen management, including with artificial ventilation. We also look forward to the use of other antiviral drugs, such as favipiravir.

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is an antiviral agent that selectively inhibits the ribonucleic acid (RNA)-dependent RNA polymerase of RNA viruses, and has been stockpiled in Japan for the treatment of severe influenza[12]. Since the catalytic domain of RNA-dependent RNA polymerase is conserved among various types of RNA viruses, this mechanism of action suggests a broader virus spectrum, including SARS-CoV-2. In fact, favipiravir has shown significant efficacy for COVID-19 treatment compared with anti- human immunodeficiency viruses drugs in an open-label study[13]. However, its efficacy depends on the severity of the disease, its effects remain unclear, and its treatment strategy remains controversial.

Moreover, as mentioned above, the effects of cytokine storms and hyperimmunity on the clinical pathology of COVID-19 are confirmed to be greater than those in influenza. Sequences of anti-immune drugs and immunomodulators are effective, and these are characteristically proposed as powerful drug candidates. The recommendation of steroids as being efficacious against infectious disease in severe cases of respiratory failure is a first for acute respiratory infections[14]. We are currently waiting for the establishment of infectious disease treatment as an "antiviral + immunomodulator + anti-thrombul" treatment regimen/bundle, and this may provide clues for new treatments for infectious disease (Table 2).

Table 2 Combination/bundle of the candidate drugs for coronavirus disease 2019 treatment based on the pathophysiological characteristics

Drugs	Pathophysiological characteristics
Antiviral drugs	
	Remdesivir for moderate to very severe patients
	Favipiravir for mild to severe patients
Immnomodulators	
	Corticosteroids for moderarte to very severe patients
	Tocilizumab for hospitalized COVID-19 patients
	Jak/Stat signaling inhibitors for hospitalized COVID-19 patients
Anticoaglnat drugs	
	Heparin
	DOAC

COVID-19: Coronavirus disease 2019; DOAC: Direct oral anticoagulant.

PREVENTION AND INFECTION CONTROL

To combat viral infections, prevention *via* vaccines or other measures, or infection control so that infections do not spread, are much more important than treatment. The appearance of an effective new SARS-CoV-2 vaccine that is equal or superior to influenza or pneumococcal vaccines, and practical vaccinations that steadily become a reality in general clinical practice, will be considered a breakthrough. Novel vaccines, including mRNA and viral vector-based types, have been in practical use for COVID-19 prevention. By contrast, inactivated vaccines have been available for influenza prevention (Table 3)[15-17]. COVID-19 vaccines to date have been shown to cause mild side effects in small numbers of individuals after the first or second dose, including pain, redness or swelling at the injection site, fever, and headache; however, vaccination might help prevent people from contracting COVID-19 or experiencing a severe case and developing serious complications. In fact, the novel COVID-19 vaccines have shown an efficacy of around 90%, compared with the standard influenza vaccine, which shows an efficacy of around 60%. The COVID-19 pandemic may be said to have in fact brought about dramatic advances in the treatment and management of infectious diseases.

More than previous anti-influenza measures, hand-washing and masks have been demonstrated to be extremely effective against the spread of respiratory viruses, which are mainly transmitted through droplets. Further responses should be advanced based on standard preventive measures and anti-droplet infection measures that have proven effective for other infectious diseases[18].

The term "social distancing" has become well known in dealing with COVD-19. Social distancing is an intervention that arose from measures to prevent "clusters" when the abovementioned special "3C" conditions are met[3]. If the 3Cs can be avoided, the spread of infections can be minimized in many cases. An age has come in which we can advance infectious disease responses that protect ourselves and our communities with a "new lifestyle" that implements these new techniques and ways of thinking.

CONCLUSION

In conclusion, we described the differences between influenza and COVID-19. SARS-CoV-2 has been found to have epidemiological and clinical characteristics with the pathophysiological conditions, including cytokine storm and severe angiopathy. Novel anti-viral agents and vaccines might be available soon. The responsibilities of doctors and health-care workers who support community medicine and infectious disease treatment are likely to continue to grow.

Table 3 Comparison of the novel coronavirus disease 2019 vaccines in clinical trials

Vaccine	Clinical trials
Pfizer vaccine (Name: BNT 162b2)	
	Type: mRNA
	Age: ≥ 16 years old
	Dose: 30 μg (0.3 mL) twice (21 d interval)
	Efficiency (95%CI): 95.0% (90.3-97.6)
Moderna vaccine (Name: mRNA-1273)	
	Type: mRNA
	Age: ≥ 18 years old
	Dose: 100 μg (0.5 mL) twice (28 d interval)
	Efficiency (95%CI): 94.5% (86.5-97.8)
AstraZeneca vaccine (Name: ChAdOx1)	
	Type: Virus vector
	Age: ≥ 18 years old
	Dose: Low doss: 2.2×10^{10} virus particle andStandard dose: 5×10^{10} virus particle, twice (28 d interval)
	Efficiency (95%CI): 90.0% (67.4-97.0)

mRNA: Messenger ribonucleic acid; CI: Confidence interval.

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META-ANALYSIS

Epidemiological characterization and geographic distribution of human immunodeficiency virus/acquired immunodeficiency syndrome infection in North African countries

Mohamed A Daw, Mohamed O Ahmed

ORCID number: Mohamed A Daw 0000-0003-1312-5956; Mohamed O Ahmed 0000-0002-1930-5707.

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Mohamed A Daw, Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Tripoli, Tripoli cc82668, Tripoli, Libya

Mohamed O Ahmed, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli cc82668, Tripoli, Libya

Corresponding author: Mohamed A Daw, BM BCh, BPharm, FTCDI, MD, PharmD, PhD, Academic Fellow, Professor, Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Tripoli, Alfrnaj Street, Tripoli cc82668, Tripoli, Libya.
mohamedadaw@gmail.com

Abstract

BACKGROUND

Human immunodeficiency virus (HIV) infection is a major global public health concern. North African countries carry a disproportionate burden of HIV representing one of the highest rates in Africa.

AIM

To characterize the epidemiological and spatial trends of HIV infection in this region.

METHODS

A systematic review was carried out on all the published data regarding HIV/acquired immunodeficiency syndrome in North African countries over ten years (2008-2017) following the PRISMA guidelines. We performed a comprehensive literature search using Medline PubMed, Embase, regional and international databases, and country-level reports with no language restriction. The quality, quantity, and geographic coverage of the data were assessed at both the national and regional levels. We used random-effects methods, spatial variables, and stratified results by demographic factors. Only original data on the prevalence of HIV infection were included and independently evaluated by professional epidemiologists.

RESULTS

A total of 721 records were identified but only 41 that met the criteria were included in the meta-analysis. There was considerable variability in the prevalence estimates of HIV within the countries of the region. The overall

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prevalence of HIV ranged from 0.9% [95% confidence interval (CI) 0.8-1.27] to 3.8% (95%CI 1.17-6.53). The highest prevalence was associated with vulnerable groups and particularly drug abusers and sexually promiscuous individuals. The dense HIV clustering noted varied from one country to another. At least 13 HIV subtypes and recombinant forms were prevalent in the region. Subtype B was the most common variant, followed by CRF02_AG.

CONCLUSION

This comprehensive review indicates that HIV infection in North African countries is an increasing threat. Effective national and regional strategies are needed to improve monitoring and control of HIV transmission, with particular emphasis on geographic variability and HIV clustering.

Key Words: North Africa; Human immunodeficiency virus/acquired immunodeficiency syndrome; Epidemiological analysis; Geographic distribution; Meta-analysis; Risk factors

Core Tip: North Africa is a unique geographical region located on the southern Mediterranean basin and represents the largest region of Africa. Human immunodeficiency virus (HIV) infection is an increasing threat in this region. Previous studies analyzed mainly the risk factors associated with risk groups at a national level and no single study has yet analyzed the actual epidemiological situation of HIV/acquired immunodeficiency syndrome (AIDS) in the whole region. This review aims to analyze and characterize the epidemiological and geographic variation of HIV/AIDS in North African countries and to highlight the strategies needed to combat this epidemic at the national and regional levels.

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INTRODUCTION

Human immunodeficiency virus (HIV) is one of the most important viruses and has had demographic, economic, social, and even political consequences. Since its discovery in the 1980s, HIV/acquired immunodeficiency syndrome (AIDS) has remained an important public health concern worldwide. Its prevalence has rapidly increased, particularly in developing countries[1,2]. As of 2012, over 70 million people have become infected with HIV, of whom over 35 million have died. Africa is the most severely affected geographical area, and over 70% of the people infected with HIV reside in Africa[3,4].

A variety of factors have contributed to the spread of HIV/AIDS. These factors vary from one region to another and even within districts in the same region. Recent reports indicate that homosexuality (men who have sex with men, MSM) has become the dominant mode of transmission among newly diagnosed HIV infections in North America, East, Southeast and South Asia, and Latin America[5-7].

North Africa is a vast region representing over 30% of the African continent, with a coast extending from the Atlantic Ocean to the Mediterranean basin facing the southern part of the European Union[8]. The region has experienced major political and demographic challenges, particularly in the previous decade after the Arab spring. This has been complicated by wars, lack of security, major population displacements, weakening of public health systems, and the influx of immigrants, particularly from western and sub-Saharan Africa[9-11].

The incidence of HIV has been increasing more rapidly in North Africa than in any other global region, and AIDS-related mortality has almost doubled in the past decade. Comparable to West and Central African regions, HIV transmission rates in the region

rose by over 10% during this decade, with a substantial increase in HIV morbidity and mortality. This has been faced by ignorance and polemic thinking even among health professionals[12,13]. Too often, patients living with HIV face denial of care, stigma, discrimination, and breach of confidentiality[14].

Furthermore, HIV infection has not been well addressed as a public health challenge in the region, and the hidden pandemic is believed to be driven by risky behaviors (such as sexual and drug-related factors) that are not well tolerated in society. The epidemiology of HIV in the region remains poorly defined. This has been complicated by the lack of accurate surveillance data, and even the existing data are prone to underestimation biases that mask the real picture of new HIV infections in the region[12].

Few studies have been conducted on the prevalence of HIV and its associated factors in North Africa. These studies present inconclusive findings on the prevalence of HIV and its associated factors[15]. Therefore, this systematic review was conducted to assess the prevalence of HIV and its associated factors in the region based on the published evidence (http://www.prisma-statement.org/). The findings of this review could be useful for designing strategies to reduce the prevalence of HIV and implement effective programs to combat its consequences.

MATERIALS AND METHODS

Data sources and search strategy

A systematic search was conducted using PubMed, Medline, Google Scholar and Embase to identify studies on HIV in North African countries published between January 1st, 2008 and December 31st, 2017 without language restriction using the search terms HIV or AIDS, OR ''human immunodeficiency virus'' OR ''acquired immunodeficiency syndrome'' prevalence, incidence in "Northern Africa", and in every country within the region. The search was restricted to North African countries and encompassed Egypt, Libya, Tunisia, Algeria, Morocco, Mauritania and Sudan, which share historical, sociocultural, linguistic and religious characteristics. Reports from the World Health Organization were also included. To minimize publication bias, we retrieved the reference lists and manually searched for relevant studies that met our inclusion criteria, in addition to regional and country-level scientific databases. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses, http://www.prisma-statement.org/) guidelines were followed to aggregate the data[16,17].

Inclusion and exclusion criteria

Studies of any design that estimated the prevalence or incidence of HIV in North African countries were initially included. The inclusion criteria were based on whether the study provided sufficient information on the prevalence, incidence, and demographic and risk factors**.** Studies were accepted if descriptions of HIV testing methods were included, such as laboratory-derived HIV status using biological specimens and primary data from populations in the North African countries. To be included, studies had to have a minimum sample size of 25, detailed descriptions of the sampling procedure, HIV testing, and analytical methods. The sources included peer-reviewed journals and non-peer-reviewed publications meeting other criteria and available online in the public domain. Duplicates were identified by comparing detailed study characteristics, including author names, study period, study location, number of infected cases, and sample size. If two publications were found to be from the same data source, only the earlier publication was included. Excluded were case reports, case series, editorials, letters to editors, commentaries, literature reviews, studies reporting HIV prevalence based on self-reporting, studies on HIV status rather than biological testing, and studies that do not mention the study period/time and geographical location.

Data abstraction and quality assessment

The titles and abstracts of all the records retrieved were screened for relevance independently and categorized according to the quality of the study design and methods. The following information was extracted from all the eligible studies: first author, publication year, study location, study period, sampling method, sample size, and the laboratory testing method for HIV infection. The studies were categorized according to countries and the type of population. Details of each study were entered into a database by one investigator and rechecked in full. Quality assessment was

carried out using a standard procedure[6].

Two trained epidemiologists (MD, MO) independently reviewed these publications and examined the sample size, sampling method, testing procedures, results, and interpretation of the data. Disagreements were resolved by consensus or arbitration by MD. A quality score between 1 and 5 was calculated for every paper based on these criteria.

Statistical analysis

Statistical analysis was carried out by calculating the percentages and confidence intervals (CI), random-effects model, crude odds ratios (OR), sensitivity analysis, and cut-off *P* value ($P < 0.1$). Heterogeneity was tested by the chi-square test. The prevalence of HIV was calculated as an average of the pooled infection prevalence of each country weighted by the ratio of the country's population to the study's sample size. The risk of bias in reporting the prevalence and cumulative incidence was independently calculated by the authors. Publication bias was assessed by inspection of a funnel plot and Egger's test. Analyses of the aggregated prevalence rates of each country were performed with metan, which is an average of the individual study results weighted by the inverse of their variances using a fixed/random model[18]. Geographic mapping and spatial variables were carried out using the national data in each country and localized clusters of spots are reported as previously published[19,20].

RESULTS

A total of 721 records were identified during the 10-year period (2008-2017). Following the elimination of duplicates, 646 studies remained. When the titles and abstracts were screened, 598 not fulfilling the selection criteria were excluded and only 48 were assessed for eligibility. Seven of these were excluded and only 41 were finally included in the meta-analysis. The steps of study selection are illustrated diagramattically in Figure 1. There was a steady increase in the number of publications and HIV records in North Africa with a slight increase in the last two years. The highest population and HIV data notification were reported in Morocco, Egypt, Sudan and to lesser extent in Libya and Tunisia. It was very low in Algeria and Mauritania. The characteristics of the studies included in the analysis are presented in Table 1.

Study quality assessment showed that seven studies were of a low quality and no full text was available, 28 had moderate quality, and only six high-quality studies were identified. However, after analysis according to quality assessment, no significant difference was noted between studies of high/medium quality and those with low/medium quality.

Temporal trends of HIV/AIDS in North Africa

During the study period, there was an increasing trend in the prevalence of HIV/AIDS in the North African region with much variation among the countries (Figure 2). In 2008, the highest prevalence was reported in Sudan (1.3%) followed by Algeria (1.2%) and Mauritania (1.3%)[21-23]. However, it was less than 1% in Tunisia (0.9%), Morocco (0.3%), Egypt (0.2%) and Libya (0.2%)[24-27]. Ten years later, the overall prevalence increased significantly by more than four-fold ($P \leq 0.001$). In 2017, the highest prevalence rate was reported in Sudan (4.3%), followed by Mauritania (2.3%), Algeria (2.2%), Egypt (1.8%), Morocco (1.6%), Tunisia (1.2%) and Libya (0.9%). The overall prevalence of HIV in North Africa varied not only among the countries but also within the population of the same country, as illustrated in the Forest plot (Figure 3). There was a clear relationship between the prevalence of HIV and attributable risk factors. The general population and ordinary patients had low HIV prevalence comparable to other studied groups. For instance, studies on blood donors and pregnant women in Egypt, Mauritania, Sudan and Morocco, and those carried out on the general population in Libya, Tunisia and Algeria showed a low HIV prevalence, but the prevalence was elevated in the risk groups within the same country. The overall odds ratio in the meta-analysis demonstrated a statistically significant variation among the populations studied, and the association appears stronger in the studies related to high-risk groups. The test of heterogeneity showed significant variation among the studies, indicating the nature and quality of these studies.

Demographic features of HIV/AIDS in North Africa

Figure 4 illustrates the sex- and age-specific distribution of HIV/AIDS in North African countries. Between 2008 and 2017, 76.9% of the reported cases were males and

Table 1 Characteristics of human immunodeficiency virus studies included in the meta-analysis (2008-2017)

Characteristics	Studies, *n*
Country	
Algeria	4
Egypt	7
Libya	6
Mauritania	2
Morocco	11
Sudan	7
Tunisia	4
Study design	
Cross-sectional	30
Cohort study	11
Data collection	
Prospective	32
Retrospective	9
Study area/site	
Hospital oriented	19
Population oriented	22
Sampling method	
Random	21
Consecutive	14
Not reported	6

25.1% were females, giving a male to female ratio of 3.1:1. Although his trend was found in most North African countries, the number of infected females was similar to that of males in Morocco. Among the HIV/AIDS cases reported, the prevalence of infection was highest among the 21-30 years age group (45%) compared to the 31-40 years age group (30%). A marked increase in the number of HIV/AIDS cases was reported among those aged < 20 years, particularly in Sudan, Algeria and Morocco. The prevalence of HIV/AIDS infections among those aged above 40 years was similar among all countries at 20%, apart from Libya, which showed a higher rate of up to 30%. A few studies from Libya, Morocco and Sudan have reported on the relation between HIV/AIDS and educational level and marital status. Most of the infected cases were found among unmarried individuals who were mainly illiterate or had a low level of education[28-31].

Prevalence of HIV among high-risk populations

Figure 5 shows the prevalence of HIV/AIDS among high-risk groups in North African countries. The highest prevalence was reported among injection drug users (IDUs), with an estimated median of 8% and a range from 3.80% (95%CI 2.46-4.67) to 15.7% (95%CI 9.46-18.67). This was particularly high in Morocco, Egypt, Sudan and Libya.

HIV/AIDS among sexually promiscuous individuals was reported to average 2.8% (1.7%-11.3%). It was reported to be 4.9% in Morocco, Algeria, Tunisia and Egypt and > 10.5% in Tunisia and Sudan[32-35]. Among prisoners, it was reported to be high in Libya, Sudan, Morocco and Egypt, but less in Mauritania and Algeria. HIV was also reported to be high in hospital care settings in North African countries, with a range of 0.8% to 9.7%[36-40]. The highest prevalence rates were reported in Mauritania, Algeria and Egypt, followed by Sudan, Tunisia, Morocco and Libya.

Distribution of HIV-1 subtypes

Based on our data, the genotype distribution of HIV in the seven North African countries is shown in Figure 6. Analysis of HIV-1 subtype distribution is scanty,

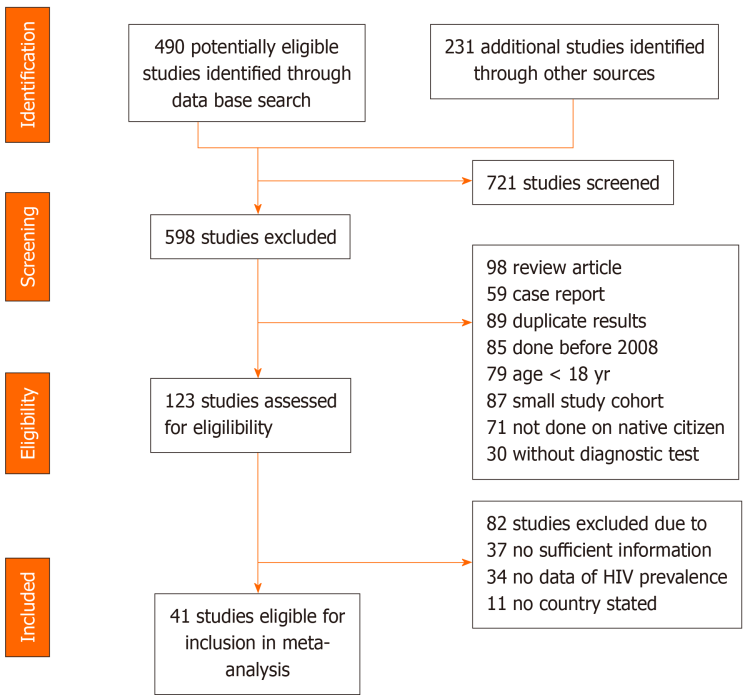

Figure 1 Selection of studies of human immunodeficiency virus/acquired immunodeficiency syndrome epidemiology in North Africa, 2008-2017, for inclusion in the meta-analysis. HIV: Human immunodeficiency virus.

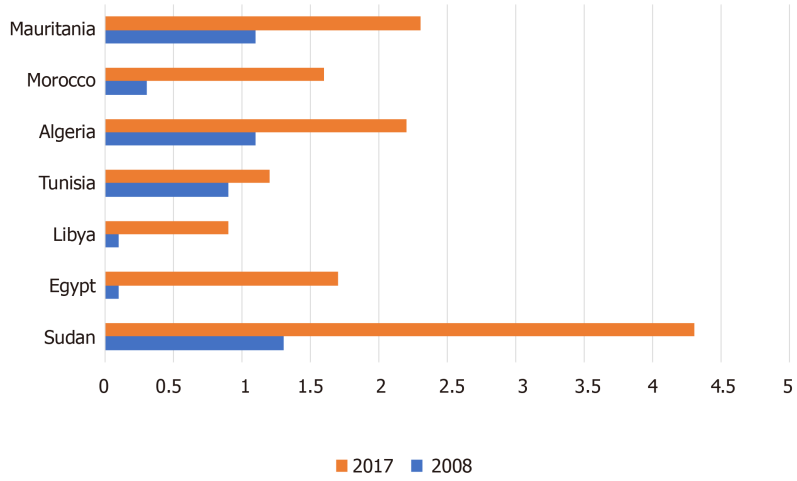

Figure 2 Prevalence of human immunodeficiency virus/acquired immunodeficiency syndrome in North African countries in 2008 and 2017.

particularly in Algeria and Mauritania, where only one study was reported in each of these two countries[41,42]. The Tunisian sequences belong to six HIV-1 subtypes (B, A1, G, D, C, and F2), five circulating recombinant forms (CRF02_AG, CRF25_cpx, CRF43_02G, CRF06_cpx and CRF19_cpx) and 11 unique recombinant forms. Subtype B (46.4%) and CRF02_AG (39.4%) were the predominant genetic forms[43]. Genetic analysis of HIV-1 strains in Libya demonstrated low subtype heterogeneity with the evolution of subtype B, which represents 74%, followed by CRF_20 AG (18%) and HIV-1 subtype A (8%). In Sudan, 50% were subtype D and 30% were subtype C. Subtypes A and B and three unique recombinants were also found, some partially unclassifiable[44-46]. In Morocco, subtype B was the predominant subtype (76.7%), followed by a high diversity of non-B subtypes, especially CRF02_AG recombinant (15%), and to a smaller extent subtype A (1.0%) and F strains (0.5%). In Egypt, the commonly isolated strains of subtype B comprise 95%, followed by CRF01_AE and A (1%). In Algeria, there was considerable HIV-1 diversity with a predominance of the B subtype followed by CRF02_AG and CRF06_cpx.5,6. Studies have indicated that the

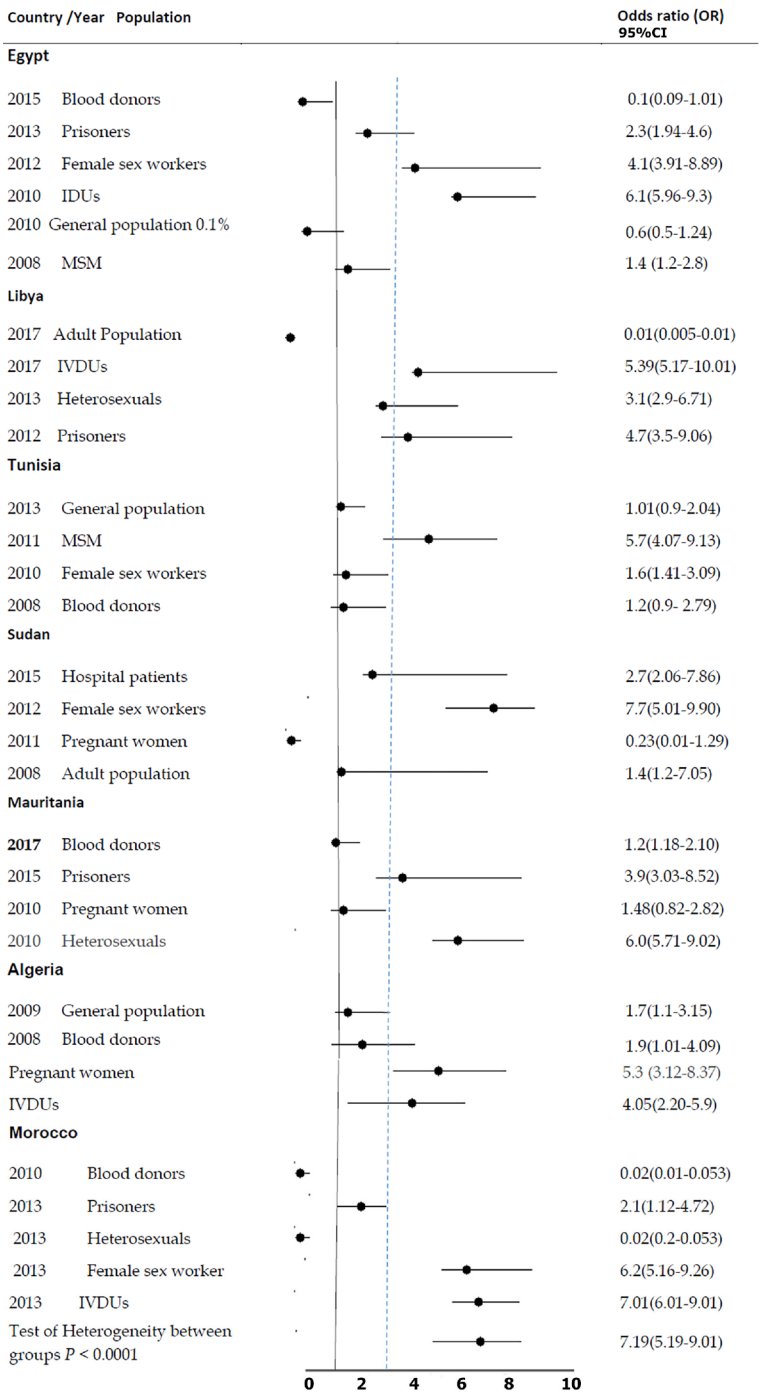

Figure 3 Forest plot of the seroprevalence of human immunodeficiency virus/acquired immunodeficiency syndrome among different populations in Northern Africa 2008-2017. IDUs: Injection drug users; MSM: Men who have sex with men; IVDUs: Intravenous drug users; CI: Confidence interval.

diversity was maintained, but CRF06_cpx became widely predominant. Phylogenetic analysis of different strains in Mauritania revealed that CRF02_AG (64.6%) was the predominant strain followed by B variants with a predominance of 10%.

Geospatial distribution of HIV/AIDS in North Africa

HIV/AIDS infections are reported to be high in the capital coastal cities in comparison to the other regions of North African countries. Figure 7 shows the spatial distribution of HIV-seropositive individuals living in North Africa between 2008 and 2017[47-50]. There is a clear change in the regional patterns of HIV with significant spatial heterogeneity within each country. The substantial variability ranged from 0.01% to 5%, with no clear regional patterning of the space-time interaction. A higher level was reported in Sudan, Morocco and Algeria and to a lower extent Mauritania and Tunisia.

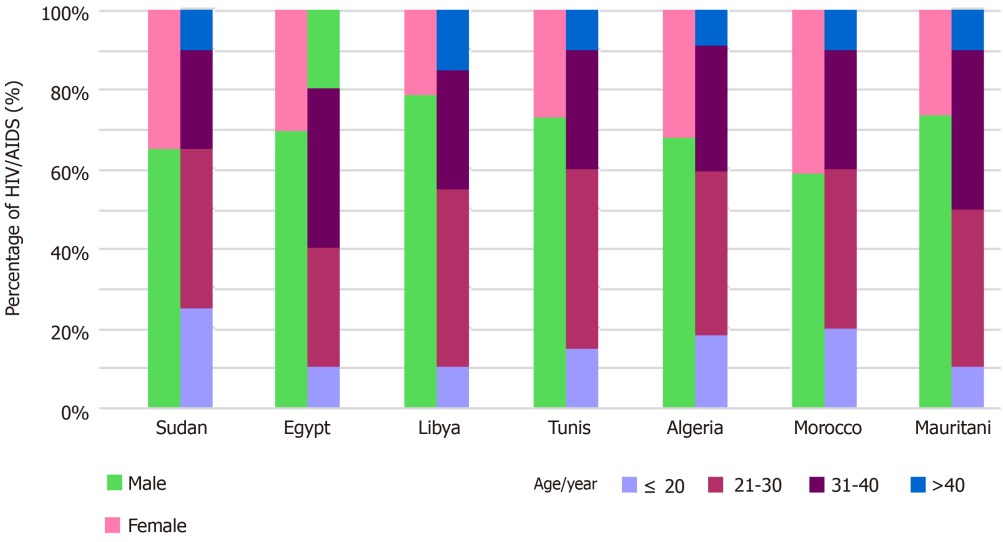

Figure 4 Gender and age-specific distribution of new human immunodeficiency virus/acquired immunodeficiency syndrome patients reported in North African countries from 2008 to 2017. HIV: Human immunodeficiency virus; AIDS: Acquired immunodeficiency syndrome.

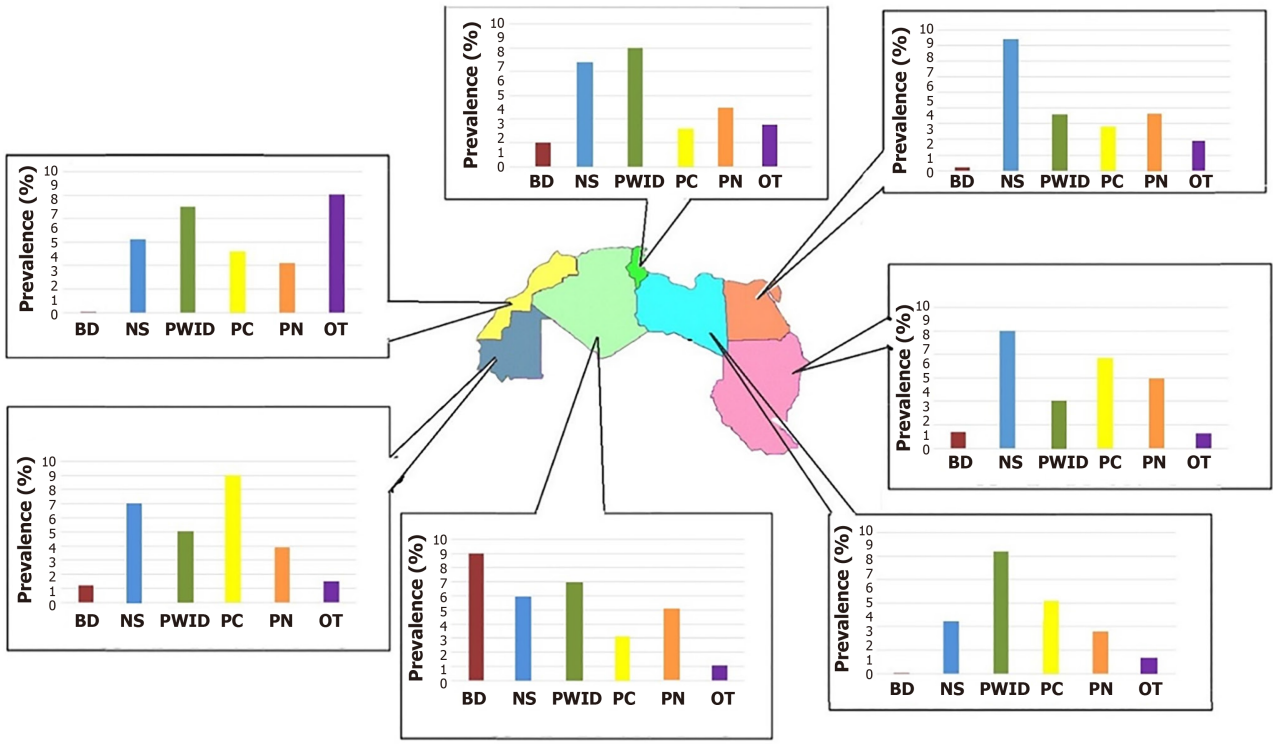

Figure 5 The prevalence of human immunodeficiency virus/acquired immunodeficiency syndrome among different population groups within North African countries, including blood donors, nosocomial infection, injection drug users, sexually promiscuous individuals, and others. BD: Blood donors; NS: Nosocomial infection; IDUs: Injection drug users; PC: Promiscuous individuals; OT: Others.

However, patterning persisted in Libya and Egypt. In Sudan, the HIV patterns reached the highest in southern regions. It is estimated that HIV prevalence in the 10 states that now make up South Sudan was 3.0%, ranging from zero in Northern Bahr el Ghazal to 7.2% in Western Equatoria State, followed by Kassala State in Eastern Sudan (0.2%-3%), Khartoum (0%-5.7%), Gadarif State (0.1%-0.4%), and Kosti (0.1%-0.7%). In Morocco, the highest was reported in Agadir Souss-Massa-Drâa in the south, Fes and Rabat in the central region, followed by Nador and Tanger in the north, and Marrakech in the southwest. In Egypt, the prevalence was high in east Cairo, followed by Alexandria and South Sinai. In Libya, it was high in both the eastern and western coastal regions, followed by the central south part of the Sebha area. In Tunisia, the

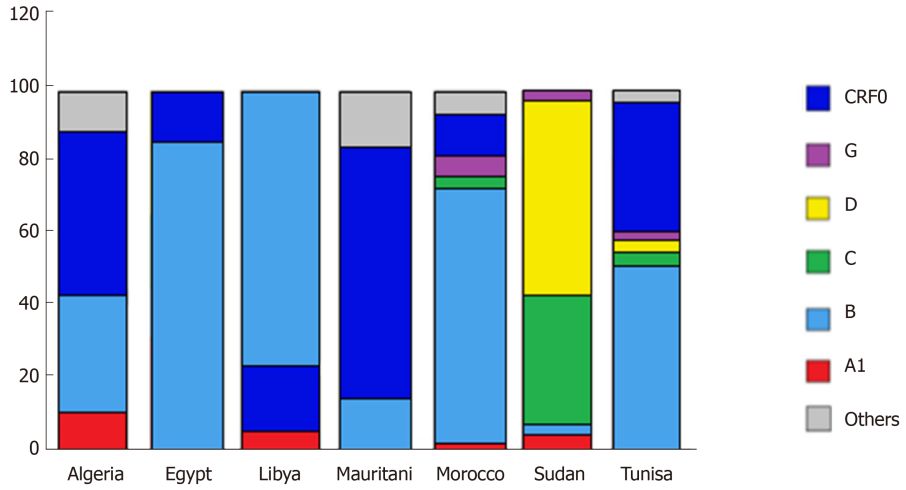

Figure 6 Distribution of human immunodeficiency virus genotypes in North African countries.

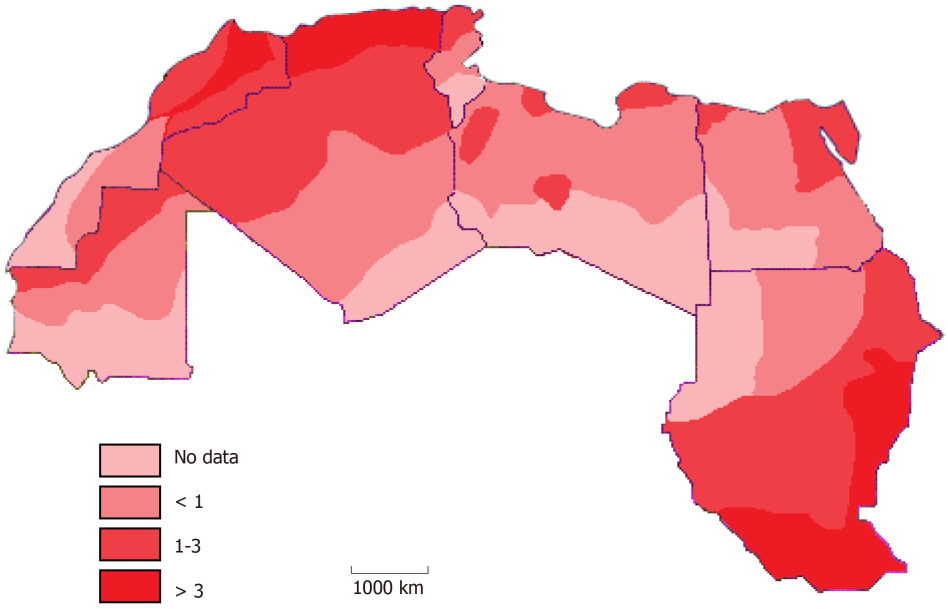

Figure 7 Geographic variation of the incidence of human immunodeficiency virus/acquired immunodeficiency syndrome in North African countries from 2008 to 2017.

prevalence was highest in the capital, Bizerte and Hammamet, followed by the other coastal cities of Sousse and Sfax, but it was lower in the middle and southern regions of the country. In Algeria, the northeastern region reported the highest HIV prevalence, particularly in the area neighboring Tunisia, followed by Oran and Sidi Bel Abbes. The prevalence was low in the central and southern regions of Algeria. In Mauritania, HIV prevalence reached its highest (1%-2%) in the Nouakchott area, followed by the central region. However, no data are available on most of the eastern and western Sahara regions.

North African countries showed spatial variation in HIV/AIDS cases during the 10-year study period. Figure 8 shows the HIV spatial clustering in the region, with dark red areas indicating statistically significant hotspots of higher than expected rates. The results of the spatiotemporal analysis suggest a special characteristic in the temporal and spatial distribution of HIV/AIDS incidents. A total of 11 statistically significant high-risk areas at different times were reported in several regions and provinces of the seven countries. In Morocco, which experienced two clusters, the largest cluster area was located in Agadir Souss-Massa-Drâa in the south in 2011 and Fes and Rabat in the central region, followed by Nador and Tanger. The second was reported in the north

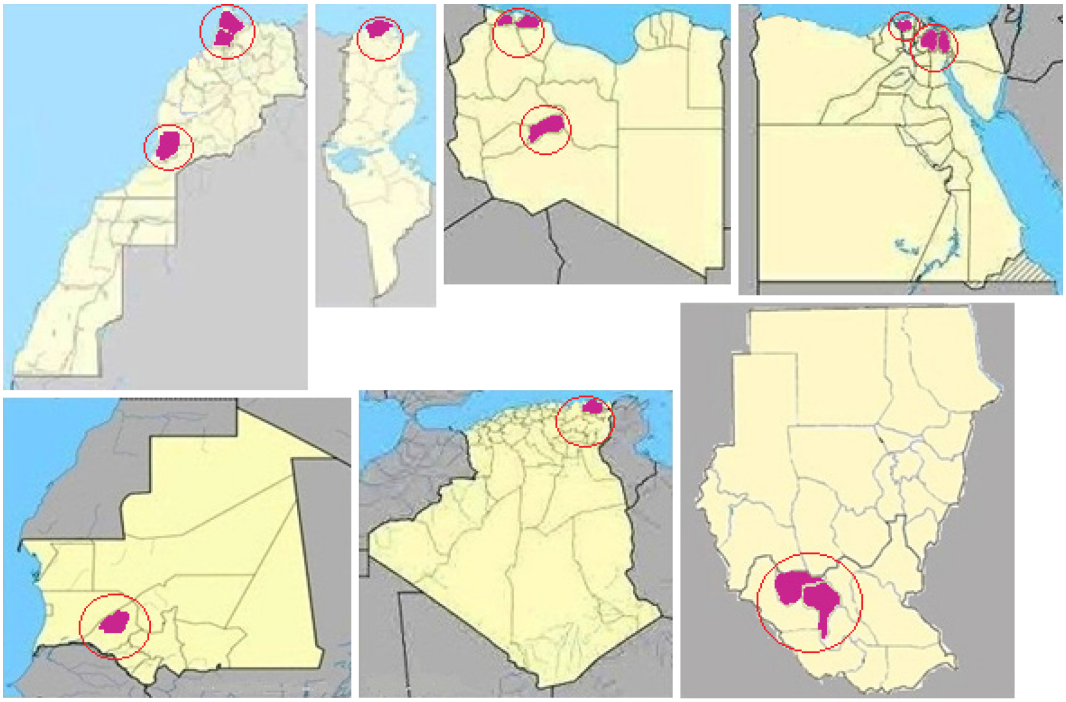

Figure 8 Spatiotemporal clusters of human immunodeficiency virus/acquired immunodeficiency syndrome in North African countries 2008-2017.

in 2012 and Marrakech in the southwest in 2014. The main clusters were reported among IDUs followed by MSM and female sex workers (FSWs). For IDU networks (6%; 95%CI 3-10), most transmission appears to have been in northern Morocco. The epidemic in the commercial heterosexual networks and MSM appears to have been most intense in the south of Morocco, especially in Souss-Massa-Drâa. The largest contribution to HIV incidence was among clients of FSWs (25%; 95%CI 14-37), followed by MSM (22%; 95%CI 12-35), stable heterosexual couples (corresponding to HIV serodiscordant couples; 22%; 95%CI 12-34), and FSWs (11%; 95%CI 6-18).

In Sudan, the biggest cluster was reported in Southern Sudan in the Western Equatoria State in 2012. Among 420 antenatal clinic attendees, HIV seropositivity was 10.7% (95%CI 8.0-14.2), and among 388 voluntary counseling and testing attendees, HIV seropositivity was 13.1% (95%CI 10.0-17.0), indicating high HIV prevalence in Western Equatoria State. In Libya, three clusters were reported during 2008-2017. The first cluster, which occurred during 2008-2012, consisted of 203 cases in Tripoli in the western region. The second one was reported in Musrata (the largest city in the central region) and consisted of 406 HIV cases detected between 2013 and 2017. The third cluster was detected in Sebha (the largest city in the south) between 2013 and 2017 and consisted of 317 HIV cases.

In 2011, Egypt experienced a concentrated epidemic among MSM and IDUs in the east Cairo sector, Alexandria, and southern Sinai. The HIV prevalence ranged from 5.4% to 6.9%. It was 6.9% among MSM and 6.7%-7.7% among IDUs. Minor clusters were reported in Algeria, Tunisia and Mauritania. In a study carried out in 2013 in two hospitals in northeastern Algeria, HIV was reported to be high among pregnant women, in whom the prevalence rate reached up to 5.3/1000. In Tunisia, a small cluster was reported in 2011 in the north of the country, particularly within the capital Tunis and mainly associated with FSWs and MSM. In Mauritania, a minor cluster was reported among blood donors in December 2015 in the Hodh El Gharbi region located 800 km from Nouakchott (the capital) in the south-east of the country.

DISCUSSION

There are insufficient data on HIV prevalence in the North African region and no formal, national, population-based surveillance studies have been reported apart from those in Libya and Morocco[19,25]. Worryingly, health planners and strategists in this region are inadequately attentive to the ongoing HIV epidemic situation. It seems that

cultural understanding and social perception are still not fully aware of the serious consequences of the epidemic. Infected individuals, particularly women, often face denial of care, stigmatizing attitudes, discrimination, and breaches of confidentiality[51].

In this review, we comprehensively analyzed the actual situation of HIV prevalence in North African countries. HIV prevalence has increased significantly in the region over the last ten years. The overall prevalence increased from 0.2%-1.4% in 2008 to 4.4% in 2017. The highest prevalence was reported in Sudan, Algeria, Mauritania and Egypt, followed by Morocco, Tunisia and Libya. However, there are concerns over the reliability of the data in certain countries. For instance, the prevalence in Egypt, which has the largest population in the region (over 100 million), Algeria and Tunisia was much lower than in Sudan and Morocco, and even in Mauritania (which has a population of only four million). However, the HIV epidemic seems to be alarming particularly in Algeria, Sudan, Mauritania and Morocco.

In the region, there is inadequate research on marginalized groups such as MSM and sex workers, particularly in Algeria, Egypt and Mauritania. The data on IDUs are even sparser, particularly in Libya, Mauritania and Algeria. However, emerging data from Morocco and Sudan indicate that HIV prevalence is significantly higher in these groups than in the general population. Hence, further studies at the national and regional levels are needed[52].

Different demographic factors have been reported to influence the prevalence of HIV among the North African populations. The highest prevalence was reported among younger individuals. Over 45% of HIV cases were aged 21-30 years, followed by the middle-aged group (31-40 years) at 30%. The infected individuals were predominantly unmarried individuals who were illiterate or had a low level of education. However, with the exception of Sudan, no country reported HIV infection among children younger than five years despite evidence of mother-to-child transmission. Furthermore, a steady increase among the older age group has been reported, particularly in Libya. The epidemic is no longer confined to males in the region. A considerable number of females are also affected. In Morocco, Sudan and Tunisia, the prevalence of HIV among women seems to be exceeding that of men. Studies in sub-Saharan Africa have shown that girls and young women have up to eight-fold higher rates of HIV infection compared to their male peers[53-55]. However, there remains a gap in young women employing HIV prevention technologies.

The patterns of HIV transmission have evolved and the epidemic concentration varies from one country to another in North Africa. Cases among blood donors have been eliminated in Morocco, Tunisia and Libya and decreased in Egypt, but they are still reported in Mauritania, Sudan and Algeria.

The prevalence was higher in healthcare settings in most of the countries. However, the epidemic is currently concentrated among IDUs and FSWs, particularly in Morocco, Egypt, Tunisia and Algeria, but to a lesser extent in Libya, Mauritania and Sudan, where incarceration was the main factor. This demographic variability is similar to that in other nations, such as in sub-Saharan Africa[56,57]. These findings indicate that drug injection might be the major risk factor for HIV transmission in the region. This is probably due to shared drug paraphernalia[58]. Hence, understanding the HIV dynamics in North Africa is an important step towards facing the challenges of this epidemic.

HIV sequences from cases identified in North African countries have been published sporadically, providing a mosaic overview of the molecular epidemiology of HIV in the region. The epidemics in Algeria, Morocco, Mauritania and Tunisia have been dominated by subtype B and CRF02_AG, as well as CRF06-cpx in Algeria. In Libya, 75% of the reported cases were subtype B and 18% were CRF 20 AG. On the other hand, in Sudan, 50% of the cases were subtype D and 30% were subtype C, while in Egypt subtype B represented 95% of cases. Given the wide distribution of subtypes in the region, HIV-1 was probably introduced multiple times in these countries. The broad array of subtypes/CRFs indicates that the epidemic is more complicated than in many other regions of the world, where one subtype usually predominates. Studies in North America and Southern Africa have shown that the main circulating subtypes represent more than 95% of all HIV-1 infections in these regions[59,60]. Furthermore, there is a lack of studies on phylodynamics in tracing the origins and transmission routes of HIV infection in the region. This is a particularly important aspect of the HIV outbreak among Libyan children associated with the Bulgarian Nurses saga[61].

Our study indicates that the spread of HIV varies greatly within North African countries and shows much geographical variation in the prevalence of HIV infection. However, there seems to be considerable inter- and intra-country variability ranging from 0% in some parts of Libya and Tunisia to over 3% in the coastal areas of Morocco, Algeria and Southern Sudan. These observed spatial variations highlight the clustering

of HIV across North African countries, indicating that a generalized epidemic may be evident in this region[62]. Certain pockets within the region harbor the threat of a generalized epidemic, as the virus spreads from the most-at-risk to the general population. Over 11 clusters have been reported. The driving factors of the dynamics of these clusters were mainly FSWs and IDUs, particularly in Morocco, Tunisia and Egypt. The rates of incidence of reported cases in Algeria, Mauritania and Sudan were associated with pregnant women and blood donation antenatal clinics. These findings should be considered in future research and clinical practice[63]. Public health policymakers should give careful consideration to the substantial variation in the spread of HIV through populations and communities within each country when formulating HIV control measures. This is especially important if one considers that there is no single global HIV epidemic. The Joint United Nations Program on HIV/AIDS has adopted the mantra ''Know your epidemic; know your response"[64].

The findings of this review shed light on key features of the epidemic in North Africa, but several gaps remain. There is a major gap in the data on HIV-related mortality in the region, particularly among HIV-infected children, and no studies have been carried out on mother-to-child transmission. Hence, emphasis needs to be placed on diagnosing and treating HIV infection in pregnant women to prevent perinatal transmission, early screening for HIV infection in infants born to HIV-infected mothers, and treating those who are infected before they develop more advanced disease as the world moves toward the goal of eliminating mother-to-child transmission of HIV[65]. Furthermore, most of these countries are plagued by internal conflicts and have persistent difficulty in addressing healthcare needs. Thus, immigrants, refugees, internally displaced persons and insurgent groups may play a role in the spread of HIV in North Africa. Further studies are needed in North Africa as data from sub-Saharan African countries suggest that reconstruction periods after conflict might be a more vulnerable time for HIV transmission than during conflict[66-68].

This meta-analysis has some limitations. One of the challenges was the fact that the studies adopted different methods in categorizing population groups and used different durations. Quality assessment of the studies included in this review showed that most studies obtained a medium-quality rating and few of them obtained a high score, which indicates that more rigorous research is needed. Moreover, we did not analyze some contributing factors, namely, the level of education and personal income of the HIV infected individuals. Furthermore, FSWs and MSM cannot be followed or even mentioned in some countries, and particularly in Libya and Mauritania, as these acts are considered crimes that may lead to the death penalty[69,70].

CONCLUSION

HIV is at an alarming status in North African countries, which face serious epidemics. Sudan, Egypt, Morocco and Algeria have concentrated epidemics, but HIV seems less concentrated in Mauritania, Libya and Tunisia. Furthermore, HIV seems to be moving towards concentrated clusters and measures have been challenged and hampered by massive population displacement associated with chaotic economic and sociopolitical situations. The endemicity of HIV in these countries is complicated by a lack of registry data and follow-up programs, particularly in the Saharan and countryside areas. The Joint United Nations Programme on HIV/AIDS (UNAIDS) definition of a generalized epidemic is an HIV prevalence of more than 1% in the general adult population (15-59 years) and more than 5% in vulnerable adult groups[71]. Hence, generalized epidemics persist in Sudan in particular and even in Algeria, Morocco and Egypt. There is an urgent need to establish a standardized epidemiological platform at both the national and regional levels that can reliably quantify individual differences in risk and understand the chain of HIV transmission and geographic clusters of HIV. Few studies have examined how geographic disparities may impact trends in HIV seropositive cases in North African countries. Identifying such social and geographic factors is important for better screening and treatment, and thus for reducing the burden among high-risk populations[19,72,73].

ARTICLE HIGHLIGHTS

Research background

Acquired immunodeficiency syndrome (AIDS) is a serious health problem in Africa but few studies have highlighted the epidemiological and spatiotemporal patterns of this infection, particularly in the North African region. Evidence is increasing regarding the magnitude of this problem and its social and economic impact in these countries. Analyzing the epidemiological situation of human immunodeficiency virus (HIV)/AIDS infection in this region has become one of the necessities for better understanding the current situation and for future planning.

Research motivation

There has been a dearth of information on the epidemiological status of HIV/AIDS in North African countries. This raises a serious concern regarding the impact of this infection in the region and how it could be controlled.

Research objectives

The aim of this comprehensive review was to analyze and characterize the epidemiological and geographic variation and clustering of HIV/AIDS in North Africa and outline the policies needed to combat this problem at both the national and regional levels.

Research methods

This is a comprehensive review of published data on different aspects of HIV/AIDS in North African countries in the last ten years (2008-2017). Every reported study was analyzed and all epidemiological parameters and risk factors associated with the spread of HIV in the region were determined. This will alert healthcare professionals and researchers to act immediately to implement proper policies to overcome this increasing problem.

Research results

The results indicate an increasing spread of HIV/AIDS in North African countries, with certain variations in prevalence, clustering and HIV subtypes between the countries and within regions of the same county. Higher prevalence rates have been reported among vulnerable populations.

Research conclusions

Based on the evidence of the collected data, North African countries are facing an intensifying problem of HIV infection. There are not enough reliable data to determine the magnitude of this problem and no clear policy to combat the infection in the region.

Research perspectives

We strongly suggest that specific and well-designed epidemiological studies should be conducted at national and regional levels to quantify the magnitude of the problem. Furthermore, clearly defined policies should be implemented to overcome this increasing problem.

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MINIREVIEWS

Angiotensin-converting enzyme 2 receptors, chronic liver diseases, common medications, and clinical outcomes in coronavirus disease 2019 patients

Wattana Leowattana

ORCID number: Wattana Leowattana 0000-0003-4257-2480.

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Wattana Leowattana, Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

Corresponding author: Wattana Leowattana, BSc, MD, MSc, PhD, Associate Professor, Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajavithi Road, Rachatawee, Bangkok 10400, Thailand. wattana.leo@mahidol.ac.th

Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for coronavirus disease 2019 (COVID-19), enters affected cells through the angiotensin-converting enzyme 2 (ACE2) receptor, which is highly expressed in type II alveolar cells, enterocytes, and cholangiocytes. SARS-CoV-2 infection causes fever, dry cough, and breathing difficulty, which can progress to respiratory distress due to interstitial pneumonia, and hepatobiliary injury due to COVID-19 is increasingly recognized. The hepatobiliary injury may be evident at presentation of the disease or develop during the disease progression. The development of more severe clinical outcomes in patients with chronic liver diseases (CLD) with or without cirrhosis infected with SARS-CoV-2 has not been elucidated. Moreover, there is limited data related to common medications that affect the disease severity of COVID-19 patients. Additionally, ACE2 receptor expression of hepatobiliary tissue related to the disease severity also have not been clarified. This review summarized the current situation regarding the clinical outcomes of COVID-19 patients with chronic liver diseases who were treated with common medications. Furthermore, the association between ACE2 receptor expression and disease severity in these patients is discussed.

Key Words: SARS-CoV-2; COVID-19; Hepatobiliary tissue; Angiotensin converting enzyme 2; Chronic liver disease; Common medications; Clinical outcome

Core Tip: With more than 100 million confirmed cases worldwide, hepatobiliary injury has been reported in many coronavirus disease 2019 (COVID-19) patients. The

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association between COVID-19 and hepatobiliary injury refers to any hepatobiliary damage during disease progression and treatment in COVID-19 patients with or without chronic liver diseases or common medications. Angiotensin-converting enzyme 2 receptor may be a significant factor in hepatobiliary derangement due to its high expression in cholangiocytes, and it is also an entry point of severe acute respiratory syndrome coronaviruses 2. Moreover, drug-induced liver injury and cytokine storm may be an added risk in severe clinical outcomes. Close monitoring of liver function in COVID-19 patients is mandatory.

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INTRODUCTION

Knowledge of the fundamental physiology of angiotensin-converting enzyme 2 (ACE2) has cumulated more than 20 years since its discovery in 2000 and has greatly increased our understanding of the renin-angiotensin system (RAS)[1,2]. The RAS is an essential hormone system with critical roles in blood pressure regulation, vascular biology, nervous system, electrolyte homeostasis, tissue injury, and lipid homeostasis[3,4]. ACE is the key-driven enzyme in classical RAS. On the other hand, the protective RAS is regulated by ACE2 and counterbalances many of the classical deleterious effects of the RAS[5,6]. ACE2 has definite roles ranging from catalytic activities with numerous substrates, as the receptors for severe acute respiratory syndrome coronaviruses (SARS-CoV) and SARS-CoV-2, and as an amino acid transporter[7-10]. ACE2 regulates the RAS by converting angiotensin (Ang) I and II into Ang 1-9 and Ang 1–7, respectively. Clinical and animal studies demonstrated a physiological and pathophysiological aspect of ACE2 in cardiovascular disease (CVD), and activating ACE2 may evoke protective outcomes against hypertension and CVD[11-13].

Since the end of 2019, ACE2 has amassed interest as the cellular receptor of SARS-CoV-2, the causative virus of the coronavirus disease 2019 (COVID-19) pandemic that emerged from Wuhan, China. It has rapidly spread through China, crossed the global borders of 221 countries, and infected 101529722 people, with 2186606 deaths resulting in a 2.15% mortality rate[14]. The clinical manifestations of COVID-19 patients include cough, fever, sore throat, diarrhea, and loss of sense of taste or smell. More than 80% of infected patients have mild symptoms, 14% have severe symptoms, and 5% have a critical illness. Older patients and those with medical co-morbidities are at risk of a severe disease course[15]. Previous studies demonstrated liver damage in nearly 60% of patients suffering from SARS. They also found SARS-CoV virus particles in the hepatocytes of patients[16]. Moreover, SARS-CoV-2 is associated with hepatic dysfunction ranging from 14% to 53% with abnormal levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) without known liver disease[17-19]. Patients with severe or critical outcomes showed higher frequency and degree of liver dysfunction, while in milder patients, the liver injury was transient[20]. Liver injury in COVID-19 patients included psychological stress, systemic inflammation response, drug toxicity, the progression of pre-existing chronic liver diseases (CLD), and other factors[21]. Hence, three possible scenarios have been postulated. Firstly, patients with CLD and pre-existing co-morbidity diseases may be more prone to the severe clinical outcomes of COVID-19, including oxygen desaturation and hypoxemia due to severe pneumonia or the cytokine storm. Secondly, liver enzyme abnormalities are the consequence of drug toxicity. Thirdly, SARS-CoV-2 directly or indirectly causes liver injury[22-24]. Although ACE2 receptors are abundantly present in type 2 alveolar cells, they are also expressed in the gastrointestinal tract, vascular endothelium, hepatocytes, and cholangiocytes and may be the significant factors in disease severity. This review will clarify the relationship between CLD, common medications, and the expression of ACE2 with the clinical outcomes in COVID-19 patients.

ACE2 RECEPTOR

Physiology of ACE2 receptor

ACE2 receptor resembles the ACE receptor and plays a crucial role in the renin-angiotensin-aldosterone system (RAAS), including blood pressure control and electrolyte homeostasis. The liver produced angiotensinogen, which is cleaved by renin from the kidney, results in Ang I. After that, ACE catalyzes the conversion of Ang I to Ang II. Ang II is the significant active RAAS portion and exerts its effects *via* Ang II type 1 receptor. Furthermore, Ang II's main effects include vasoconstriction, renal sodium reabsorption, potassium excretion, aldosterone synthesis, blood pressure elevation, and induction of pro-inflammatory and pro-fibrotic pathways. ACE2 splits Ang II to Ang (1-7) and Ang I to Ang (1-9). Furthermore, Ang (1-9) is cleaved by ACE to Ang (1-7). Ang (1-7) exerts vasodilatation, anti-inflammatory, and anti-fibrotic effects through the Mas receptor to counterbalance Ang II's action. Notably, ACE2 functionally counteracts the physiological role of ACE and creates the tissue balance of ACE and ACE2, which determines the pro-inflammatory, pro-fibrotic, or anti-inflammatory and anti-fibrotic pathways[25,26] (Figure 1). The common drugs prescribed for RAAS blockade in several disease conditions can affect this balance. Moreover, many dietary factors (high sodium, high fat, and high fructose intake) can also shift the ACE/ACE2 balance towards pro-inflammatory and pro-fibrotic[27-29].

Expression of ACE2 receptor in hepatobiliary tissue

In 2004, Hamming *et al*[30] investigated the immuno-localization of ACE2 in 93 human specimens and found that ACE2 was present in endothelial cells from small arteries, large arteries, and veins in the studied tissues. Marked ACE2 immuno-staining was found in type I and typed II alveolar epithelial cells in normal lungs. ACE2 was abundantly demonstrated in enterocytes of all small intestine but not in the enterocytes of the large intestine. ACE2 was not found in lymphoid tissues and hepatocytes. Recently, Xu *et al*[31] investigated ACE2 expression in the oral cavity mucosa and various organs, including the intestine, kidney, stomach, bile duct, liver, lungs, thyroid, esophagus, bladder, breasts, uterus, and prostate. They found that ACE2 could be expressed in various organs. The mean expression of ACE2 in the liver, bile duct, and lungs was 6.86 ± 1.35, 7.23 ± 1.16, 5.83 ± 0.71, respectively. This result demonstrated that the expression of ACE2 in the lungs and the liver was not different. Moreover, Zhao *et al*[32] identified ACE2 expression sparsely in cholangiocytes of human liver ductal organoids cells. Anti-ACE2 immuno-staining further confirmed the presence of ACE2 receptors on those cells. Furthermore, Li *et al*[33] explored the underlying liver injury mechanism by profiling ACE2 expression with CLD expression data. They found that the liver tissues with chronic diseases, such as cirrhosis, non-alcoholic steatohepatitis, simple steatosis, and dysplasia, could express higher levels of ACE2 than normal liver tissues.

The relationship between common medications and ACE2 expression

Sinha *et al*[34] performed *in vitro* and *in vivo* studies to identify the clinically approved drugs that could modify ACE2 expression. They found that ACE inhibitors (ACEIs) but not angiotensin II type-I receptor blockers (ARBs) tend to upregulate ACE2 expression, and anti-adrenergic drugs other than alpha/beta-blockers tend to down-regulate ACE2 expression. Moreover, calcium channel blockers (CCBs) do not significantly change ACE2 expression, consistent with the finding that they do not act on the RAAS. This evidence provides preliminary *in vitro* support for the use of CCBs as an alternative to ACEIs in COVID-19 patients with hypertension. They also studied the 13 approved anti-diabetic drugs related to ACE2 expression, and they could not demonstrate that the drugs significantly altered ACE2 expression. Surprisingly, they reported that intravenous dexamethasone injection could increase ACE2 expression. They also demonstrated the effect of vancomycin, which increased an ACE2 expression. Saheb SharifAskari *et al*[35] studied the effect of common medications on the expression of ACE2 receptors in human primary hepatocytes. They found that the top three drugs that increased ACE2 expression were penicillamine, ethambutol, and vitamin A. The top five drugs that decreased ACE2 expression were colchicine, acetaminophen, sulindac, diazepam, and nimesulide. The top five drugs that did not change ACE2 expression were ibuprofen, lornoxicam, mefenamic acid, meloxicam, and methyltestosterone.

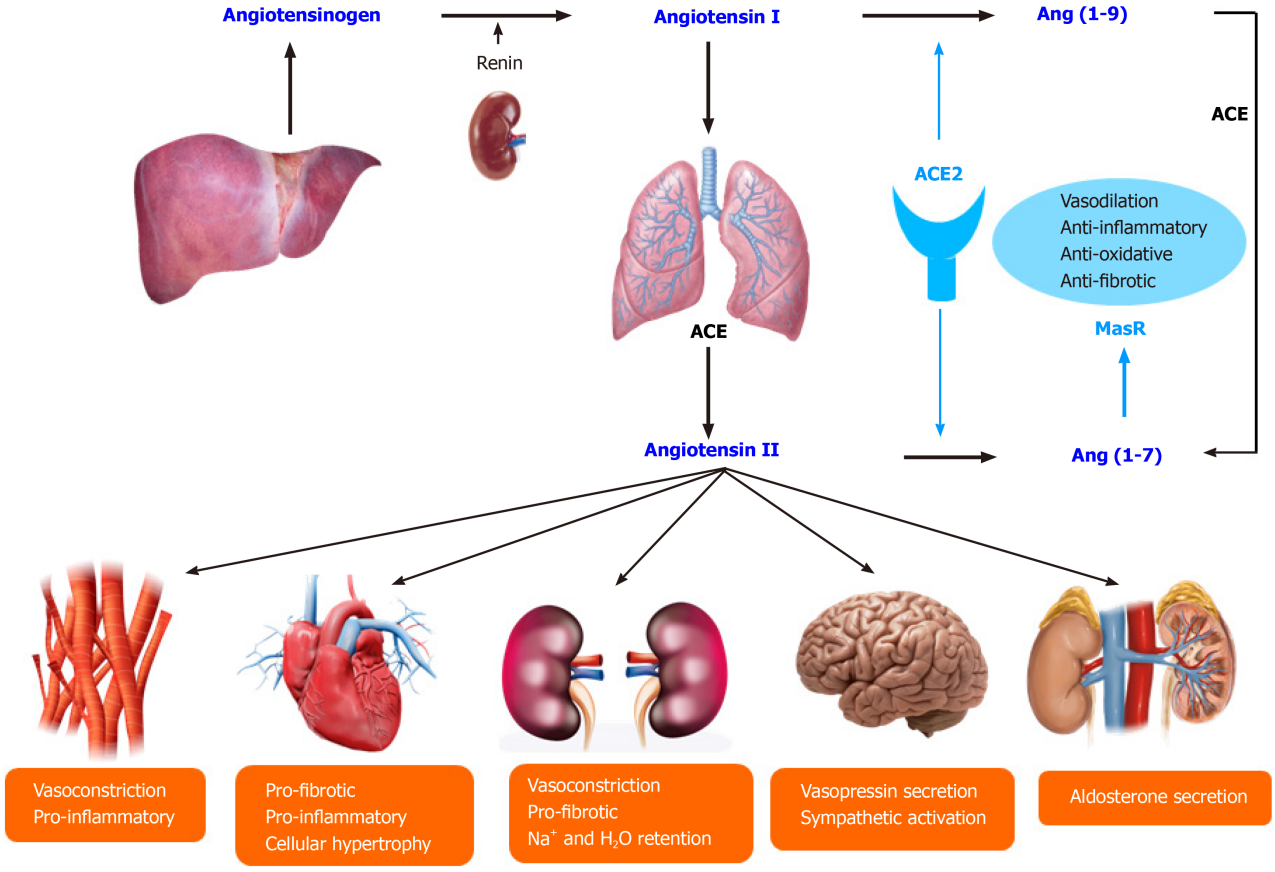

Figure 1 The renin-angiotensin-aldosterone system and the physiology of angiotensin-converting enzyme 2. ACE: Angiotensin-converting enzyme.

COVID-19 AND HEPATOBILIARY INJURY

Laboratory evidence of hepatobiliary injury

Previous studies have shown that nearly 60% of SARS patients developed a hepatobiliary injury and that SARS-CoV antigens were detected in liver tissues by reverse transcription-polymerase chain reaction[36,37]. Hepatobiliary injury in COVID-19 patients was also demonstrated by abnormal transaminase levels linked to the disease severity and the clinical outcome. Abnormal liver enzymes in COVID-19 patients were first reported by Chen *et al*[38]. They analyzed data of 99 COVID-19 patients from Wuhan and found that 43 cases (43.4%) had elevated ALT, AST, and lactic dehydrogenase. Most of them had a mild elevation of AST and ALT, and only one patient had very high ALT levels of 7590 U/L and AST levels of 1445 U/L. Recently, Kulkarni *et al*[39] conducted a systematic review with meta-analysis to evaluate the liver manifestations and clinical outcomes in 20874 COVID-19 patients. They found that the pooled incidence of elevated AST and ALT in COVID-19 was 23.1% (19.3%-27.3%) at initial presentation. Moreover, 24.4% (13.5%-40%) of the patients developed elevated AST and ALT during the illness. They also reported the prevalence of underlying CLD as 3.6% among the 15407 COVID-19 patients. The pooled incidence of drug-induced hepatobiliary injury was 25.4% (14.2%-41.4%). They found that the development of severe COVID-19 in CLD patients had an odds ratio (OR) of 0.81 [95% confidence interval (CI): 0.31-2.09] compared with non-CLD patients. Furthermore, COVID-19 patients with elevated AST and ALT had increased risk of mortality (OR = 3.46, 95%CI: 2.42-4.95, $P < 0.001$) and severe disease (OR = 2.87, 95%CI: 2.29-3.6, $P < 0.001$) when compared with the patients without elevated AST and ALT.

Recently, Del Zompo *et al*[40] conducted a systematic review with meta-analysis to elucidate the prevalence of hepatobiliary injury in 20724 COVID-19 patients with or without pre-existing CLD. They found that the pooled prevalence of abnormal liver function tests (LFTs) on admission was 46.9% [AST 26.5%, ALT 22.8%, gamma-glutamyl transferase (GGT) 22.5%, alkaline phosphatase (ALP) 5.7%, and total bilirubin (tBIL) 8.0%]. The elevation of ALT, AST, and tBIL were independent predictors of disease severity and in-hospital mortality. Wong *et al*[41] conducted

another systematic review with meta-analysis to evaluate the prevalence and degree of liver injury in 5961 severe and non-severe COVID-19. They found that the OR for elevated ALT was 2.5, AST was 3.4, hyperbilirubinemia was 1.7, and hypoalbuminemia was 7.1, which were higher in critical COVID-19. They concluded that hepatobiliary injury is more common in COVID-19 patients with severe clinical outcomes than in COVID-19 patients with non-severe clinical outcomes.

Mao *et al*[42] conducted another meta-analysis to evaluate the prevalence and prognosis of gastrointestinal symptoms and hepatobiliary injury in 6686 patients with COVID-19. They found that the pooled prevalence of liver co-morbidities was 3%, including chronic hepatitis and liver cirrhosis. The pooled prevalence of liver injury from 12 studies (n = 1267) was 19%. The prevalence of elevated ALT was 18%, AST was 21%, tBIL was 6%, and decreased albumin was 6%. They also reported a higher risk of abnormal LFT in patients with severe COVID-19 than those with the non-severe disease.

Kumar-M *et al*[43] conducted another meta-analysis to evaluate the overall prevalence, stratified prevalence based on severity, estimated risk ratio (RR), and estimated standardized mean difference (SMD) of liver function parameters in severe compared to non-severe COVID-19 patients with a total number of 28659 subjects. They found that the most frequent abnormalities were hypoalbuminemia (61.27%), elevated GGT = 27.94%, elevated ALT = 23.28%, and elevated AST = 23.41%. Furthermore, the relative risk (RR) of these abnormalities was higher in the patients with severe COVID-19 when compared to non-severe disease (hypoalbuminemia RR = 2.65; GGT RR = 2.31; AST RR = 2.30; and ALT RR = 1.76). The pooled prevalence and RR of CLD as a pre-existing co-morbidity were 2.64% and 1.69%, respectively. They concluded that the most frequent hepatobiliary injury was hypoalbuminemia followed by elevated GGT, elevated AST, and elevated ALT, which were more common in severe COVID-19 patients.

Youssef *et al*[44] conducted a meta-analysis of 3428 COVID-19 patients to elucidate the relationship between hepatobiliary injuries and the severity of COVID-19 disease. They found that the patients who had severe presentations of COVID-19 had hypoalbuminemia (SMD = 0.68), elevated AST (SMD = 0.36), elevated ALT (SMD = 0.44), and elevated tBIL (SMD = 0.40). They also reported that severe COVID-19 patients had a higher OR of developing acute hepatobiliary injury (OR = 1.93). They concluded that hepatobiliary injury was related to a critical outcome of COVID-19 patients. Close monitoring of the development of liver dysfunction is beneficial in early warning of unfavorable outcomes.

Wang *et al*[45] conducted a meta-analysis to evaluate the association of liver injury and gastrointestinal symptoms (GIS) with the progression of COVID-19 in 3024 patients. They found that 53% of patients had a hepatobiliary injury, and the degree of hepatobiliary damage was associated with disease severity. The prevalence of GIS was relatively low and was not associated with disease progression, with diarrhea of 9.1%, nausea/vomiting of 5.2%, and abdominal pain of 3.5%.

Wu *et al*[46] conducted a meta-analysis to explore the probable clinical severity and mortality of COVID-19 patients and their liver dysfunction in 3722 COVID-19 patients. They found a significant connection between hepatobiliary dysfunction and mortality in COVID-19 patients with a pooled OR of 1.98. There was a significant association between elevated AST and severity of COVID-19 with a pooled OR of 4.48 and a pooled weighted mean difference of 3.35. They also found a significant difference between elevated tBIL and severe COVID-19 (pooled OR = 1.91 and pooled weighted mean difference = 1.18). They concluded that the mortality and severity of COVID-19 patients are significantly associated with hepatobiliary dysfunction.

Samidoust *et al*[47] conducted a meta-analysis study to investigate the incidence of liver injury among 4191 COVID-19 patients. They found that the pooled prevalence of liver injury was 19.5%. They concluded that hepatobiliary system is the most frequently damaged outside of the respiratory system. Wu *et al*[48] conducted the meta-analysis to explore the incidence, risk factors, and prognosis of abnormal liver biochemical tests in 7228 COVID-19 patients. They found that the pooled prevalence of any abnormal liver biochemistry parameters on admission and during hospitalization was 27.2% and 36%, respectively. The most common prevalence was hypoalbuminemia followed by GGT, AST, ALT, tBIL, and ALP (39.8%, 35.8%, 21.8%, 20.4%, 8.8%, and 4.7%). Moreover, severe or critical patients had a significantly higher pooled incidence of abnormal liver biochemistry parameters on admission than mild or moderate patients. Non-survival patients also had a significantly higher incidence of abnormal liver biochemical indicators than survival patients (RR = 1.34). They concluded that abnormal liver biochemical tests are common and are closely related to the severity and prognosis of COVID-19 patients.

Mantovani *et al*[49] conducted the meta-analysis to assess the overall prevalence of CLD among 2034 COVID-19 patients. They found that the overall prevalence of CLD at baseline was 3%, and patients with severe COVID-19 disease had relevant increases of liver enzymes and coagulation profile due to the innate immune response against the SAR-CoV-2 virus. Sultan *et al*[50] conducted the meta-analysis to summarize international data on the gastrointestinal (GI) and liver manifestations of SAR-CoV-2 infection and treatment in 10890 COVID-19 patients. They found that elevated AST, elevated ALT, and elevated tBIL are observed in approximately 15%-20% of COVID-19 patients. These findings inform that the clinician should perform a careful evaluation of patients with new-onset GI symptoms for classic and atypical symptoms of COVID-19. All hospitalized COVID-19 patients may benefit from liver enzyme monitoring, particularly in drug treatment with known hepatotoxic potential.

Pathological finding of hepatobiliary injury

Xu *et al*[51] reported the first post-mortem findings of a patient who succumbed to severe COVID-19. They found that the liver histology showed moderate microvesicular steatosis and mild inflammatory infiltrates in the hepatic lobule and portal tract. They do not know whether these changes were from the direct viral injury or drug toxicity. Wichmann *et al*[52] conducted a prospective cohort study to perform the autopsies of 12 consecutive COVID-19 deaths, including post-mortem computed tomography and histopathologic and virologic analyses. The median patient age was 73 years (52 to 87 years), 75% of patients were male, and death occurred in the hospital (n = 10) or outpatient department (n = 2). They did not report the histopathology of the hepatobiliary system; however, they could demonstrate the detection of SARS-CoV-2 ribonucleic acid in the lungs of 12 patients (1.2×10^4 to 9×10^9 copies/mL) and the pharynx of nine patients. In five of these patients, viral ribonucleic acid was also detected in the heart, liver, and kidney. They concluded that SARS-CoV-2 might spread *via* the bloodstream and infect other organs, including the hepatobiliary system. Tian *et al*[53] performed post-mortem needle core biopsies of lung, liver, and heart in four patients who died of COVID-19 pneumonia. They found that the liver histopathology showed mild lobular infiltration by small lymphocytes, centrilobular sinusoidal dilatation, focal macrovesicular steatosis, and patchy hepatic necrosis in the periportal and centrilobular areas. Tabary *et al*[54] reviewed multiple organs, including lung, GI tract, liver, kidney, skin, heart, blood, spleen, lymph nodes, brain, blood vessels, and placenta, in COVID-19-related pathological alterations. The liver found hepatocyte degeneration with lobular focal necrosis, congestion of hepatic sinuses with microthrombus, fibrosis of portal tract, the proliferation of portal vein branches, mononuclear leukocyte, and neutrophil infiltration within the portal area and moderate microvascular steatosis. Yao *et al*[55] conducted another histopathology of the hepatobiliary system. They found that the liver exhibits mild sinusoidal dilation, with mildly increased small lymphocytes infiltration in sinusoidal spaces. Mild to moderate steatosis and multifocal hepatic necrosis have been reported. These findings confirmed that the hepatocellular injury in COVID-19 patients should be considered as a significant factor in disease severity.

CLD AND CLINICAL OUTCOME

The COVID-19 patients with pre-existing CLD usually face a relatively high risk of poor clinical outcomes. Li *et al*[33] established that patients with CVDs could express higher ACE2 expression than those without heart diseases. Furthermore, ACE2 was upregulated in patients with type 2 diabetes (T2D) compared to the individuals without T2D. For CLD such as cirrhosis, non-alcoholic steatohepatitis, and simple steatosis, ACE2 could express higher levels than normal liver tissues. The upregulation of ACE2 expression in patients with CLD may result in greater susceptibility to SARS-CoV-2 infection of hepatobiliary tissues. Sarin *et al*[56] conducted The APASL COVID-19 Liver Injury Spectrum Study (APCOLIS Study) to evaluate the liver injury patterns of SARS-CoV-2 in 185 CLD patients without cirrhosis compared with 43 CLD patients with cirrhosis. They found that pre-existing CLD, like metabolic associate fatty liver disease, obesity, and diabetes, was present in nearly 80% of the patients. Moreover, SARS-CoV-2 infection produces acute liver injury in 43% of CLD patients without cirrhosis. Nearly half of decompensated cirrhosis patients develop liver-related complications, which were more severe and had higher mortality. The liver injury pattern in CLD patients was mostly a hepatocellular injury. Notably, elevated serum ALP and elevated GGT were detected, indicating virus-related injury to hepatobiliary

tissue due to the overexpression of ACE2 on cholangiocytes. They also found acute, chronic liver failure (ACLF) or acute decompensation in 20% of the cirrhotic patients, which indicated that SARS-CoV-2, a non-hepatotropic virus, can directly precipitate a severe hepatic injury to cause liver failure in cirrhotic patients. They concluded that pre-existing CLD is an added risk in severe COVID-19 patients. Liver-related complications, overall complications, and clinical outcomes correlated with the existing hepatic reserve. Moreover, acute liver injury is more severe and more progressive with higher mortality in COVID-19 patients with decompensated cirrhosis.

Marjot *et al*[57] conducted an international registry study to evaluate the impact of COVID-19 on patients with pre-existing CLD. They recruited 745 patients with CLD who were infected with SARS-CoV-2 (386 with cirrhosis and 359 without cirrhosis) and compared them to non-CLD patients with SARS-CoV-2 infection. They found that the mortality rate was 32% in COVID-19 patients with cirrhosis compared to 8% in those without cirrhosis. Mortality in cirrhosis patients increased according to Child-Pugh Class [A (19%), B (35%), and C (51%)] and 71% of death was an acute respiratory distress syndrome. Compared to COVID-19 patients without CLD (n = 620), the propensity-score-matched analysis revealed a significant increase in mortality in those with Child-Pugh B cirrhosis (+ 20.0%) and Child-Pugh C cirrhosis (+ 38.1%). Acute hepatic decompensation developed in 46% of cirrhosis patients, of whom 21% had no respiratory symptoms. Half of those with hepatic decompensation had ACLF. They concluded that baseline liver disease and alcohol-related liver disease are independent risk factors for death from COVID-19. Another group of investigators from Korea conducted a multicenter study to evaluate the clinical outcomes in 1005 COVID-19 patients related to pre-existing CLD and the predictors of disease severity and mortality. They found that liver cirrhosis was more common in COVID-19 patients with severe pneumonia than in non-severe pneumonia (4.5% *vs* 0.9%). The overall survival rate significantly decreased in COVID-19 patients with liver cirrhosis than in those without liver cirrhosis. The presence of liver cirrhosis was found to be an independent predictor of severe clinical outcome. They suggested that more robust personal protection and more intensive treatment for COVID-19 with pre-existing CLD should be highly recommended[58].

Del Zompo *et al*[40] conducted the meta-analysis to elucidate the prevalence of hepatobiliary injury in COVID-19 patients with or without pre-existing CLD. They explored 36 studies, including 20724 patients with SARS-CoV-2 infection, and found that LFTs alterations were reported in up to 47% of unselected patients with COVID-19 and were associated with severe clinical outcomes or in-hospital mortality. COVID-19 was associated with a high risk of liver decompensation or mortality. Váncsa *et al*[59] conducted the meta-analysis to evaluate the prognostic value of on-admission LFTs and pre-existing CLD on the clinical course of COVID-19. They evaluated 50 studies with 17205 COVID-19 patients. They reported that the decreased platelet count, elevated ALT, elevated AST, increased C-reactive protein, and the presence of acute or CLDs at the time of admission could predict severe clinical outcomes of COVID-19 patients. Significantly, the pre-existing CLD or acute liver injury combined with SARS-CoV-2 infection was an important factor in predicting mortality rate.

COMMON MEDICATIONS TREATMENT AND CLINICAL OUTCOME IN COVID-19 PATIENTS

Several publications reviewed the role of RAS inhibitors in COVID-19 patients and found that there is no definitive evidence indicating harmful effects of RAS inhibitors. Because ACE and ACE2 are different enzymes, ACEIs do not inhibit ACE2, making this class' harmful effect unlikely[60-62]. Other common anti-hypertensive drugs are ARBs, which have been shown to upregulate ACE2 in animal studies, but these findings do not translate into clinical observations related to COVID-19[63]. Drager *et al*[64] summarized that the available clinical evidence points to a neutral or even beneficial effect on clinical outcomes in COVID-19 patients who received ACEIs or ARBs. Luo *et al*[65] conducted a retrospective analysis to compare the outcome of metformin users and non-users in 283 hospitalized COVID-19 patients with diabetes (104 used metformin, and 179 did not use metformin). They found that in-hospital mortality was significantly lower in the metformin group [3/104 (2.9%) *vs* 22/179 (12.3%), P = 0.01]. They concluded that metformin might offer benefits in COVID-19 patients. However, they did not mention the relationship between metformin and hepatobiliary injury in their study. Treatment of common co-morbidities such as cardiovascular, hepatobiliary, and metabolic disorders often requires continuous use

of several medications, which may result in an additive increase in the expression of ACE2. Furthermore, the combined effect of chronic use of these medications could affect liver susceptibility in COVID-19 patients. Although the increased risk of developing severe clinical outcomes in COVID-19 patients should not be the direct effect of common medications, we should be vigilant about the possible effects of those medications.

CONCLUSION

Several factors have been associated with the alteration of ACE2 expression and COVID-19 severity and progression. Although ACE2 is widely expressed in various human tissues and most of its determinants have been well recognized, ACE2-expressing organs do not equally participate in COVID-19 pathophysiology, implicating that other factors are involved orchestrating cellular infection resulting in several organs injury. Abnormal LFTs are reported in up to half of the patients with COVID-19 infection. The disease severity, pre-existing CLD, and some common medications presented risks for hepatobiliary injury in COVID-19 patients. It has been demonstrated that SARS-CoV-2 may directly bind to ACE2 positive cholangiocytes and cause severe hepatic injury. However, pre-existing CLD and some common medications could also upregulate ACE2 expression in the hepatobiliary tissues and cause more severe clinical outcomes in COVID-19 patients. Furthermore, other contributing mechanisms such as drug-induced liver injury, activation of the immune system, and cytokine storm may be the other contributing factors in severe clinical outcomes.

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MINIREVIEWS

Impact of COVID-19 in patients with lymphoid malignancies

John Charles Riches

ORCID number: John Charles Riches 0000-0002-3425-7686.

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John Charles Riches, Centre for Haemato-Oncology, Barts Cancer Institute - a Cancer Research UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom

Corresponding author: John Charles Riches, BM BCh, MA, MRCP, FRCPath, PhD, Clinical Senior Lecturer and Honorary Consultant Haemato-oncologist, Centre for Haemato-Oncology, Barts Cancer Institute - a Cancer Research UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, 3rd Floor John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom. j.riches@qmul.ac.uk

Abstract

The first cases of coronavirus disease 2019 (COVID-19) were detected in Wuhan, China, in December 2019. Since this time a concerted global effort of research and observational data gathering has meant that a great deal has been learnt about the impact of COVID-19 in patients with lymphoid malignancies. Approximately one-third of patients with lymphoid malignancies who acquire COVID-19 and have it severely enough to require hospital assessment will die from this infection. Major risk factors for a poor outcome are age and co-morbidities, but when these are taken into account lymphoma patients have a slightly greater than 2-fold increased risk compared to the general population. Notably, despite early concerns regarding the particular vulnerability of lymphoma patients due to the immunosuppressive effects of therapy, active treatment, including B-cell depleting agents such as rituximab, do not appear to be associated with an increased risk of a poorer outcome. Indeed, some treatments such as ibrutinib may be beneficial due to their modulation of the potential fatal hyperinflammatory phase of infection. There are risks associated with hemopoietic stem cell transplantation, but the collective experience is that these can be minimized by preventive strategies and that the majority of transplant recipients with COVID-19 infection will survive. Many questions remain including those regarding the outcome of COVID-19 infection in the rarer lymphoid malignancies and the efficacy of COVID-19 vaccines in lymphoma patients. This review aims to discuss these issues and present a summary of the current knowledge of the impact of COVID-19 in lymphoid malignancies.

Key Words: COVID-19; Lymphoma; Leukemia; Chemoimmunotherapy; Hemopoietic stem cell transplantation; Vaccination

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Core Tip: Patients with lymphoid malignancies who have coronavirus disease 2019 (COVID-19) severely enough to require hospital assessment have an approximately one-third chance of dying from the infection, representing a slightly greater than 2-fold increased risk compared to the general population. Despite initial concerns, treatment for lymphoma is not associated with increased risk for poor outcome. Current evidence for the efficacy of COVID-19 vaccines in patients with lymphoid malignancies is extremely limited, so it will be crucial to conduct studies to address this issue over the coming months.

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INTRODUCTION

The first cases of coronavirus disease 2019 (COVID-19) were detected in Wuhan, China, in December 2019. The disease, caused by a novel RNA beta coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was initially reported as predominantly causing a pulmonary syndrome, typified by fevers in combination with breathlessness and cough[1]. However, it is now appreciated that COVID-19 can cause a wide range of symptoms of variable severity, including fatigue, myalgia, headache, anosmia, pharyngitis, coryza, nausea and diarrhoea[2]. Since initial detection of the virus, more than 130 million cases of COVID-19 have been confirmed worldwide, with more than 2.8 million deaths[3]. Initial reports from China have indicated that COVID-19 has an overall mortality rate of 1.4%. However, the prognosis varies widely between groups, with those people over the age of 60 years and those with underlying conditions, including hypertension, diabetes, cardiovascular disease, chronic respiratory disease and cancer, at a significantly higher risk for severe disease and death[4].

There has been a great deal of concern that patients with lymphoid malignancies such as lymphomas and lymphoid leukemias would be at particular risk from COVID-19. The initial reports from China showed that patients with cancer were over-represented among individuals who developed severe COVID-19 after contracting the virus[5]. Patients with lymphoid malignancies could be expected to be at increased risk of adverse outcomes from this viral infection, both due to being immuno-compromised as a consequence of the underlying cancer, and due to the myelosuppressive and lymphodepleting effects of therapy. A number of retrospective studies have reported outcomes of patients with lymphoid malignancies who became infected with SARS-CoV-2 during or shortly after treatment[6-21]. These were pooled into a large meta-analysis of 3377 patients with hematological malignancies who developed COVID-19 with a primary outcome of risk of death[22]. Among all blood cancers the overall risk of death was 34%, rising to 39% when combining data for hospitalized patients. Within this the pooled risk of death was also calculated by hematologic malignancy subtype with lymphomas including/excluding chronic lymphocytic leukemia (CLL) having a risk of death of 32%, with CLL specifically having a risk of 31%. This was comparable to myeloproliferative neoplasms (34%) and plasma cell dyscrasias (33%), but somewhat less than acute leukemias (41%) and acquired bone marrow failure syndromes (53%). Notably the primary risk factor for COVID-19 mortality was age with patients aged 60 years and older having a significantly higher risk of death than patients under 60 years. While these "headline" figures are rather high, one of the major limitations of these retrospective studies was that almost all of them focused on patients who were either assessed in hospital, or were actually hospitalized for their COVID-19. Invariably, these patients had more severe infections than those who remained at home, who were not necessarily detected and included in these studies, making these mortality statistics an over-estimation. Ascertaining the true mortality rates remains challenging and governments around the world continue to advise patients with mild COVID-19 symptoms to self-isolate at home. At the time of our own study the United Kingdom was focused on hospital-based testing for suspected COVID-19, representing a comparable group of patients to the meta-

analysis[23]. This allowed an estimation of a crude case fatality rate of 14% suggesting that blood cancer patients have a 2-2.5 -fold greater risk of dying from COVID-19 than the general population. The largest single study to date also likely has the best estimate of true population mortality risk from COVID-19 for hematological cancer patients as they used population-based data from a countrywide Ministry of Health database[18]. This reported a risk of death 14%, which was twice that of a control population in their study (7%) and was comparable to the estimated risk of death of 13% in patients with all cancers[24]. A further study from Italy of 536 patients with hematologic malignancies and COVID-19 reported a mortality rate 37%, with a standardized mortality ratio for of 2.04 increased risk when compared with the impact of COVID-19 in the general Italian population[13]. Taken together, these studies have fairly consistently demonstrated that approximately one-third of patients with hematological malignancies who acquire COVID-19 and have it severely enough to require hospital assessment and/or admission will die from this infection. The major risk factors are age and co-morbidities, but when these are taken into account patients with blood cancers have a slightly greater than 2-fold increased risk compared to the general population.

IMPACT OF COVID-19 BY LYMPHOMA SUBTYPE

Many of the larger studies have pooled all patients with hematological cancers together. While this is useful, clearly there is very significant heterogeneity within this group of diseases, in respect of pathophysiology, clinical characteristics, and the type and intensity of treatment. Therefore, studies which have included patients with a single disease/disease group can give more "granularity" and aid physicians in informing their patents. At the time of writing, the lymphoid malignancy with the most data in this regard is CLL. Patients with this leukemia could be hypothesized to be particularly vulnerable to SARS-CoV-2 infection. This is due to the fact that CLL is frequently accompanied by an immunodeficiency which can be further aggravated by therapy, and also that it typically effects older adults (median age at diagnosis 70 years) who are higher risk due to their age[25,26]. A number of studies have now looked at the impact of COVID-19 in CLL patients specifically. Perhaps, due to the geography of the pandemic one of the first reports was from an Italian group who assessed 47 symptomatic CLL patients were found to be positive for COVID-19[27]. Of the 46 evaluable patients, 14 died, equating to a morality rate of 30.4%. The median age of these patients was 75 years, meaning that the mortality rate of this group was only a little higher than the mortality rate of 25.5% in 70-79-year-olds in the general Italian population at the same time. The European Research Initiative on CLL group reported outcomes of 190 CLL patients who presented in the first wave of the pandemic. 151 (79%) presented with severe COVID-19 (requiring oxygen and/or intensive care admission) which was associated with more advanced age (≥ 65 years) with a mortality rate of 36.4%[15]. Mato *et al*[12] reported data from a further international (predominantly United States) multi-center cohort of 198 patients. This again revealed a relatively high rate of severe disease and hospital admissions with an overall case fatality rate of 33%. This rose to 37% in those requiring admission, a remarkably similar figure to the other study. Across these two major studies the main risk factors were mainly those already known for COVID-19 itself: age and co-morbidities. Interestingly, hypogammaglobulinemia, a marker of the CLL-associated immunodeficiency, did not impact upon the outcome. It could be hypothesized that the immune defect associated with this defect could be a "double-edged" sword. On one hand, a weakened immune system may not be as capable of eliminating SARS-CoV-2, yet on the other, it might help to prevent a fatal immune and inflammatory over-reaction[28].

They have been a few reports of the outcomes of COVID-19 more specifically in patients with lymphoma. A study by Lamure *et al*[29] investigated the outcomes of 89 patients, the majority of whom had recently treated (within the last year) B-cell non-Hodgkin lymphoma. With a median follow-up of 33 d from admission, 30-d overall survival was 71%, with age ≥ 70 years and relapsed/refractory lymphoma being risk factors for a poorer outcome in a multivariate analysis. They did not see any differences in outcomes of patients with B-cell *vs* T-cell lymphomas, but they only included 7 patients in the latter group. Recent bendamustine treatment was also identified as a potential risk factor. However, the numbers of patients were few and this characteristic was strongly associated with (and probably confounded by) relapsed/refractory lymphoma. Notably they concluded that survival of patients younger than 70 years without relapsed/refractory lymphoma was comparable to that

of the general population[29]. A further Spanish study reported on 177 patients, 89% of who had non-Hodgkin lymphoma. The overall mortality rate was 34.5%, with age > 70 years, heart disease, chronic kidney disease, CURB-65 score ≥ 2 and active disease significantly increasing the risk of death in a multivariate analysis. Interestingly they did also note that the persistence of a positive polymerase chain reaction for SARS-CoV-2 after week 6 was significantly associated with mortality, suggesting that longer term viral suppression is an important component of recovery[30].

Not unexpectantly current published data is limited to small case series and case reports when it comes to the rarer forms of lymphoma. A Parisian study reported outcomes for 13 patients with primary central nervous system lymphoma. The mortality rate was 23% in this group, 11 (85%) of whom were undergoing chemotherapy at the time of infection. Two additional patients (15%) required mechanical ventilation, but two patients (15%) had no COVID-19 symptoms. A medical history of diabetes mellitus was more common in patients with severe disease. Chemotherapy was resumed after COVID-19 recovery in nine patients (69%) after a median delay of 16 d with no unusual chemotherapy complications nor incidents of SARS-CoV-2 reactivation[31]. Gonzaga *et al*[32] reported on the outcome of 2 patients with Sezary syndrome who acquired COVID-19. Unfortunately, both patients died, one attributable to COVID-19 and the other due to progressive disease. In contrast another patient who was receiving treatment for lymphoma type adult T-cell leukemia-lymphoma recovered after developing severe COVID-19 pneumonia with favipiravir therapy. Interestingly, there have also been a few reports of COVID-19 being beneficial to lymphoma patients, presumably due to an "immunostimulatory effect". Challenor and Tucker[33] reported the case of a 61-year-old man who went into remission after SARS-CoV-2 infection without treatment. Sollini *et al*[34] also report a case of a patient with follicular lymphoma, who having achieved a partial remission after bendamustine-based therapy, went onto achieve a complete remission after asymptomatic COVID-19. In addition, Pasin *et al*[35] report an interesting case of a patient with natural killer (NK)/T-cell lymphoma who having been refractory to previous immuno-chemotherapy, subsequently developed a transient remission at the time if SARS-CoV-2 infection. As NK cells express angiotensin converting enzyme 2, the binding site for this virus, they hypothesize that a direct oncolytic effect of the virus combined with production of proinflammatory cytokines led to NK-cell apoptosis, something seen with other RNA viruses. Clearly, more data needs to be collected on these and other types of lymphoid malignancies, something that will almost certainly occur as the pandemic progresses.

INTERACTION OF COVID-19 AND TREATMENT OF LYMPHOMA

While a large part of this involves the management of bacterial infections, particularly in the context of concurrent neutropenia, infection with and re-activation of viruses are also a feature of the clinical course of many lymphoma patients on treatment. Prolonged symptoms from seasonal "flu" and "cold" viruses and reactivation of viruses such as hepatitis B and varicella zoster are common complications of treatment, particularly after depletion of the B-cell compartment with anti-CD20 monoclonal antibodies such as rituximab. Given that most effective lymphoma therapies are also lymphodepleting it could be expected that anti-lymphoma drugs would compromise the normal immune response to SARS-CoV-2 leading to prolonged and more severe infection. However, even in the early stages of the pandemic it was clear that this was not so straightforward. The infection typically begins with relatively mild symptoms, which if the infection is not controlled, then can become more severe at around day 10 associated with a cytokine-induced inflammatory storm as the "adaptive" immune response takes off. Therefore, it could also be hypothesized that the immunosuppressive effect of many lymphoma treatments could actually be beneficial at this stage by limiting this hyperinflammation, thereby avoiding severe pneumonitis and thrombotic sequelae. In light of this, a number of guidelines, consensus statements and recommendations regarding the management of lymphoma(s) were published at the start of the pandemic[36-43]. They invariably recommended a common-sense approach. Patients with aggressive lymphoma were to be treated as usual, while minimizing time in the hospital by use of measures including the wider use of granulocyte colony stimulating factor prophylaxis and subcutaneous administration of rituximab. In contrast, the advice for patients with more indolent lymphomas was to continue expectant management where possible and to use oral regimens where reasonable. In all cases virtual consultations were to be

encouraged, particularly for patients in complete remission or for those in which no immediate change in therapy was expected. However, there was a clear concern that patients with lymphoid malignancies were going to be at particular risk from COVID-19 due to the combined immunosuppression from their underlying disease and its treatment.

Interestingly, multiple studies have consistently reported little or no negative impact of therapy on outcomes from COVID-19. The large meta-analysis of over 3000 patients with hematological cancers showed no association of poorer outcome with concurrent treatment, as have many smaller studies[17,22]. Similarly, in the two largest lymphoma-specific COVID-19 studies, there was no association of active treatment with poor outcome[29,30]. In particular there was no excess mortality identified with anti-CD20 treatment despite the anticipated risk of depleting the B-cell compartment and inhibiting humoral immunity. While, there have been several reports of prolonged viral shedding and/or pneumonia symptoms, and failure of SARS-CoV-2 antibody responses in patients treated with rituximab, this has not translated into a significant impact on survival in the larger studies[44-46]. It is possible that modulation of the "hyperinflammatory" phase of COVID-19 is playing a role; it is also possible that the relative sparing of T-cell responses may be enough to control the virus. As a consequence, most expert bodies are recommending continuing treat lymphoid malignancies as usual whilst highlighting the importance of a risk-benefit analysis in each individual patient scenario. While there does not appear to be any additional risk from treatment *per se*, COVID-19 does pose a significant risk to lymphoma patients in itself, particularly those who are older with multiple co-morbidities. Therefore, infection with SARS-CoV-2 needs to be avoided in lymphoma patients who should generally be regarded as clinically vulnerable and advised to "shield". Visits to hospital (and hence potential exposure to the virus) should be reduced by choosing oral regimens over infusional ones where possible (*e.g.*, ibrutinib or acalabrutinib for the treatment for CLL) and avoiding treatments with marginal benefit (*e.g.*, maintenance rituximab for follicular lymphoma), particularly when COVID-19 infection rates in the general population are high.

There has been particular focus regarding the potential of ibrutinib as a potential immuno-modulator of COVID-19. Ibrutinib is used for the treatment of several B-cell disorders, including CLL, mantle cell lymphoma and Waldenstrom macroglobulinemia (WM)[47]. In addition to its inhibition of B-cell receptor signaling by Burton's tyrosine kinase (BTK) it is also known to inhibit interleukin-2 inducible T-cell kinase (ITK) modulating T-cell responses[48]. There were early reports of ibrutinib potentially having a beneficial effect in SARS-CoV-2 infection, protecting against pulmonary injury, both in the context of treatment for CLL and WM[49,50]. The effect has been hypothesized to be due not only to "off-target" inhibition of ITK, but also of inhibition of Src family kinases and attenuation of M1 macrophage polarization with the net effect of reducing viral entry and inflammatory cytokine responses in the lungs[51,52]. Whether or not the anti-platelet effect of ibrutinib could also help combat the pro-thrombotic events associated with severe COVID-19 has not been explored. Interestingly, a small clinical study has suggested that BTK inhibition could be the most important component of ibrutinib's immunomodulatory activity. Roschewski *et al*[53] assessed the efficacy of 19 patients without hematological malignancies who were hospitalized with severe COVID-19 (11 on supplemental oxygen and 8 on mechanical ventilation), 18 of whom had increasing oxygen requirements at baseline. Acalabrutinib is a more selective inhibitor of BTK and should not have any effect on ITK and Src kinases. Analysis revealed a rapid normalization of inflammatory markers such as C-reactive protein and interleukin-6 with a temporal correlation with improved oxygenation. These results suggested that targeting excessive host inflammation with a BTK inhibitor is a therapeutic strategy in severe COVID-19 and has led to an ongoing international prospective randomized controlled clinical trial. A protective effect of BTK inhibition was also observed in the European study of outcomes of CLL patients with SAR-CoV-2 infection, with lower rates of hospitalization rate for severe COVID-19 for patients on ibrutinib *vs* those on other regimens or off treatment[15]. However, an effect was not seen in the Mato *et al*[12] report, although in many cases therapy was withheld once COVID-19 was diagnosed. Again, further work is required to investigate this, but it would seem reasonable to continue BTK inhibitors in patients who are diagnosed with COVID-19 on the basis of the available evidence. Certainly, discontinuation of effective anti-lymphoma therapy has its own risks, particularly in patients with more aggressive lymphoma subtypes, as exemplified by a report of patient who developed rapid progression of their mantle cell lymphoma after ibrutinib was discontinued for intercurrent COVID-19[54].

Further questions remain around the use of other immunomodulatory drugs for lymphoid malignancies in the context of COVID-19. Immune checkpoint blockade with drugs targeting programmed cell death 1 and other immuno-inhibitory molecules is widely used in the solid cancer field where they "release the brakes" of immune tolerance mechanisms leading to effective anti-tumor responses[55]. These agents are less commonly used in lymphoma where the main indications are in relapsed Hodgkin lymphoma and Richter syndrome. Again, the potential impact of immune checkpoint blockade in patients with COVID-19 could be hypothesized to be double-edged, with these agents potentially enhancing immunological control of the viral infection, yet also contributing to inflammation and aggravating the clinical course of COVID-19. Reports of these drugs in lymphoma are currently limited to a single case report. O'Kelly *et al*[56] report a case of a 22 year-old female with multiply relapsed Hodgkin lymphoma having pembrolizumab who developed severe COVID-19 requiring high levels of oxygen supplementation but not intubation, who subsequently recovered. A recently published study of 35 patients receiving immune checkpoint blockade in solid cancers concluded that COVID-19 related mortality in this population did not appear to be higher than previously published mortality rates for patients with cancer suggesting that this type of treatment does not increase the risk[57]. Another class of anti-lymphoma drugs that could be hypothesized to have an impact on the course of COVID-19 are the immunomodulatory imide drugs such as thalidomide and lenalidomide. While being used most commonly in the treatment of multiple myeloma, lenalidomide is well known to have activity in lymphomas including follicular lymphoma, mantle cell lymphoma and CLL[58,59]. At the time of writing the reports of the impact of these drugs on COVID-19 outcomes in myeloma patients remain equivocal; there are no reports of the outcome of COVID-19 with intercurrent use of these drugs in lymphoma. The potential mechanisms by which treatments for lymphoma may modulate COVID-19 infection is summarized in Figure 1.

A discussion of the general principles of managing severe COVID-19 in lymphoid malignancies is beyond the scope of this review. However, one aspect that might be expected to be specifically relevant to these cancers is the use of convalescent plasma to treat COVID-19, given the hypogammaglobulinemia that frequently observed, particularly in CLL. As intravenous immunoglobulin replacement is indicated to prevent infections in these patients, it is reasonable to hypothesize that plasma containing anti-SARS-CoV-2 antibodies might be of particular benefit in these patient groups. Several studies have now looked at the efficacy of convalescent plasma in the general population. Initial randomized trials of convalescent plasma in patients with COVID-19 focused on hospitalized patients who were already moderately to severely ill, with these trials providing little evidence of clinical efficacy[60,61]. Subsequent observational studies have been more positive but generally the clinical benefits have been modest[62]. However, a recent randomized study has suggested that this "passive immunotherapy" can be effective if the right plasma is used for the right patients, with early administration of high-titer convalescent plasma against SARS-CoV-2 to mildly affected older adults reducing the progression of COVID-19[63]. While there have been no randomised studies investigating the use of convalescent plasma in patients with lymphoid malignancies, there have been several case reports and observational case series reporting efficacy in this patient group[64-70]. As a consequence, it seems reasonable to use convalescent plasma for high risk individuals in this patient group as long as the plasma contains high titers of SARS-CoV-2 antibodies and is given early enough in the patient's course of infection.

IMPACT OF COVID-19 ON HEMOPOIETIC STEM CELL TRANSPLANTATION OF LYMPHOMA

High-dose chemotherapy with autologous hemopoietic stem cell transplantation (HSCT) represents a standard of care for many lymphoid malignancies, with allogeneic HSCT being potentially curative for other particular indications. Both types of transplantation are scenarios where COVID-19 infection could be expected to lead to particularly severe consequences, given the state of immune suppression that they induce. As a consequence, transplant organizations such as the European Society for Blood and Marrow Transplantation (EBMT) have been regularly issuing and updating recommendations regarding all aspects of transplantation during the pandemic[71]. The EBMT has been collecting data regarding the impact of COVID-19 on HSCT recipients and also those undergoing treatment with chimeric antigen receptor (CAR)

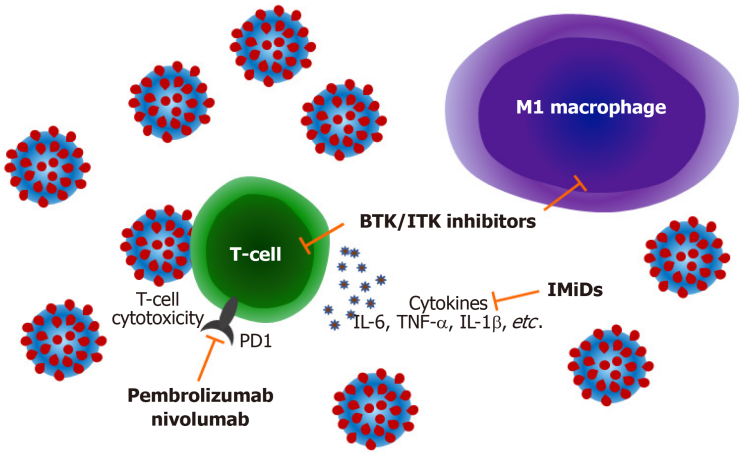

Figure 1 Mechanisms by which lymphoma treatments may modulate coronavirus disease 2019 infection. Inhibition of Burton's tyrosine kinase and interleukin-2 inducible T-cell kinase modulates T-cell immune responses decreasing production of pro-inflammatory cytokines such as interleukin-6, tumour necrosis factor α and interleukin-1b and also attenuating M1 macrophage polarization reducing pulmonary inflammation. Immune checkpoint blockade with drugs targeting programmed cell death 1 may improve antiviral cytotoxic T-cell responses. Immunomodulatory imide drugs can also block cytokine responses and improve T-cell function. BTK: Burton's tyrosine kinase; ITK: Interleukin-2 inducible T-cell kinase; PD1: Programmed cell death 1; IMiDs: Immunomodulatory imide drugs; IL: Interleukin; TNF-α: Tumour necrosis factor α.

T cells. While the 6-wk mortality in this patient group in the 1st wave was approximately 25%, preliminary data from the 2nd wave (August to December 2020) suggests a mortality rate slightly below 20%. This figure is not too dissimilar to that published by the group at the Memorial Sloan Kettering Cancer Center who observed that 22% of patients who had received cellular therapy (Allogeneic, 35; Auto, 37; CAR T, 5) had died after 30 d[72]. Notably the largest study published to-date did not observe any differences in 30-d overall survival when comparing recipients of allogeneic *vs* autologous HSCT[73]. Despite the theoretical risks associated with the procedure itself, the very nature of determining an individual's eligibility for transplant typically excludes those at higher risk from COVID-19, which probably explains why these figures are lower than the fatality rates seen for patients with hematological malignancies outside the transplant setting. Many of the recommendations focus on avoiding SARS-CoV-2 infection by limiting risk of exposure to infected individuals as much as possible and strictly adherence to prevention practices such as hand hygiene and social distancing—something that applies to the donor as well as the recipient in allogeneic transplants[74]. The challenging question is what to do in patients that develop COVID-19 during preparation for transplantation? This includes those that acquire COVID-19 immediately before transplantation and those that develop and recover but have a persistently positive polymerase chain reaction test. Generally, the decision to proceed has to be assessed on a case-by-case basis weighing in the risks from COVID-19 infection *vs* the risks from delaying the transplant. The grade of lymphoid malignant (indolent *vs* aggressive) and availability of alternative salvage therapy will clearly play into these decisions. In addition to ongoing data collection by the bone marrow transplant registries there are now several published case reports and case series of patients successfully completing a bone marrow transplant despite intercurrent SARS-CoV-2 infection, including one report where all 11 patients survived without oxygen supplementation or mechanical ventilation[72,73,75-78]. Despite this, risks for lymphoma patients remain, with one study reporting a higher risk of mortality in autologous HSCT recipients when the indication was for lymphoma compared to myeloma—likely reflecting the increased intensity of the multi-agent high-dose chemotherapy used in lymphoma autograft conditioning[73]. Other potential factors identified as being predictive of poorer outcomes in HSCT include older age, being on steroids at the time of diagnosis of COVID-19, and COVID-19 infection within 1 year of HSCT[16].

IMPACT OF LYMPHOMA ON VACCINATION FOR COVID-19

The enormous societal and economic impact of the pandemic made it a global emergency to develop effective vaccines. In a testament to human ingenuity the first SAR-CoV-2 vaccine trails were being reported less than a year after the virus was

initially identified[79-81]. A number of vaccines are in production with efficacy against laboratory-confirmed infection typically greater than 90%. Not surprisingly, the trials have excluded patients on treatment with immunosuppressive therapy or those diagnosis with an immunocompromising condition, which includes all patients with lymphoid malignancies. Therefore, at the time of writing there is no data on the efficacy of any of the leading SARS-CoV-2 vaccines in patients with lymphoid malignancies. As discussed above patients with these cancers could be expected to fail to mount an immune response to these vaccines. This is due both to the immune defects associated with the diseases themselves and also due to the impact of treatments. While little is known about the efficacy of COVID-19 vaccines in lymphoma patients, plenty of studies have demonstrated reduced rates of sero-conversion in patients vaccinated for other viruses in the past. Furthermore, one-third of CLL patients who had COVID-19 failed to mount a persistent antibody response in one study[69]. Therefore, it will be vital to design studies to assess their efficacy in patients with lymphoid malignancies, as even if current vaccines achieve the ideal of "herd immunity", the presence of SARS-CoV-2 mutant strains will likely mean that lymphoma patients still require direct protection[82]. A further consideration is perhaps the opposite problem. As vaccines are widely rolled-out some patients with lymphoid malignancies will receive one or more doses during therapy. We have seen several cases at our centre when vaccination results in an increase in glycolytic lymphadenopathy as part of the normal immune response, something that can mimic lymphoma progression on fludeoxyglucose positron emission tomography/computed tomography[83].

CONCLUSION

The COVID-19 pandemic has been a challenge for all sections of society across the world. Despite this, a great deal has been learnt about this virus in a very short space of time, including its impact in patients with hematological malignancies. Multiple studies have consistently demonstrated that approximately one-third of patients with blood cancers who acquire COVID-19 and have it severely enough to require hospital assessment will die, representing a slightly greater than 2-fold increased risk compared to the general population. Perhaps surprisingly, several studies have shown little or no negative impact of concurrent or recent anti-cancer therapy on outcomes from COVID-19, with reports of agents such as the BTK inhibitors actually having a protective effect. This is important as it means that treatment should be initiated and continued as required, rather than being delayed due to concerns regarding the risks from COVID-19. Instead, the focus needs to be stopping lymphoma patients from acquiring SARS-CoV-2 in the first place, by advising them to shield and taking steps to reduce hospital visits. However, a great deal still remains unknown about the impact of this infection in patients with lymphoid malignancies. Particular questions remain around the outcomes of COVID-19 in rarer lymphomas, and about the interaction between lymphoma-associated and treatment-induced immunosuppression and vaccine responses. While it can be anticipated that these gaps in our knowledge will start to become filled over the coming months, the presence of novel SARS-CoV-2 mutants will almost certainly mean that many years of work lie ahead.

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EVIDENCE-BASED MEDICINE

Association between population vitamin D status and SARS-CoV-2 related serious-critical illness and deaths: An ecological integrative approach

Dimitrios T Papadimitriou, Alexandros K Vassaras, Michael F Holick

ORCID number: Dimitrios T Papadimitriou 0000-0002-6083-3560; Alexandros K Vassaras 0000-0001-5777-0768; Michael F Holick 0000-0001-6023-9062.

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Dimitrios T Papadimitriou, Pediatric - Adolescent Endocrinology and Diabetes, Athens Medical Center, Marousi 15125, Greece

Dimitrios T Papadimitriou, Endocrine Unit, Aretaieion University Hospital, Athens 11528, Greece

Alexandros K Vassaras, Neurology Department, Papageorgiou Hospital, Aristotle University of Thessaloniki, Thessaloniki 56429, Greece

Alexandros K Vassaras, Neuroimmunology Department, Democritus University of Thrace, Alexandroupoli 68100, Greece

Michael F Holick, Section Endocrinology, Nutrition and Diabetes, Department of Medicine, Boston University Medical Center, Boston, MA 02118, United States

Corresponding author: Dimitrios T Papadimitriou, MD, MSc, PhD, Director, Pediatric - Adolescent Endocrinology and Diabetes, Athens Medical Center, 58, Kifisias av., Marousi 15125, Greece. info@pedoendo.net

Abstract

BACKGROUND

Vitamin D population status may have possible unappreciated consequences to the coronavirus disease 2019 (COVID-19) pandemic. A significant association between vitamin D sufficiency and reduction in clinical severity and inpatient mortality from COVID-19 disease has recently been shown, while a recent study has claimed lower COVID-19 cases in European countries with a better vitamin D status. Low serum 25-hydroxyvitamin-D [25(OH)D] was identified as an independent risk factor for COVID-19 infection and hospitalization, and administration of 0.532 mg (21280 IU) of calcifediol or 25(OH)D, followed by 0.266 mg on days 3 and 7 and then weekly until discharge or intensive care unit admission significantly reduced the need for intensive care unit treatment.

AIM

To elucidate the role of vitamin D European population status in the COVID-19 pandemic, data from the Worldometer were analyzed.

METHODS

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Linear regression explored the correlation between published representative-standardized population vitamin D concentrations and the number of total cases/million (M), recovered/M, deaths/M and serious-critically ill/M from COVID-19 for 26 European countries populated > 4 M (Worldometer). Life expectancy was analyzed with semi-parametric regression. Weighted analysis of variance/analysis of covariance evaluated serious-critical/M and deaths/M by the vitamin D population status: Deficient < 50, insufficient: 50-62.5, mildly insufficient > 62.5-75 and sufficient > 75 nmol/L, while controlling for life expectancy for deaths/M. Statistical analyses were performed in XLSTAT LIFE SCIENCE and R (SemiPar Library).

RESULTS

Linear regression found no correlation between population vitamin D concentrations and the total cases-recovered/M, but negative correlations predicting a reduction of 47%-64%-80% in serious-critical illnesses/M and of 61%-82%-102.4% in deaths/M further enhanced when adapting for life expectancy by 133-177-221% if 25(OH)D concentrations reach 100-125-150 nmol/L, sustained on August 15, 2020, indicating a truthful association. Weighted analysis of variance was performed to evaluate serious-critical/M ($r^2 = 0.22$) by the vitamin D population status and analysis of covariance the deaths/M ($r^2 = 0.629$) controlling for life expectancy ($r^2 = 0.47$). Serious-critical showed a decreasing trend ($P < 0.001$) from population status deficient ($P < 0.001$) to insufficient by 9.2% ($P < 0.001$), to mildly insufficient by 47.6% ($P < 0.044$) and to sufficient by 100% (reference, $P < 0.001$). For deaths/M the respective decreasing trend ($P < 0.001$) was 62.9% from deficient ($P < 0.001$) to insufficient ($P < 0.001$), 65.15% to mildly insufficient ($P < 0.001$) and 78.8% to sufficient ($P = 0.041$).

CONCLUSION

Achieving serum 25(OH)D 100-150 nmol/L (40-60 ng/mL) (upper tolerable daily doses followed by maintenance proposed doses not requiring medical supervision, Endocrine Society) may protect from serious-critical illness/death from COVID-19 disease.

Key Words: COVID-19; SARS-CoV-2; Vitamin D status; Vitamin D concentrations; 25-hydroxyvitamin-D; Immunity

Core Tip: To elucidate the role of vitamin D in the coronavirus disease 2019 (COVID-19) pandemic, we examined associations between published representative and standardized European population vitamin D data and the Worldometer COVID-19 data. Linear regression found no correlation between population vitamin D concentrations and the total cases-recovered/million (M), but negative correlations predicting a reduction of 47%-64%-80% in serious-critical illnesses/M and of 61%-82%-102.4% in deaths/M further enhanced when adapting for life expectancy by 133-177-221% if 25-hydroxyvitamin-D concentrations reach 100-125-150 nmol/L. Weighted analysis of variance/analysis of covariance showed a decreasing trend ($P < 0.001$) evaluating serious-critical/M ($r^2 = 0.22$) and the deaths/M ($r^2 = 0.629$) after controlling for life expectancy ($r^2 = 0.47$), by vitamin D population status, respectively.

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INTRODUCTION

Vitamin D deficiency and insufficiency is a global health issue affecting probably many more than 1 billion children and adults worldwide, with institutionalized elderly being at higher risk of exhibiting lower 25-hydroxyvitamin-D [25(OH)D] blood concentrations. According to a systematic review of vitamin D status in populations worldwide, 37.3% of the studies reported 25(OH)D mean concentrations < 50 nmol/L in newborns and institutionalized elderly, who are at higher risk of exhibiting lower 25(OH)D concentrations[1]. Public health policy development is needed to reduce risk for potential health consequences of an inadequate vitamin D status[1], with consequences that should not be underestimated, especially now with this unprecedented pandemic of coronavirus disease 2019 (COVID-19)[2]. The initial universal lockdown for a period of 2-3 mo and the consequent repeated lockdowns along with the social distancing measures would further reduce the incidental solar vitamin D3 production, worsening the population's vitamin D status[3]. Strong evidence supports the role of vitamin D particularly in preventing rickets and osteomalacia[4]. While circulating 25(OH)D concentrations below 30 nmol/L (12 ng/mL) are associated with an increased risk of rickets/osteomalacia, 25(OH)D concentrations between 50-125 nmol/L (20-50 ng/mL) appear to be safe and sufficient to promote skeletal health in the general population[5]. A serum 25(OH)D concentration of at least ≥ 50 nmol/L at the end of winter (10-20 nmol/L higher at the end of summer, to allow for seasonal decrease) is required for optimal musculoskeletal health[6]. Supplements of vitamin D in low doses together with calcium, alone or in combination with antiresorptive drugs may prevent hip or any type of fracture and have been evaluated in osteoporotic and osteopenic patients for primary as well as secondary prevention[7-9]. However, the role of vitamin D in innate and adaptive immunity remains rather underappreciated, with possible consequences and public health implications, leading to an increased risk for infectious diseases, autoimmune disorders and cancers[10]. Even if a recent randomized control trial (RCT) did not show lower incidence of invasive cancer in men ≥ 50 years or women ≥ 55 receiving 2000 IU of vitamin D3 daily up to 5 years[11], the study did report a statistically significant 25% reduced risk for cancer mortality. The study, however, had several limitations. Only 13% of the participants were vitamin D deficient [25(OH)D < 50 nmol/L], and 42%-45% of the participants were receiving a vitamin D supplement and multivitamins at inclusion. The participants, including the placebo group, were permitted to take up to 800 IU of vitamin D daily. This is the likely explanation why the mean baseline blood concentration of 25(OH)D was 74.5 nmol/L for the participants in this study[11]. The optimal 25(OH)D concentration is at least 75 nmol/L (30 ng/mL), which is what the mean baseline level was for the participants in the VITAL study. Secondary analyses from the VITAL study should be also considered as they indicate that the vitamin D dose was too low, since significant benefits were found for cancer incidence for those with body mass index (BMI) < 25 kg/m^2 and almost as significant for blacks. In fact, the authors speculated that the possible trial regimen–associated effects on cancer incidence among normal-weight participants and suggestive effects among black participants, which contrast with the null cardiovascular findings in these groups, may be explained by different vitamin D requirements for these outcomes. The Endocrine Society, which made its recommendations in 2011 for the treatment and prevention of vitamin D deficiency, concluded that to guarantee bone health, a blood level of 25(OH)D of at least 75 nmol/L (30 ng/mL) is required (https://www.endocrine.org/clinical-practice-guidelines/vitamin-d-deficiency)[12]. Beyond musculoskeletal health however, it has been found that vitamin D supplementation significantly reduced the risk of cancer death by 15% in a systematic review and meta-analysis of 52 trials with a total of 75454 participants[13], and it has been suggested that better health outcomes may occur in the range of 100-150 nmol/L[10]. The largest meta-analysis ever conducted of all studies published between January 1, 1966 and January 15, 2013 dealing with all-cause mortality related to serum 25(OH)D showed that 25(OH)D < 75 nmol/L was associated with higher all-cause mortality, its reduction being maintained with 25(OH)D ≥ 175 nmol/L (70 ng/mL), without a U-shaped curve as previously reported[10]. Achieving such concentrations with supplements and sensible sun exposure for a normal weight adult requires 2000–5000 IU daily intake of vitamin D2/D3, practically all year long except maybe during sunny vacations[14]. With vitamin D adequacy relying mainly (80%-90%) on sun exposure rather than on dietary sources (10%-20%), if not on supplementation, these doses should be adapted accordingly during lockdowns. It should also be recognized that sensible sun exposure has many additional health benefits not only in the immune system but also in

improving the feeling of well-being[15]. At this time, neither the World Health Organization nor any other public health authority has issued any official advice or recommendation on vitamin D, or any other nutrients, to the best of our knowledge.

A quadratic relationship was found between vitamin D deficiency in countries affected by COVID-19 and the latitudes, implying a possible relation[16]. When mortality/ million (M) is plotted against latitude, all countries below 35 degrees North, above which people do not receive sufficient sunlight to retain adequate 25(OH)D concentrations during winter, have relatively lower mortality, implying a role for vitamin D status in outcomes from COVID-19[17]. Vitamin D is strongly affected by ozone variability, since ozone filters ultraviolet B, an important factor for vitamin D synthesis. A statistically significant link between ozone concentration and incidence of COVID-2019 disease in 34 countries was established[18]. Going back to the 1918-1919 influenza pandemic, substantial correlations were found for associations of July ultraviolet B dose in the United States with case fatality rates and rates of pneumonia[19]. A significant association between vitamin D sufficiency and reduction in clinical severity and inpatient mortality was very recently shown[20]. Thus, to elucidate further the possible role of vitamin D population status in the COVID-19 pandemic, we examined the associations between published representative and standardized population vitamin D data on European population vitamin D status and the Worldometer COVID-19 data.

MATERIALS AND METHODS

Accessing data on European countries at the Worldometer, on June 19, 2020, we analyzed the 28 countries populated > 4 M (Table 1). For months, Swedish public health authorities have defended their controversial decision not to lock down the country in response to the global COVID-19 pandemic, with the country experiencing dramatic casualties. Thus, Sweden was excluded from analysis. The remaining 27 European countries adopted a defensive strategy during the current pandemic, even with delays and hesitations, as in the United Kingdom. Moldova was also excluded as no published vitamin D status data were found. For the remaining 26 countries, we used linear regression to explore the correlation between reported representative and standardized population vitamin D concentrations[21-28] and the number of total cases/M and recovered/M until June 19, 2020 as well as the deaths/M and the serious-critically ill/M from COVID-19 on that date (Table 1). Since mortality of COVID-19 disease has been shown to increase rapidly in respect to age, life expectancy (LE), an age-related index, was analyzed using a semi-parametric regression approach using Worldometer data. Weighted (https://doi.org/10.13094/SMIF-2015-00001) analysis of variance (ANOVA)/analysis of covariance (ANCOVA) was performed to evaluate serious-critical/M and deaths/M by the vitamin D population status - categorized as deficient (D) < 50, insufficient (IN) 50-62.5, mildly insufficient (MIN) > 62.5-75 and sufficient (S) > 75 nmol/L – while controlling for LE for deaths/M. To test whether these correlations withstand at another completely different momentum of this pandemic, which would be an indication of a truthful association, although still not a proof of causality, we also checked the above correlations and the differences between consecutive points of the same variables on August 15, 2020. All statistical analyses were performed in XLSTAT LIFE SCIENCE version April 1, 2020 (copyright Addinsoft 1995-2020) and R (R Core Team 2017), with the use of the SemiPar library.

RESULTS

From the 26 European countries included in the analysis, populated 714.661 M in total, nine (54.17%, 387.15 M) had a vitamin D deficient status, eight an insufficient status (33.58%, 240.022 M), eight a mild insufficiency status (11.48%, 82.023 M) and only one country, Slovakia, a sufficient status (0.76%, 5.459 M). There was no correlation between the total cases/M nor the recovered/M and the European population vitamin D concentrations. Negative correlations were recognized regarding the total deaths/M (Figure 1A), predicting a reduction of deaths/M by 20% if the 25(OH)D concentration reaches 50 nmol/L (related to the number calculated at 25), by 40% at 75, by 61% at 100, by 82% at 125 and by 102.4% at 150 nmol/L and the serious-critical/M (Figure 1B), predicting a reduction of serious-critically ill/M by 16% if 25(OH)D concentration reaches 50 nmol/L (related to the number calculated at 25), by 31% at 75, by 47% at 100, by 64% at 125 and by 80% at 150 nmol/L.

Table 1 European coronavirus disease 2019 data from the Worldometer on June 19, 2020, compared to life expectancy and to available representative and standardized data on the European population vitamin D status[21-28]

	Country	Total cases/M	Total recovered	Serious critical	Deaths/M	Life expectancy in yr	Population 25(OH)D in nmol/L	Population, M
1	Russia	3899	324406	2300	54	72.99	39.7	145.93
2	Germany	2273	174400	396	107	81.88	50.1	83.77
3	United Kingdom	4447	N/A	379	626	81.77	47.4	67.87
4	France	2431	73887	752	454	83.13	60.0	65.26
5	Italy	3939	180544	168	571	84.01	45.0	60.46
6	Spain	6253	N/A	617	580	83.99	59.9	46.75
7	Ukraine	800	16033	343	23	72.50	29.0	43.74
8	Poland	827	15698	87	35	79.27	32.0	37.84
9	Romania	1216	16555	184	77	76.50	65.0	19.24
10	Netherlands	2885	N/A	57	355	82.78	64.7	17.13
11	Belgium	5219	16751	55	837	82.17	49.3	11.58
12	Czechia	968	7472	9	31	79.85	62.5	10.70
13	Greece	311	1374	10	18	82.80	54.3	10.42
14	Portugal	3772	24477	67	150	82.65	55.4	9.66
15	Sweden	5550	N/A	272	500	83.33	68.7	9.44
16	Hungary	422	2581	15	59	77.31	60.6	83.33
17	Belarus	6067	35275	92	36	75.20	72.0	9.00
18	Austria	1918	16141	7	76	82.05	51.7	8.73
19	Serbia	1454	11511	18	30	76.47	65.7	8.65
20	Switzerland	3,608	28900	17	226	84.25	46.0	6.94
21	Bulgaria	529	1941	13	27	75.49	38.7	5.79
22	Denmark	2139	11282	6	104	81.40	65.0	5.54
23	Finland	1287	6200	2	59	82.48	67.7	5.45
24	Slovakia	289	1447	0	5	78.00	81.5	5.41
25	Norway	1,609	8138	5	45	82.94	71.0	4.93
26	Ireland	5137	22698	28	347	82.81	56.4	4.10
27	Croatia	555	2142	0	26	79.02	46.9	4.03
28	Moldova	3249	7525	455	111	72.30	N/A	10.09

25(OH)D: 25-hydroxyvitamin-D; M: Million.

Population vitamin D concentrations *vs* life expectancy exhibits a non-linear relationship (Figure 2): Higher life expectancy until approximately 77 years of age is characterized by better vitamin D concentrations, while practically reaching a plateau at 82 years, and then by a decline as expected in the elderly. There is a non-linear relationship between life expectancy and deaths/M with a dramatic increase in deaths/M after approximately 80 years (Figure 3). LE (*i.e.* age) seems to interfere with the effect of a better vitamin D concentration to the total number of deaths/M, rendering the vitamin D benefit even more important than the unadjusted one: A reduction in total deaths/M by 44% if 25(OH)D concentration reaches 50 nmol/L (related to the number calculated at 25), by 88% at 75, by 133% at 100, by 177% at 125 and by 221% at 150 nmol/L. The analytical form for the model on the deaths/M accounting for a potential non-linear effect of LE is year = -2675-4.111*vitamin D + f(LE), where f(.) is a non-linear smooth function of life expectancy. The *P* value for the

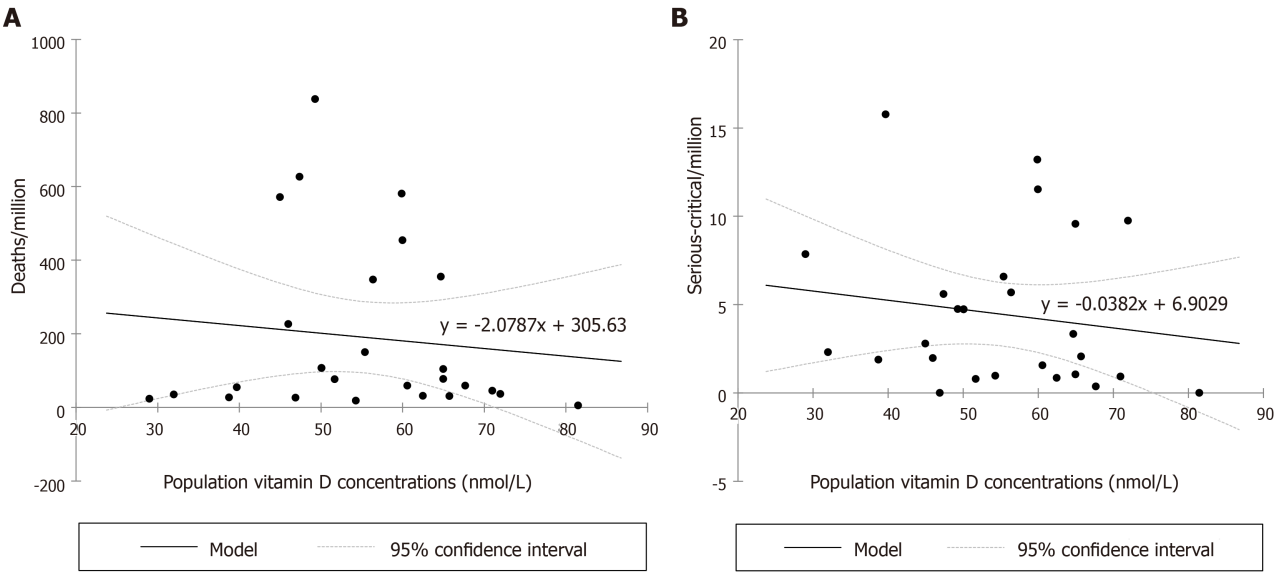

Figure 1 Linear regression on June 19, 2020 related to available representative and standardized data on the European population vitamin D concentrations (x axis, nmol/L). A: Of the total deaths/million (M); B: Of the serious-critical cases/M.

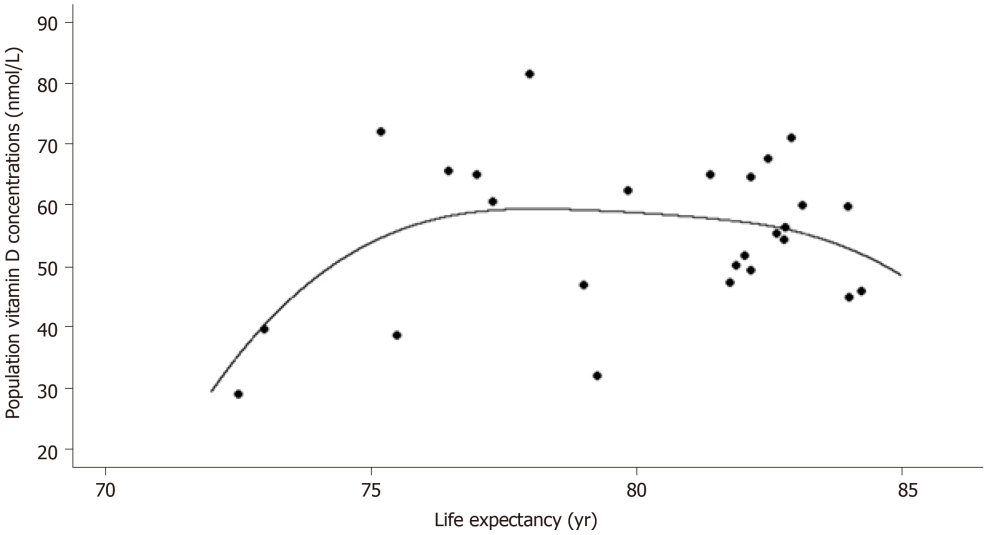

Figure 2 Population vitamin D concentrations *vs* life expectancy exhibits a non-linear relationship.

term f(LE) was estimated *via* likelihood ratio test to be $P = 0.042$, indicating a statistically significant effect of life expectancy on deaths/M after adjusting for vitamin D concentration.

Weighted (https://doi.org/10.13094/SMIF-2015-00001) ANOVA was performed to evaluate serious-critical/M and ANCOVA for deaths/M by the population vitamin D status while controlling for LE. Given the r^2, about 22% of the variability of the dependent variable serious-critical/M could be explained by the population vitamin D status. A decreasing trend from population status D [β = 8.684, standard error (SE) = 2.196, 95% confidence interval (CI): 4.372/12.996, $P < 0.001$], IN (β = 7.883, SE = 2.205, 95%CI: 3.553/12.213, $P < 0.001$), MIN (β = 4.548, SE = 2.252, 95%CI: 0.126/8.169, $P = 0.044$) to S (LE mean 0.0, SE 2.181, 95%CI: -4.282/4.282, $P < 0.001$) was found with an average reduction of serious-critical/M of 9.2% from vitamin D status deficient to insufficient, of 47.6% from deficient to mildly insufficient and 100% from deficient to sufficient (reference, Figure 4). Regarding deaths/M (Figure 5), given the r^2, about 63% of the variability of the dependent variable deaths/M could be explained by the two variables, LE alone accounting for 47%. A decreasing trend from population status deficient (β = 150.375, SE = 8.859, 95%CI: 132.982/167.768, $P < 0.001$), insufficient (β = -72.514, SE = 10.336, 95%CI: -150.170/-55.866, $P < 0.001$), mildly insufficient (β =

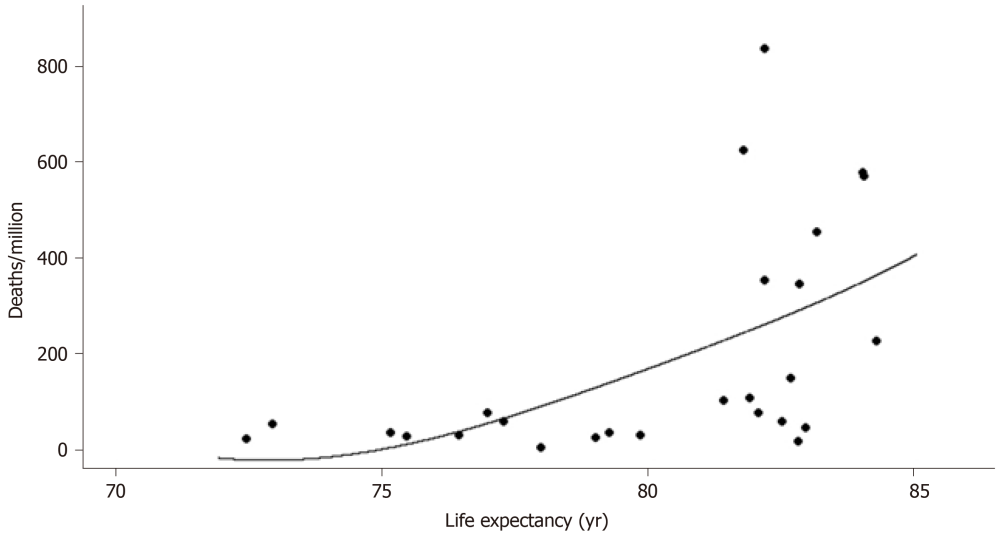

Figure 3 Non-linear relationship between life expectancy and deaths/million.

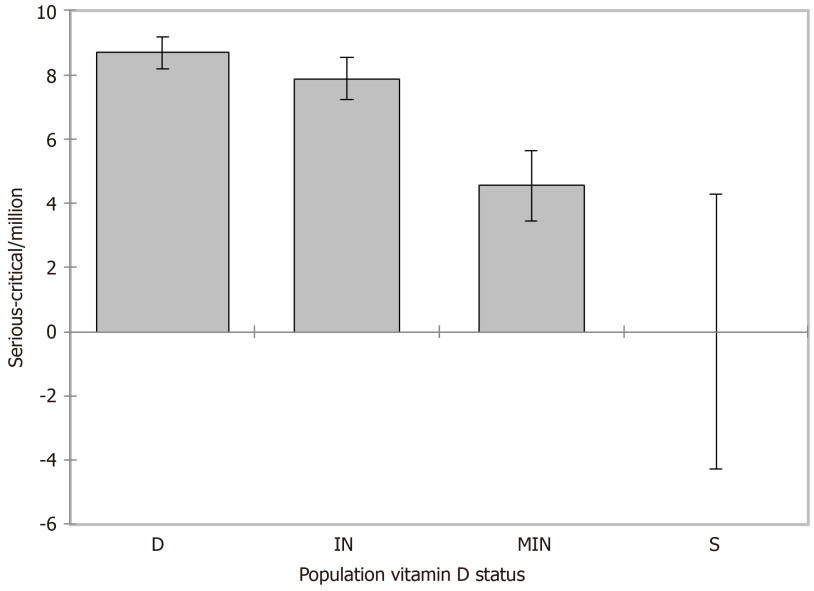

Figure 4 Least square means of serious-critical/million for factor population vitamin D status. D: Deficiency; IN: Insufficiency; MIN: Mild insufficiency; S: Sufficiency.

-80.518, SE = 12.556, 95%CI: -105.170/-55.866, $P < 0.001$) to sufficient (β = -129.122, SE = 62.915, 95%CI: -252.644/-5.599, $P = 0.041$) was found with an average reduction of deaths/M of 62.9% from vitamin D status deficient to insufficient, of 65.15% from deficient to mildly insufficient and 78.8% from deficient to sufficient.

On August 15, 2020, the above correlations were sustained and the differences between consecutive points for the two variables serious-critical/M and deaths/M in the two time points were correlated, not proving causality but suggesting a truthful association.

DISCUSSION

We explored any possible correlation between the population vitamin D status - influenced by various factors - and COVID-19 disease, in particular total cases, serious-critical illness and deaths. In contrast to a recently published study[29], we found no association between the vitamin D status of the European populations and the total confirmed cases/M of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

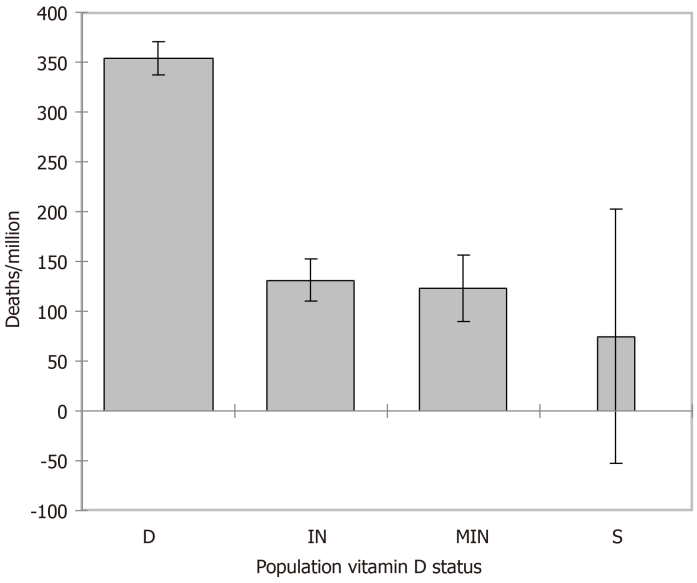

Figure 5 Least square means of deaths/million for factor population vitamin D status. D: Deficiency; IN: Insufficiency; MIN: Mild insufficiency; S: Sufficiency.

infections when we analyzed data from the Worldometer on June 19, 2020 on 26 European countries populated > 4 M. However, the negative correlations that we found between population vitamin D status and serious-critical/M and deaths/M show a clear tendency, even if they do not prove causality, namely after adjusting for LE, underlining the importance of an optimal vitamin D status especially in the elderly[30]. On August 15, 2020, at a completely different time point of this pandemic, before the second wave even had started, the above associations were sustained, suggesting a truthful correlation. Since the risk of COVID-19 disease increases rapidly with respect to age, an age-related index, such as LE, was found, as expected, to be a more important predictor of death rates. Thus, according to our results, a higher 25(OH)D concentration may protect from serious-critical illness and death from COVID-19 disease even more in the elderly but does not seem to prevent SARS-CoV-2 from spreading, in contrast to a recent study[29], which however reported also a negative correlation between the mean population vitamin D concentrations of 20 European countries and deaths/M from COVID-19 on April 8, 2020. Our findings also coincide with a recent study from Maghbooli *et al*[20] showing that vitamin D sufficiency [a serum 25(OH)D > 75 nmol/L (30 ng/mL)] reduced risk for adverse clinical outcomes in patients with COVID-19 infection: 6.3% of the patients who had a blood 25(OH)D concentration of at least 100 nmol/L (40 ng/mL) succumbed to the infection compared to 9.7% and 20% who died and had a circulating blood level above and below 75 nmol/L (30 ng/mL), respectively[20,31], suggesting that a blood level of at least 100 nmol/L (40 ng/mL) may be optimal for obtaining vitamin D's immunomodulatory benefit.

Various parameters played a significant role in the spread of the current pandemic. Among them, air travel and direct connections with China and particularly Wuhan, where the epidemic started. Then, health policymaking with mass quarantine was instituted in most countries, influencing the course of the disease, but with no central coordination of the measures taken during the first wave of the pandemic, not even in the core of the European Union itself. Timing of the lockdowns, at least in the first wave, seemed to have been the main factor affecting the number of the cumulative deaths – although this has been strongly debated (https://thefatemperor.com/published-papers-and-data-on-lockdown-weak-efficacy-and-lockdown-huge-harms/), along with travel and border restrictions. Recent research emphasizes the importance of face masks while self-protection measures seem to be better implemented by populations with higher educational levels. Temperature also appears to have a small but statistically significant impact on the viral transmission rate as countries with daily average temperatures below 20 °C had a faster transmission rate. Most probably, genetic predisposition must have played a fundamental role in the susceptibility in SARS-CoV-2 infection[32,33]. The recent discovery of robust genetic signals relating to key host antiviral defense mechanisms and mediators of inflammatory organ damage

in COVID-19 may lead to targeted treatment with existing drugs[33]. Most recent evidence show that angiotensin-I converting enzyme-2 (ACE2) expression and/or polymorphism could also influence both the individual susceptibility to SARS-CoV-2 infection and the outcome of the COVID-19 disease[34]. Thus, the integrity of our immune system and its ability to fight back with a coordinated way, keeping asymptomatic or within the subclinical spectrum most of the people infected and saving the lives of the severely infected, is a crucial factor. And there is significant evidence that vitamin D deficiency may compromise both innate and acquired immunity responses, leading to increased vulnerability to infections as to autoimmune responses and disorders[35].

The vitamin D status of a population is dependent on a variety of factors including supplementation and food fortification strategies, latitude of the country, season as well as on the local nutritional and sun exposure habits, especially in the non-institutionalized elderly[36]. The vitamin D status in the winter is even lower[1,37,38], with underappreciated consequences to the immune function[39,40]. Ideally, we should be able to analyze data on vitamin D status of the elderly in winter. Thus, a major limitation of our ecological approach is that we had to rely on published - but perhaps not always completely representative - data on the vitamin D status of the populations in Europe. However, data analyzed are based mainly on "Current vitamin D status in European and Middle East countries and strategies to prevent vitamin D deficiency: A position statement of the European Calcified Tissue Society" recently published in the European Journal of Endocrinology[21] – presenting not only representative nationally or regionally as possible but also standardized population vitamin D concentrations -, a systematic review of vitamin D status in southern European countries[22], and a very important study applying the protocols developed by the National Institutes of Health-led international Vitamin D Standardization Program to serum 25(OH)D data from representative childhood/teenage and adult/older (we chose data from older adults) European populations, representing a sizable geographical footprint, to better quantify the prevalence of vitamin D deficiency in Europe[28]. Keeping in mind that the population vitamin D status reflects that of the elderly, which by default will be worse, we tried to analyze the most recently validated and representative data possible, whereas from the available data for each country we chose data from older adults in winter where provided, and in any case from Caucasian descent. Ideally, we should be able to analyze data on 25(OH)D concentrations of the patients as in an interesting recent report from Switzerland, which found significantly lower circulating 25(OH)D concentrations [27.75 nmol/L (11.1 ng/mL), $P = 0.004$] in polymerase chain reaction-positive for SARS-CoV-2 patients compared with negative patients [61.5 nmol/L (24.6 ng/mL)], even after stratifying patients according to age > 70 years[41]. Another important issue would be the differences in assessment mainly of the COVID-19 deaths in the various European countries. However, the World Health Organization had already issued the "International guidelines for certification and classification (coding) of COVID-19 as cause of death, April 20, 2020" 2 mo earlier to our analysis, allowing us to assume that they must had already been adopted by the European Countries responsible public health authorities. Furthermore, Worldometer.info mainly collects data from official reports, directly from governmental communication channels. An additional important limitation is the true evaluation of the number of affected subjects in the variable countries: Since not all patients infected with COVID-19 are symptomatic, the cases/M are dependent upon the percentage of the population tested and the consistency of the frequency of testing during the disease period evaluated, not to mention that several patients or carriers have been tested several times. Furthermore, the definition of case includes a carrier as well as a patient. Unfortunately, this limitation could not be overcome with the publicly available COVID-19 data at the time of our analysis. However, we had to report the absence of any correlation between total cases/M and the population vitamin D status in the sample we analyzed, in contrast to a recently published study with the opposite results[29]. Assessment of serious-critical cases in the European countries may also have been limited at some points by the shortcoming of intensive care unit (ICU) beds as well as the introduction of different drugs and "cocktail" treatments from country to country. Albeit, on June 19, 2020, the first wave of the pandemic in Europe was kind of winding down, and not particularly effective new or repurposed medication had at least been qualified at that point as such to change significantly the clinical course of the serious-critical patients, other than the accumulated experience of the health workers fighting on the frontline.

Independent researchers increasingly call for optimization of vitamin D status for enhanced immune protection against COVID-19 at least in older adults, hospital inpatients, nursing home residents and other vulnerable groups, extending this

recommendation to the general population[42]. The elderly (> 65 years) have a higher risk for vitamin D deficiency due to decreased sun exposure and reduced ability for cutaneous synthesis[38], whereas aging exerts significant effects on all cells of the innate immune system[40], making vitamin D sufficiency even more valuable in this group. Early nutritional supplementation in non-critically ill patients hospitalized for COVID-19 has been implemented in hospital protocols providing 50000 UI/wk if 25(OH)D < 50 nmol/L and 25000 UI/wk if 25(OH)D < 75 nmol/L aiming at improved immunologic recovery with reduced levels of inflammation, immune activation, and increased immunity against pathogens[43].

The COVID-19 pandemic presents a puzzling challenge without specific treatment yet with timely administration being crucial for all current regimens on clinical trial or use. This is also the case for vitamin D, and this might be the reason why in a recent RCT, a single enteral dose of 540000 IU of vitamin D3 or matched placebo started late within 12 h after the decision to admit the critically ill (unrelated to COVID- 19) vitamin D deficient patient to an intensive care unit, had no benefit at a 90-d all-cause, all-location mortality[44]. Regarding vitamin D, we know that respiratory viruses downregulate vitamin D receptor expression in human bronchial epithelial cells, while improvement in vitamin D status increases antiviral defenses *via* cathelicidins and innate interferon pathways[45]. Vitamin D has a 12% overall protective effect against bacterial and viral acute respiratory tract infection, increased to 19% in those individuals on daily or weekly regimen compared to those on monthly boluses and up to 70% when vitamin D deficiency is corrected with daily supplementation[46]. Bioavailable 25(OH)D is inversely associated with illness severity in critically ill ICU patients associated with increased mortality and morbidity[47]. Calcitriol [1,25$(OH)_2D_3$] alleviates lipopolysaccharide induced acute lung injury and prevents the adult respiratory distress syndrome by minimizing the alveolar damage[48]. Vitamin D is also a negative endocrine regulator of the renin-angiotensin system. The mechanism for SARS-CoV-2 infection is the requisite binding of the virus to the membrane-bound form of ACE2 and internalization of the complex by the host cell. Recognition that ACE2 is the main host receptor by SARS-CoV-2 to infect human has prompted new therapeutic approaches to block the enzyme or reduce its expression to prevent cellular entry of SARS-CoV-2 in tissues expressing ACE2 (lung, heart, kidney, brain, and gut). Thus, it seems that both stimulation of the immune system and inhibition of renin-angiotensin system are mechanisms by which vitamin D may play a beneficial role in COVID-19 infection[49]. Vitamin D repletion in critical illness with a more aggressive dosing is showing similarly promising results with vitamin C repletion in septic shock[50] and may be able to prevent the cytokine storm that seems to be killing people rather than the virus itself[51]. C-reactive protein is a surrogate marker for unregulated inflammation and cytokine storm and is associated with vitamin D deficiency. Retrospective data and indirect evidence also show a possible role for vitamin D in reducing complications attributed to and the cytokine storm itself[52]. Moreover, recent research revealed that vitamin D receptor signaling in macrophages regulates a shift between proinflammatory and anti-inflammatory activation during ER stress-induced inflammation[53]. Thus, supplementation within recommended upper safety limits, for specific nutrients such as vitamins C and D, warrants optimal nutritional status to insure a well-functioning immune system protecting against viral infections[54].

Recent research demonstrated that low serum 25(OH)D was an independent risk factor for COVID-19 infection and hospitalization analyzing data from 14,000 members of Leumit Health Services in Israel[55]. A very recent pilot randomized clinical study demonstrated that administration of a relatively high dose (0.532 mg-21280 IU) of calcifediol or 25(OH)D, followed by 0.266 mg on days 3 and 7, and then weekly until discharge or ICU admission, significantly reduced the need for ICU treatment of patients requiring hospitalization due to proven COVID-19 disease[56]. In a single-center, retrospective cohort study concerning 489 patients, likely deficient vitamin D status was associated with increased COVID-19 risk[57].

Our analysis took place at two completely different time points during the beginning and the end of the first wave of this pandemic. We needed to confirm our first results at a completely different time point of the first wave. One could not attempt to extend this type of approach to the second wave or the third wave, which is now hitting Europe, first because the virus has significantly spread into the European populations. Secondly, after extended lockdowns and limited - if any - summer vacations and with no public health authority having officially advised supplementation with vitamin D even aiming to protect musculoskeletal health, one can hypothesize that the European population vitamin D status had to be worse, and this could also be one of the main reasons why the second and third waves appeared more

deadly than the first, at least in several European countries, even in sunny countries as Greece. One of the main outcomes of our analysis though is that vitamin D does not prevent SARS-CoV-2 from spreading, while it may protect from serious-critical illness and death form COVID-19 disease, with significant and substantial protection being obtained at a 25(OH)D concentration of 100-150 nmol/L (40-60 ng/mL).

CONCLUSION

At this time and despite the ongoing debate on "The Big Vitamin D Mistake"[15], referring to the statistical error in the estimation of the Recommended Dietary Allowance of vitamin D discovered by Veugelers and Ekwaru[58] in 2014 and confirmed by Heaney *et al*[59]: About 4000 IU/d (3385) are needed to ensure 50 nmol/L in 97.5% of the population, about 6000 IU/d (6201) are needed to achieve the Endocrine Society's recommendation of 75 nmol/L and about 9000 IU/d (9122) to reach 100 nmol/L, and even if the vitamin D deficiency pandemic is still being questioned[60], no one should confuse the global consensus on the minimum vitamin D doses needed to prevent nutritional rickets[61], with the doses needed to exert all of its extra-skeletal health benefits[62], particularly those related to our immune system. Apart from the known disagreement between the Endocrine Society and the Institute of Medicine (IOM) but also the discrepancy between the IOM and the Scientific Advisory Committee on Nutrition in Great Britain, two equally respectable government advisory committees, who after reviewing the same evidence, ended up with a twofold difference in target concentrations in serum 25(OH)D and similarly divergent conclusions for intakes of vitamin D[12], one can notice that differences concerning upper tolerable limits for vitamin D administration are limited. The more conservative IOM advises up to (upper tolerable limit) 1500 IU daily in infants < 1 year, 2500 IU in children 1-3 years, 3000 IU in children 4-8 years and up to 4000 IU for everybody after 9 years of age; where the Endocrine Society advices are up to 2000 IU for infants < 2 years, up to 4000 IU for children 1-18 years and up to 10000 IU for adults, adult pregnant and lactating women as well as the elderly, underlining that obese people may need up to two to three times more, as it may be needed to correct vitamin D deficiency or to treat specific conditions such as rickets, osteomalacia, hyperparathyroidism, malabsorption syndromes or if on medications affecting vitamin D's metabolism. However, the doses that the Endocrine Society practice committee characterizes as not requiring medical supervision are practically identical to the IOM's upper tolerable limits. Thus, supplementation with vitamin D within recommended safety limits, with doses that do not require prior measurement of the 25(OH)D concentration or medical supervision, apart from the already established protective role in bone mineral density[63], may also assure a well-functioning immune system[64].

In 2011, the Endocrine Society published the Endocrine Society Practice Guidelines on vitamin D, recommending how to treat and prevent vitamin D deficiency in children and adults. Based on the literature these recommendations were related to maximizing musculoskeletal health. However, in 2011 there was not enough scientific evidence for the Committee to recommend improvement in vitamin D status for reducing risk of many chronic illnesses or improving immune function. During the past decade, however, numerous studies have been conducted demonstrating that improvement in vitamin D status reduces risk for upper respiratory tract viral infections as well as having a wide variety of effects on both innate and acquired immunity[39,65]. A recent randomized controlled double-blind clinical trial assessed the impact of vitamin D supplementation on calcium metabolism and non-calcemic broad gene expression by relating them to the individual's responsiveness to varying doses of vitamin D3[66]. Thirty healthy adults were randomized to receive 600, 4000 or 10000 IU/d of vitamin D3 for 6 mo. Circulating parathyroid hormone (PTH), 25(OH)D, calcium and peripheral white blood cells broad gene expression were evaluated. The investigators reported dose-dependent increase in circulating 25(OH)D concentrations, decreased PTH concentrations and no change in serum calcium levels. A plateau in circulating PTH levels was achieved at 16 wk in the 4000 and 10000 IU/d groups. There was a dose-dependent 25(OH)D alteration in broad gene expression with 162, 320 and 1289 genes up- or down-regulated in their white blood cells, respectively. Thus, improvement in vitamin D status does have a dramatic effect on immune cell activity. However, can it therefore be expected that everyone who improves their vitamin D status would experience the same genomic influences on their immune system if they raised their blood level of 25(OH)D to the same degree? Carlberg and

Haq[67] gave daily 3200 IU of vitamin D3 to 71 prediabetic patients for 5 mo and found robust changes in total gene expression in peripheral blood mononuclear cells only in about half the subjects. Shirvani *et al*[66] observed in healthy adults who were vitamin D deficient and who received this same dose of vitamin D and raised their blood concentrations of 25(OH)D to the same degree, marked differences in the level of expression of the same genes. They reported that 60% of the healthy vitamin D deficient adults who received 10000 IU daily for 6 mo had a robust response in gene expression compared to the other 40% who had minimum to modest responses even though these subjects raised their blood concentrations of 25(OH)D in the same range of 60-90 ng/mL (150-225 nmol/L).

With all of this compelling information, it is reasonable for all responsible Public Health Authorities to consider advising their populations to enhance their immune system by improving their vitamin D status by encouraging sensible sun exposure and by taking vitamin D supplements (if not already on adequate supplementation or medically prohibited due to a vitamin D hypersensitivity disorder) at the doses which, as proposed by the Endocrine Society Guideline Committee in 2011, do not require previous laboratory testing nor medical supervision. To prevent nutritional rickets, daily doses of 400-1000 IU in infants, 600-1000 in children and 1500-2000 in teenagers (should be treated as adults) and adults, are needed. However, to achieve higher circulating concentrations of 25(OH)D at the range of 100-150 nmol/L (40-60 ng/ml), appearing according to our analysis to be necessary for substantially improving immune function and protect from COVID-19 disease, without any risk of toxicity[68], higher doses can be used. As mentioned above, the Endocrine Society Practice Guidelines recommends the safe upper limit for infants < 1 year is 2000 IU daily, children 1-18 years 4000 and adults (including elderly and adult pregnant-lactating women) 10000 IU, unless they are obese, requiring two to three times more. Thus, after a necessary initial repletion for up to 2 mo with these upper tolerable doses, the Endocrine Society's Committee's maintenance proposed doses, which can be safely given without medical supervision to prevent vitamin D deficiency and are practically identical with the IOM's upper tolerable limits, can be continued: *i.e.* up to 1000 IU/d for infants aged < 6 m, 1500 for age 6 m - 1 year, 2500 for 1-3 years, 3000 for children 4-8 years and 4000 for children > 8 years, with adults, pregnant/lactating women and adolescents requiring a daily intake of 4000-5000 (8000-10000 if obese) to maintain circulating concentrations of 25(OH)D at the range of 100-150 nmol/L. For teenagers and adults on a weekly scheme, these doses translate to about 50000 or if obese 100,000 IU, this being equivalent to approximately 6000 IU daily and 12000 IU for obese, respectively.

These doses will achieve blood concentrations of 25(OH)D of at least 75 nmol/L (30 ng/mL) aiming at the preferred range of 100-150 nmol/L (40-60 ng/mL), without any risk of toxicity[68]. It has been estimated that once a blood concentration of 25(OH)D reaches 50 nmol/L (20 ng/mL) that for every 100 IU ingested, the blood concentration increases by approximately 0.6-1 ng/mL. A good example of this dosing was reported by Shirvani *et al*[66] who demonstrated that circulating concentrations of 25(OH)D were maintained in the range of 24.3 ± 4.1, 40.8 ± 3.8 and 78.6 ± 13.5 ng/mL, in vitamin D deficient adults who ingested 600, 4000 and 10000 IU daily for 6 mo. These data are supported by a population based Canadian study demonstrating that some adults taking up to 20000 IU daily for more than a year maintained a blood concentration of 25(OH)D in the range of 60-80 ng/mL without any evidence of toxicity[69]. This study also nicely demonstrated the effect of BMI on vitamin D status. The authors observed that those who had a BMI > 30 kg/m^2 needed to ingest 2.5 times more vitamin D to maintain the same blood level as a normal weight adult.

Achieving circulating concentrations of 25(OH)D in the range of 100-150 nmol/L (40-60 ng/mL) appears to optimize vitamin D's effect on improving immune function, thereby substantially reducing the risk for serious-critical infections, particularly from SARS-CoV-2 according to our study, and possibly modulating the immune response, helping to prevent the dangerous cytokine storm often leading to COVID-19 related deaths. The COVID-19 pandemic is an unprecedented medical emergency for the modern world, and we may not possess the luxury, the time nor even the ethical argument to wait the definite results on RCTs while people are dying[70], while prospective well designed studies are needed to conclude on the impact of the vitamin D status on COVID-19 morbidity and mortality[71]. These trials are hopefully awaited, but before a medical emergency of this magnitude we need to remember that Evidence Based Medicine is not necessarily synonymous to RCTs. We do know that vitamin D enhances immune function. We know the extent of vitamin D deficiency, and we know that restrictions and lockdowns have probably worsened the populations' vitamin D status. Thus, until then, decisions are taken based on and adapted to the best available

evidence. And, as far as vitamin D, the evidence is there[51], justifying even the use of vitamin D as a possible adjuvant therapy for COVID-19 disease[72]. A preponderance of evidence does suggest that vitamin D deficiency increases mortality. Our findings predict a striking reduction of serious-critical illness and deaths from COVID-19 if 25(OH)D concentrations reach 100-150 nmol/L (40-60 ng/ml), and very recently SARS-CoV-2 positivity was found to be strongly and inversely associated with circulating 25(OH)D concentrations irrespective of latitudes, races/ethnicities, both sexes and age ranges[73]. Slovakia, at five deaths/M, having the lowest mortality rate in Europe from COVID-19 disease at the time of our analysis, a 125-fold lower than in the UK where official advice remains that 25(OH)D deficiency is < 25 nmol/L (https://www.nice.org.uk/advice/es28/evidence/evidence-review-pdf-8777674477), is a characteristic paradigm, being practically the only country in Europe with a 25(OH)D status meeting the Endocrine Society's recommended level of sufficiency > 75 nmol/L (30 ng/mL).

From a public health perspective, given the established safety of even high doses, and the potential benefits in enhancing innate and adaptive immunity[74], mitigating also the inflammatory response[3], the recommendation of intensive supplementation with vitamin D as possible prophylaxis with safe doses that do not require prior measurement or medical supervision, must be seriously considered, especially now that the world is facing the third deadly wave of this pandemic, forcing populations into repeated new lockdowns without the broad availability of specific medications yet and while awaiting for vaccinations to be widely available and plausible.

There is no need to require a measurement of serum 25(OH)D before recommending treatment and/or supplementation with vitamin D. This is supported by the observation that ingesting 50000 IU of vitamin D every 2 wk for up to 6 years is not associated with any toxicity[75]. Furthermore, this study was conducted in a clinical setting and all patients were prescribed this vitamin D therapy without the knowledge of their baseline serum 25(OH)D concentration. After completion of the study, the baseline levels were measured. Some of the patient's had a blood concentration of 25(OH)D of 125 nmol/L (50 ng/mL) and after being on 50000 IU of vitamin D once every 2 wk, their 25(OH)D concentration reached 200 nmol/L (80 ng/mL) without any evidence of toxicity[75].

There is essentially no vitamin D naturally occurring in the diet apart from oily fish, cod liver oil and sun-dried mushrooms. The modern way of life deprives us from sun exposure together with the warning to avoid all direct sun exposure by the national and international Dermatology Societies contributing to the worldwide vitamin D deficiency pandemic: Approximately 40% of the world's population is vitamin D deficient, *i.e.* 25(OH)D < 50 nmol/L (20 ng/mL) and 60% or insufficient *i.e.* 50-79 nmol/L (20-29 ng/mL). Therefore, we also need to consider worldwide recommendations for vitamin D food fortification that is practiced in several countries including the United States, Canada, and Finland to name a few. Most other countries either do not encourage or forbid food fortification with vitamin D. Recently, in 2017, India implemented fortification of milk and cooking oil with vitamin D2 as a means of reducing vitamin D deficiency that is common in both children and adults in this sunny Asian subcontinent.

Vitamin D is safe, not toxic and inexpensive. In the "shade" of the modern way of life, the human body cannot produce enough vitamin D from sun exposure, as our hunter gatherer forefathers did and as Maasai herders and the Hazda continue to do. Vitamin D may improve and modulate immune response against SARS-CoV-2. With all the above data, the limitations and the perspectives discussed, the possible benefit in the fight against SARS-CoV-2 should the protection against COVID-19 serious-critical illnesses and death with vitamin D prove truthful, and this without any risk of toxicity, the gain for humanity as well global public health might be just invaluable.

ARTICLE HIGHLIGHTS

Research background

Recent studies have claimed lower coronavirus disease 2019 (COVID-19) cases in European countries with a better vitamin D status and a significant association between vitamin D sufficiency and reduction in clinical severity and inpatient mortality from COVID-19 disease. Low serum 25(OH)D was identified as an independent risk factor for COVID-19 infection and hospitalization, and administration of calcifediol or 25(OH)D significantly reduced the need for intensive care unit treatment.

Research motivation

Vitamin D population status may indeed have possible unappreciated consequences to the COVID-19 pandemic, a hypothesis that needed to be further elucidated.

Research objectives

Following an ecological integrative approach, we examined the associations between published representative and standardized European population vitamin D data and the Worldometer COVID-19 data at two completely different time points of the first wave of this pandemic. If any sustained correlations were to be found, they would be an indication of a truthful association, even though they could not prove causality.

Research methods

Using linear regression, we explored the correlation between published representative and standardized population vitamin D concentrations and the number of total cases/million (M), recovered/M, deaths/M and serious-critically ill/M from COVID-19 for 26 European countries populated > 4 M. Life expectancy (LE) was also analyzed with semi-parametric regression. Weighted analysis of variance/analysis of covariance evaluated serious-critical/M and deaths/M by the vitamin D population status: deficient < 50, insufficient: 50-62.5, mildly insufficient > 62.5-75 and sufficient > 75 nmol/L, while controlling for LE for deaths/M. Statistical analyses were performed in XLSTAT LIFE SCIENCE and R (SemiPar library).

Research results

No correlation was found between population vitamin D concentrations and the total cases-recovered/M, but negative correlations were depicted predicting a reduction of 47%-64%-80% in serious-critical illnesses/M and of 61%-82%-102.4% in deaths/M, further enhanced when adapting for LE by 133%-177%-221% if 25(OH)D concentrations reach 100-125-150 nmol/L. Weighted analysis of variance evaluated serious-critical/M ($r^2 = 0.22$) by the vitamin-D population status and analysis of covariance the deaths/M ($r^2 = 0.629$) while controlling for LE ($r^2 = 0.47$). Serious-critical showed a decreasing trend ($P < 0.001$) from population status deficient ($P < 0.001$) to insufficient by 9.2% ($P < 0.001$), to mildly insufficient by 47.6% ($P = 0.044$) and to sufficient by 100% (reference, $P < 0.001$). For deaths/M the respective decreasing trend ($P < 0.001$) was 62.9% from deficient to insufficient ($P < 0.001$), 65.15% to mildly insufficient ($P < 0.001$) and 78.8% to sufficient ($P = 0.041$).

Research conclusions

A higher 25(OH)D concentration may protect from serious-critical illness and death from COVID-19 disease - even more in the elderly - but does not seem to prevent severe acute respiratory syndrome coronavirus 2 from spreading.

Research perspectives

Considering the ongoing pandemic situation, the presented results are useful for public health systems to advise their populations to enhance their immune system by improving their vitamin D status. Specifically, achieving a serum 25(OH)D concentration of 100-150 nmol/L (40-60 ng/mL) with vitamin D2/D3 supplementation using the upper tolerable daily doses for up to 2 mo (infants < 1 year 2000 IU daily, children 1-18 years 4000 and adults including elderly and adult pregnant-lactating women 10000 IU, unless they are obese requiring 2-3 times more) followed by the maintenance proposed doses not requiring medical supervision, as proposed by the Endocrine Society and being practically identical with the Institute of Medicine's upper tolerable limits (up to 1000 IU/d for infants aged < 6 mo, 1500 for age 6 mo - 1 year, 2500 for 1-3 years, 3000 for children 4-8 years and 4000 IU for children > 8 years, with adults, pregnant-lactating women and adolescents requiring a daily intake of 4000-5000 unless they are obese requiring two to three times more) may protect from serious-critical illness and death from COVID-19 disease.

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CASE REPORT

Chest radiography requirements for patients with asymptomatic COVID-19 undergoing coronary artery bypass surgery: Three case reports

Amr Salah Omar, Bassam Shoman, Suraj Sudarsanan, Yasser Shouman

ORCID number: Amr Salah Omar 0000-0001-8560-2745; Bassam Shoman 0000-0002-6432-1319; Suraj Sudarsanan 0000-0001-7325-7526; Yasser Shouman 0000-0002-2897-1533.

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Amr Salah Omar, Bassam Shoman, Suraj Sudarsanan, Yasser Shouman, Department of Cardiothoracic Surgery, Heart Hospital, Hamad Medical Corporation, Doha 3050, DA, Qatar

Amr Salah Omar, Department of Critical Care Medicine, Beni Suef University, Beni Suef 3050, DA, Qatar

Amr Salah Omar, Department of Medicine, Weill Cornell Medical College, Doha 3050, DA, Qatar

Corresponding author: Amr Salah Omar, MBChB, MSc, PhD, Professor, Department of Cardiothoracic Surgery, Heart Hospital, Hamad Medical Corporation, Alryan road, Doha 3050, DA, Qatar. a_s_omar@yahoo.com

Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2, represents a major challenge to health care systems both globally and regionally, with many opting by cancelling elective surgeries. Cardiac operations in patients diagnosed with COVID-19 have been imperative due to their emergency nature, critical condition of patients awaiting cardiac surgery, and accumulated number of cardiac surgical interventions throughout the last months.

CASE SUMMARY

Here we describe three COVID-19 positive cases who underwent coronary surgery, on an urgent basis. We did not experience worsening of the patients' clinical condition due to COVID-19 and therefore a routine post-operative chest X-ray (CXR) was not required. None of the health care providers attending the patients endured cross infection. Further trials would be needed in order to confirm these results.

CONCLUSION

While the pandemic has adversely hit the health systems worldwide, cardiac surgical patients who concomitantly contracted COVID-19 may undergo a smooth post-operative course as a routine post-operative CXR may not be required.

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Core Tip: Routine chest radiology is considered one of the core components of the post-operative care in cardiac surgery settings, there may be additional benefits in patients with associated coronavirus disease 2019 (COVID-19) infection to check the possible lung involvement. However, we found that routine chest radiology may not be required for post-operative care in COVID-19 patients undergoing cardiac surgery. This may reduce overall costs and radiographer's unnecessary exposure.

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INTRODUCTION

The World Health Organization (WHO) declared the coronavirus disease 2019 (COVID-19) as a global pandemic in March 2020, after the disease swept across the world from its epicenter in Wuhan, China. The disease represented a major challenge for the public and healthcare community globally[1]. The pandemic overwhelmed the health systems, forcing major changes in the health care practices[2]. Under the pressure from acute bed shortage, many health care facilities opted to defer elective surgical procedures[3], consequently, cardiac surgery elective services were forced to be canceled or postponed[4]. Shoman *et al*[5] reported that urgent cardiac in patients with COVID-19 without pneumonia could be carried out safely without further complications or health care associated cross infection, if strict infection control protocols would be enforced during the procedure[5].

The explosive and uncontrolled spread of COVID-19 globally made it imperative for the cardiac surgery societies to release guidelines and protocols aiming to risk assess protocols based on probabilities and resources[6]. Here we describe three COVID-19 positive cases, with no pulmonary-related symptoms, diagnosed with significant coronary artery disease and subsequently subjected to urgent coronary surgery. This manuscript also sheds light on the role of routine chest radiology in perioperative management.

CASE PRESENTATION

Chief complaints

Case 1: A 43-year-old gentleman was presented to the hospital with recent onset chest pain.

Case 2: A 50-year-old gentleman was presented to the emergency cardiac department with acute onset of severe chest pain.

Case 3: A 47-year-old gentleman came to the emergency room with typical post-prandial chest pain.

History of present illness

Case 1: The patient's 12-lead electrocardiogram (ECG) indicated a non-ST segment elevation myocardial infarction (NSTEMI). Subsequent coronary angiography revealed critical left main coronary artery distal occlusion with additional three vessels coronary artery disease (CAD), all of which were severely occluded.

Case 2: The patient's 12-lead ECG showed anterior wall ST segment elevation myocardial infarction (STEMI). Subsequent coronary angiography revealed left main coronary artery disease, left anterior descending, and left circumflex coronary artery disease. Patient's routine swab was positive for COVID-19, but no respiratory symptoms noted. Chest radiology was normal.

Case 3: The working diagnosis after evaluating his 12-ECG was NSTEMI. Coronary angiography detected significant three vessels CAD and patient was referred for urgent surgical revascularization.

History of past illness

Case 1: Patient's past medical history included type II-diabetes mellitus, smoking, and dyslipidemia.

Case 2: Unremarkable past medical history.

Case 3: Patient's medical history was significant for diabetes mellitus, hypertension, smoking, and dyslipidemia.

Physical examination

Case 1: None.

Case 2: The patient's pre-procedure examination was unremarkable. The vital signs showed temperature of 37.1 °C, blood pressure of 127/77 mmHg, heart rate of 87 beats/min regular, and oxygen saturation of 98% on supplemental oxygen flow at 2 liters/min delivered *via* nasal cannula.

Case 3: The patient pre-procedure examination was unremarkable. The vital signs showed temperature of 36.8 °C, blood pressure of 107/67 mmHg, heart rate of 77 beats/min regular, and oxygen saturation of 97% on room air.

Laboratory examinations

Case 1: Routine nasopharyngeal swab was positive for COVID-19 after admission, without respiratory symptoms or chest roentgenogram findings.

Case 2: Patient's routine swab was positive for COVID-19, no respiratory symptoms noted, and normal chest radiology.

Case 3: Similar to the previous two patients here studied, a positive swab for COVID-19 was taken, without additional clinical or radiologic manifestations.

FINAL DIAGNOSIS

Cases 1 and 3: Acute NSTEMI with three vessels disease. Patient positive for COVID-19.

Case 2: Acute STEMI with three vessel disease. Patient positive for COVID-19.

TREATMENT

Case 1: The patient subsequently underwent urgent surgical revascularization with three grafts. Full personal protective equipment (PPE) was used, with the anesthesia team taking a lead in the operating room team preparation and theatre. Patient followed a dedicated predesigned transport from and to the operating room and the cardiothoracic intensive care unit (ICU) for post-operative recovery.

Case 2: Patient underwent urgent surgical revascularization under the departmental predesigned guidelines for surgical management of COVID-19 patients. Post-operatively, patient's disposition was carried out in an isolation room of the cardio-thoracic ICU (CTICU) and extubated within six hours of admission on the same day.

Case 3: Patient underwent on-pump coronary artery bypass graft and the procedure was uneventful.

OUTCOME AND FOLLOW-UP

Case 1: Patient's post-operative course in the CTICU was uneventful, after removal of the chest drain patient was discharged to the dedicated COVID-19 high dependency unit within the hospital for a short stay, in order to optimize COVID treatment. Patient was subsequently discharged home on the seventh post-operative day.

Case 2: The patient remained in the unit until removal of the chest drain and then transferred to the dedicated isolation ward in the hospital. Later, the patient was discharged home for self-quarantine, on the eight post-operative day, and subsequently followed up by routine telephonic consultation without any reported surgical complications.

Case 3: Patient was extubated on the same operative day in the CTICU and transferred to an isolation room on the ward in the first post-operative day, where cardiac rehabilitation was completed. Patient was then discharged for self-quarantine for 14 d.

No chest radiography was required in the aforementioned three patients (Table 1).

DISCUSSION

The challenge of handling urgent surgeries alongside COVID-19 diagnosis is of limited familiarity amongst practitioners. Decision making and risk assessment protocols can define COVID-19's influence on cardiothoracic surgical outcomes. The three patients here referred are examples of patients who had been through pragmatic decision making protocols to perform such surgeries. The apparent medical stability of these patients, from a respiratory standpoint, encouraged our team to act towards treating the patient's acute coronary syndrome, reducing possible related mortality and morbidity.

Anticipating the need to operate COVID-19 patients, our department developed a protocol for perioperative management of COVID-19 patients undergoing cardiac surgery, which was reviewed by all stakeholders. Furthermore, our team followed patients with COVID-19 after cardiac surgery with a chest radiology when clinically indicated as per the CTICU protocol. This was successfully carried out for all three patients here reported, without any significant clinical issue compromising the patient's outcome.

Triaging and routine testing

Reducing unnecessary chest radiology is a widely agreed goal in the post-operative care of patients after cardiac surgery. Tolsma *et al*[7] made an observational study with 1102 patients aiming to define clear indications for chest X-ray (CXR) after cardiac surgery. This practice was safe and effective in reducing the total number of CXRs performed and also anticipated increased efficacy[7]. Similarly, Forouzannia *et al*[8] reviewed 118 patients who underwent off pump coronary surgeries and their post-operative outcome did not change when CXR were eliminated in the post-operative period[8].

In our organization, we have defined certain criteria for chest radiography during post-operative cardiac surgical care. This included clinical evaluation-based findings of fever, dyspnea, abnormal pulmonary sounds, signs and symptoms of cardiac tamponade, abnormal chest tube bleed or air leak, and doubtful position of endodontically treated teeth and vascular lines. Hypoxia on pulse oximeter (SaO_2 < 92% on regular oxygen therapy) and multiple punctures during central venous access also mandated CXR. A final clinical evaluation focused on X-ray findings. All patients were discharged 5-7 d after surgery. A 30-d follow-up included at least two visits. Patients were in constant contact with the cardiac clinic. Symptomatic patients were selectively re-examined to rule out complications.

Decision to operate

In our tertiary center, we have set up a multidisciplinary team approach before deciding to surgically operate on COVID-19 positive patients. This team involved anesthesiologists, cardiac surgeons, cardiologists, and infectious diseases specialists. Asymptomatic but serologically positive COVID-19 patients underwent management as actively infectious. To all these patients the used of full PPE was mandatory[9]. The coronary lesions' anatomical complexity in all three patients here studied were treated as meaningful and consequently conceived to be subjected to operation. Significant left main disease or acute coronary syndrome not amenable to percutaneous intervention

Table 1 Description and outcome of the studied patients

	Case 1	Case 2	Case 3
Age	43	50	47
BMI (kg/m^2)	27.4	24.7	27.1
Creatinine (micromole/L)	97	64	81
EF%	62	57	58
Additive European score	0.68%	0.8%	0.68%
CPB time (min)	86	75	85
ACC time (min)	43	30	48
Anesthesia time (min)	287	280	245
VIS	13	5	8
LOS_{ICU} (h)	49	22	18
LOV (min)	707	722	505
LOS_{hosp} (d)	18	18	22
POAF	None	None	None
AKI	None	None	None
In-hospital-mortality	None	None	None
VA-ECMO	None	None	None
Re-admission ICU	None	None	None
Re-exploration	None	None	None
PMI	None	None	None
Pulmonary complications	None	None	None
Thromoembolic complications	None	None	None
Post-operative CXR requirement	None	None	None

ACC: Aortic cross clamp; AKI: Acute kidney injury; BMI: Body mass index; CXR: Chest X-ray; CPB: Cardiopulmonary bypass; EF: Ejection fraction; $LOS_{ICU:}$ Length of stay in intensive care unit; LOV: Length of mechanical ventilation; LOS_{hosp}: Hospital length of stay; PMI: Perioperative myocardial infarction; POAF: Post-operative atrial fibrillation; VA-ECMO: Venoarterial extracorporeal membrane oxygenation; VIS: Vasoactive inotrope score; ICU: Intensive care unit.

was a prerequisite for urgent or emergent surgical intervention[10].

Practice of routine post-operative chest radiograph

Most cardiac cardiothoracic centers practice CXR in the immediate post-operative period routinely, in absence of any clinical or laboratory indication. However, the accuracy of CXR in diagnosing pulmonary opacities in the post-operative period is limited and its accuracy in visualizing and defining etiology of pulmonary opacity is moderate[11]. Moreover, management may not be changed in response to abnormal CXR findings[12]. The risks associated with radiation exposure, manpower wastage, cost incurred, possible displacement of invasive line, and endotracheal tubes are additional concerns[13].

Transport and ICU disposition

We appealed the CTICU team to be present at the operating theatre door for receiving the patient and to minimize practitioners' transportability of a possibly contaminated PPE. Patient's transfer to the CTICU after surgery was carried out with a transport ventilator and minimal essential team comprised of a single respiratory therapist, nurse, and physician. Patel *et al*[14] emphasized the value of minimal ventilator circuit interruption, reducing practitioners' presence and unnecessary ventilator transport[14]. The same principles applied when attempting to do CXRs.

The patient's preparation before transport to ICU, by covering the patient with a plastic sheet and connecting them to a portable ventilator, was done after clamping/

de-clamping technique. Patient's escorting to the isolation room of the CTICU was done by the ICU team which comprised a physician, nurse, and respiratory therapist. Doffing of the anesthesia team was done in a pre-designated area in the operation theatre. The operation room was disinfected thereof and restricted until the following morning. The protocol for managing COVID-19 positive patients was followed by the anesthesia team.

The safety of patients transported to and from the theatres needs to be customized for each hospital, considering the basic principles of minimizing exposure and maximizing communication[15]. We have transferred COVID-19 positive patients to a COVID ICU unit enclosing negative-pressure rooms with additional high-efficiency particulate air filters. We have also taken into account early possible surgical complications such as arrhythmias, myocardial injury, acute renal injury, and the respiratory complications[16,17]. None of our three patients showed early cardiac or respiratory complications and all were able to be transferred from ICU after a median of 24 h after surgery.

CONCLUSION

While the pandemic adversely has hit the health systems worldwide, cardiac surgical patients who concomitantly contracted COVID-19 infection may undergo a smooth post-operative course as a routine post-operative CXR may not be required.

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REVIEW

Hypotheses and facts for genetic factors related to severe COVID-19

Stanislav Vasilev Kotsev, Dimitrina Miteva, Stanislava Krayselska, Martina Shopova, Maria Pishmisheva-Peleva, Spaska Angelova Stanilova, Tsvetelina Velikova

ORCID number: Stanislav Vasilev Kotsev 0000-0001-8201-0242; Dimitrina Miteva 0000-0002-5931-2426; Stanislava Krayselska 0000-0002-2029-7409; Martina Shopova 0000-0003-0031-7940; Maria Pishmisheva-Peleva 0000-0001-6792-9146; Spaska Angelova Stanilova 0000-0003-1368-9081; Tsvetelina Velikova 0000-0002-0593-1272.

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Stanislav Vasilev Kotsev, Martina Shopova, Maria Pishmisheva-Peleva, Department of Infectious Diseases, Pazardzhik Multiprofile Hospital for Active Treatment, Pazardzhik 4400, Bulgaria

Dimitrina Miteva, Department of Genetics, Sofia University "St. Kliment Ohridski", Sofia 1000, Bulgaria

Stanislava Krayselska, Private Practice General Praxis, Sofia 1113, Bulgaria

Spaska Angelova Stanilova, Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Stara Zagora 6000, Bulgaria

Tsvetelina Velikova, Department of Clinical Immunology, University Hospital Lozenetz, Sofia 1407, Bulgaria

Tsvetelina Velikova, Medical Faculty, Sofia University "St. Kliment Ohridski", Sofia 1407, Bulgaria

Corresponding author: Tsvetelina Velikova, MD, PhD, Assistant Professor, Department of Clinical Immunology, University Hospital Lozenetz, Kozyak 1 Street, Sofia 1407, Bulgaria. tsvelikova@medfac.mu-sofia.bg

Abstract

Genome-wide association analysis allows the identification of potential candidate genes involved in the development of severe coronavirus disease 2019 (COVID-19). Hence, it seems that genetics matters here, as well. Nevertheless, the virus's nature, including its RNA structure, determines the rate of mutations leading to new viral strains with all epidemiological and clinical consequences. Given these observations, we herein comment on the current hypotheses about the possible role of the genes in association with COVID-19 severity. We discuss some of the major candidate genes that have been identified as potential genetic factors associated with the COVID-19 severity and infection susceptibility: *HLA, ABO, ACE2, TLR7, ApoE, TYK2, OAS, DPP9, IFNAR2, CCR2*, *etc.* Further study of genes and genetic variants will be of great benefit for the prevention and assessment of the individual risk and disease severity in different populations. These scientific data will serve as a basis for the development of clinically applicable diagnostic and prognostic tests for patients at high risk of COVID-19.

Key Words: Genome-wide association studies; Severe COVID-19; SARS-CoV-2; *ACE2*; *TLR7*; *ApoE*; *TYK2*; *OAS*; *DPP9*; *IFNAR2*; *CCR2*

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Core Tip: Understanding what contributes to the development of severe coronavirus disease 2019 (COVID-19) can be of considerable clinical and therapeutic advantage. Severe acute respiratory syndrome coronavirus 2 infection may present with different COVID-19 manifestations, where various host genetic factors influence the viral susceptibility, immune response, disease progression, and outcomes. Genome-wide association analysis allows the identification of potential candidate genes involved in the development of severe COVID-19. Hence, it seems that genetics matters here, as well.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the coronavirus disease 2019 (COVID-19) that emerged in Wuhan, China, in December 2019 and its rapid spread all over the world. COVID-19 was declared a pandemic by the World Health Organization in March 2020. Since then, it has become the leading burden for healthcare[1]. Although healthcare workers have been facing the disease for almost a year, the management of COVID-19 is still a challenge because of the clinical course it may take. On the one hand, about 40% of SARS-CoV-2 infected people present with mild or no symptoms. At the same time, moderate illness is observed in another 40% of them. On the other hand, about 15% manifest with symptoms of pneumonia that requires hospital admission and oxygen support, and 5% develop a critical illness, complicated with respiratory failure, acute respiratory distress syndrome (ARDS), sepsis and septic shock, thromboembolism, and/or multiorgan failure, including acute kidney injury and cardiac injury[2]. Regarding the World Health Organization data, since the pandemic was declared, more than 2.4 million deaths have been reported to date[3]. Some of the risk factors considered predisposing to a severe course of COVID-19 and higher mortality rates include: Advanced age and smoking, underlying chronic conditions affecting the cardiovascular system, the lungs, and the kidneys, as well as immunosuppression and cancer [4]. However, there is still a lack of predictive features and signatures for severe COVID-19.

Additionally, the clinical course of COVID-19 is closely related to the severity of the inflammatory response conducted by the immune system activation. A complex interaction involving immune cells, cytokines, and mediators leads to systemic immune reactions, which might result in immune hyperactivation or dysregulation. Hence, the cytokine storm is caused by the uncontrolled inflammatory response, and it is crucial for illness's severity and the development of ARDS, multiorgan failure, and fatal outcome[5,6]. Clinical laboratory results might serve useful functions as biomarkers in the management of COVID-19 and prediction of the probable outcome [7]. Laboratory findings in the severe course of COVID-19 usually include low lymphocytic count and hypoalbuminemia, significant elevation of liver transferase enzymes, C-reactive protein, lactate dehydrogenase, ferritin, and D-dimer, along with high levels of some cytokines[8]. However, the influence of various host genetic factors on viral susceptibility, immune response, disease progression, and outcomes has been discussed recently[9,10]. Genome-wide association analysis allows the identification of potential candidate genes involved in the development of severe COVID-19. Hence, it seems that genetics matters here, as well. Nevertheless, the virus's nature, including its RNA-genome, determines the enhanced rate of mutations leading to a new viral genome with significant epidemiological and clinical consequences. Given these observations, we herein comment on the current hypotheses about the possible role of the genes for COVID-19 severity. We discuss some of the major human candidate genes that have been identified as potential genetic factors associated with the

different COVID-19 severity and infection susceptibility.

MAIN CONVENTIONAL RISK FACTORS FOR SEVERE COVID-19

The factors that predispose to a severe course of COVID-19 are of great importance for infection confinement among people from risk groups. Age, gender, and comorbidities, particularly cardiovascular diseases, should be taken as risk factors that depend on one another[11].

In numerous recent research studies, based on the clinical course of COVID-19, age is discussed as a leading risk factor. On the one hand, most of the viral infections affect children, whereas SARS-CoV-2 infection typically occurs in people of advanced age, which might be due to the increased comorbidities as well as to the age-dependent gene expression. In a published study, the death rate among people older than 80 was 14.8%. In contrast, the percentage among those between 70-79.9 years was 8% and 3.6% among those between 60-69.6 years. Owing to the latter, provided the same comorbidities, the younger the age, the lower the death rate is[12].

Gender and its significance as a risk factor are difficult to be evaluated due to the differences in the socio-economical status, lifestyle, and quality of life between men and women. Furthermore, cardiovascular and chronic pulmonary diseases are more frequently observed in men. Moreover, tobacco and alcohol abuse are usual for the male gender and might as well cause respiratory, liver, gastrointestinal illnesses, *etc.* Alternatively, women are commonly involved in caring for sick family members at home and patients at hospital centers, as most nurses are women[13]. Therefore, females are exposed to an increased risk of COVID-19 contraction. Additional factors such as socioeconomic status, menopausal transition, pregnancy and complications during pregnancy, fertility treatment, hormone contraceptive usage, postmenopausal hormone replacement therapy, breast cancer as well as prostate cancer anamnesis are recognized to have an impact on the differences in the COVID-19 course in men and women. Recently, more pieces of evidence have been accumulated about different gender-dependent expression of proinflammatory cytokines such as tumor necrosis factor-alpha and interleukin (IL)-12, which play a significant role in the systemic inflammation and cytokine storm[14-16]. According to published data, the death rate is assessed to be 2.5% in the male gender, while in females it is 1.7%. Nevertheless, these values do not provide proof of more severe COVID-19 for men[13].

Additionally, investigations of the laboratory changes in patients with COVID-19 have shown considerably elevated levels of lactate dehydrogenase, alanine transaminase, gamma-glutamyl transaminase, C-reactive protein, IL-6, erythrocyte sedimentation rate, ferritin, coagulation factors (including D-dimer and fibrinogen), along with significant anemia and lymphopenia in patients with accompanying disease in comparison to those without. These findings suggest that underlying comorbidities increase the risk for an uncontrollable inflammatory reaction, hypercoagulation, and excessive release of tissue-damaging enzyme, hence more severe COVID-19[17].

Another critical observation has shown the majority of those diagnosed with COVID-19 had type 2 diabetes. This metabolic illness affects the whole organism and the immune system and, by misbalancing its function, predisposes to infections. Moreover, SARS-CoV-2 disturbs glucose metabolism and increases the insulin requirements of the organism. Thus, diabetes and obesity should be considered risk factors for a severe course of the coronaviral infection as well[17].

Interestingly, during the first wave of COVID-19 in the United Kingdom, younger and less burdened by comorbid illnesses patients were also admitted to intensive care units[18]. These data have only shown us that there might be other factors, including genetic background, related to the severity of COVID-19.

CHARACTERISTICS OF CRITICALLY ILL COVID-19 PATIENTS

COVID-19 manifests with various or no symptoms. Despite having no symptoms, an asymptomatic person can also be a source of the infection. In symptomatic COVID-19 cases, the symptom onset is after an average incubation period of 5-6 d (up to 14 d). However, there are no specific and pathognomonic symptoms of the illness[5,8]. COVID-19 patients usually present with fever, dry cough, appetite loss, as well as sore throat, nasal congestion, malaise, headache, diarrhea, nausea, and vomiting. Some of the patients experience anosmia and ageusia. People of advanced age may present

with qualitative and quantitative consciousness disorders and lost mobility. Dyspnea and shortness of breath are typically observed in severe cases[19].

Disease physiology includes damage of type 2 pneumocytes, viral pneumonia, cytokine storm, macrophage-activation syndrome, ARDS, disseminated intravascular coagulation, sepsis, and general immune dysregulation, all of which can be combined or present simultaneously[20].

Most of the SARS-CoV-2 infected experience mild to moderate symptoms. Fifteen percent of the patients present with pneumonia that requires hospital admission. According to published data, patients in hospitals develop dyspnea about 5 d after symptom onset. On the contrary, in severely ill patients, the disease may rapidly progress to multiorgan failure[21-23].

A typical complication of SARS-CoV-2 infection is the development of ARDS. The latter is presumed the leading cause of death in patients with COVID-19, particularly among those with underlying diseases and conditions, assessed as risk factors, smokers, and older ones. The immunological events during COVID-19 cause not only severe harm and ventilation collapse of the lung parenchyma, but perhaps, it would eventually lead to complications later in life[5]. Additionally, inflammation destroys the endothelium and contributes to the release of the plasminogen tissue activator that can contribute to COVID-19 associated thromboembolic complications consistent with a hypercoagulable disease. Although the primary cause of death in COVID-19 is thought to be ARDS, the problem associated with bradykinin B1 receptor activation in the lung endothelial cells is another serious cause for severe COVID-19, as well as sepsis-associated disseminated intravascular coagulation[24]. Thromboembolic events are among the most commonly observed complications in COVID-19. Its incidence is higher in critical illness, despite the anticoagulant administration. Thromboembolism may manifest as deep vein thrombosis, pulmonary thromboembolism or may lead to myocardial infarction or cerebral ischemia[21]. We hypothesize that complement overactivation and C1-esterase hyperproduction could be another cause of thromboembolic complication in severe COVID-19.

COVID-19 manifests as a severe illness in patients with underlying chronic conditions, including cardiovascular diseases, hypertension, diabetes, and renal disease. Moreover, the mortality rate is higher among these patients, whereas infants and children experience milder disease, and the mortality rate among them is comparatively lower[21,25,26]. Furthermore, between 3%–29% of the patients develop complications that require intensive care, and the approximate mortality rate is 38%[21,23]. Within a week after the symptoms worsen, pneumonia progresses to ARDS. Along with ARDS, critically ill patients may also develop extrapulmonary manifestations, some of which are cardiovascular, neurological, and gastrointestinal disorders, renal impairment, thromboembolism, sepsis, and septic shock[1,21].

Amongst them, the disorders of the cardiovascular system include myocardial ischemia, myocarditis, myocardial injury, arrhythmias, and cardiogenic shock. Neurological manifestations are observed in about 36% of the patients with severe COVID-19, presented as dizziness, headache, ageusia and anosmia, myalgia, or with more severe manifestations such as acute stroke, consciousness disorders, Guillain-Barré syndrome, meningoencephalitis, and necrotizing encephalopathy, which affects the brain stem and basal ganglia. Acute liver and kidney injuries (31%) are also observed, whereas gastrointestinal bleeding rarely occurs. Elevation of the liver enzymes and the bilirubin level might correlate with the severity of the disease[21].

Critically ill COVID-19 patients may develop sepsis as a result of host response dysregulation to infection, leading to organ dysfunction. It clinically presents with respiratory failure, impaired tissue oxygen supply, tachycardia, hypotension, oliguria, coagulopathy, *etc*. Septic shock occurs in extreme hypotension that is ineffectively treated with infusions and requires vasopressor application[27]. Collectively these observations have shown that a certain genetic background is required.

Besides, the recently published Genome wide association study suggests that individuals with blood group A be predisposed to a severe COVID-19, whereas those with blood group 0 might be at lower risk for developing critical illness[28].

GENETIC ASSOCIATION STUDIES AND COVID-19 HOST GENETICS INITIATIVE

In recent years, genome-wide association studies (GWAS) have offered the possibility of detecting the most common genetic variants associated with various diseases. To date, a large number of single nucleotide substitutions have been found in different

genes or regulatory regions (polymorphic variants) in the genome that can explain the severity and pathology of these diseases.

In a GWAS that involved patients with severe COVID-19 at seven hospitals in Italy and Spain and a meta-analysis of the two case-control panels, 8582968 single-nucleotide polymorphisms (SNPs) were analyzed. It was identified that the first gene cluster of chromosome 3 covers six genes (*3p21.31-SLC6A20, LZFTL1, CCR9, CXCR6, XCR1,* and *FYCO1*) that aggravate the COVID-19 disease[28]. This study showed the potential involvement of the ABO blood-group system. Other GWAS papers reported results about risk loci in chromosome 19p13.3, 12q24.13, and 21q22.1 associated with severe COVID-19[29]. Some genes belong to the type I interferon pathway and predispose to life-threatening COVID-19 pneumonia. Five common variants were identified (rs3787946, rs9983330, rs12329760, rs2298661 and rs9985159) at locus 21q22.3 within transmembrane serine protease (TMPRSS)2 that showed associations with severe COVID-19[30].

Chromosome 3p21.31

At locus 3p21.31, the association with severe COVID-19 signal spanned the genes *SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6*, and *XCR1*. A candidate in this region is SLC6A20, which encodes the SIT1 (sodium–amino acid transporter 1). It functionally interacts with angiotensin-converting enzyme 2 (ACE2), which SARS-CoV-2 uses for entering the cells[31,32]. The locus also contains genes encoding CCR9 and CXCR6 (chemokine receptors of the C-C and CXC families). They control the cell migration associated with the immune system by trafficking effector cells to the sites of inflammation, especially in the immune response to airway pathogens, including influenza viruses[28,33,34].

A meta-analysis has found a significant association between the severe COVID-19 disease and rs11385942 at locus 3p21.31and rs657152 at locus 9q34.2. Leucine zipper transcription factor-like 1 (LZTFL1) might be the most important, with the rs11385942 variant. LZTFL1 is expressed mainly in human lung cells. It encodes a protein involved in the immunologic synapse with antigen-presenting cells such as dendritic cells[35,36]. Reduced expression of CXCR6 and enhanced expression of SLC6A20 were related to the risk genotype GA of rs11385942. The frequency of the risk allele at 3p21.31 (rs11385942) was increased among patients on mechanical ventilation than those who received only oxygen supplementation. Available database variants suggest that the frequency of this risk allele varies among populations worldwide[28].

ABO locus

A genome wide association analysis has identified the locus 9q34.2 where the rs657152 is located and also includes the ABO blood group locus. A blood-group analysis demonstrated a higher risk for people with blood group A and a protective effect in people with blood group O as compared with other blood groups[28,37]. Variation in the *ABO* gene is the basis of the ABO blood group. Since the 'O' blood group is caused by a deletion of guanine-258 near the N-terminus of the protein, this results in a frameshift mutation and translation of an almost entirely different protein. This 9q34.2 locus has also been identified as a susceptibility locus for severe COVID- 19. Using the combinations of genotypes of three different SNPs, a higher risk among individuals with blood group A and a protective effect of blood group O in the Spanish and Italian analyses was reported[28]. A similar study in China in March 2020 showed that blood group A was associated with a significantly higher risk of COVID-19 compared with the other blood groups[37,38].

Human leukocyte antigen (HLA) analysis

HLA region (6p21.33) was analyzed with GWAS. The spike protein and the nucleocapsid proteins of the SARS-CoV-2 are reported to contain multiple class I epitopes with predicted HLA restrictions. Individual HLA genetic variations can explain different immune responses to different viruses across the population. Nguyen *et al* [39] reported the potential associations between the genetic variants in major histocompatibility complex class I genes (HLA A, B, and C) and the severity of COVID-19. The fewest binding peptides for SARS-CoV-2 were found for HLA-B*46:01, suggesting that individuals with this allele should be more vulnerable to COVID-19 [40]. Conversely, the highly conserved SARS-CoV-2 peptides that are shared among common human coronaviruses were detected for HLA-B:15:03, suggesting that individuals could be protected with T cell immunity[29,39]. Another published report from Italy defined other three HLA alleles-HLA-DRB1*15:01, -DQB1*06:02, and -B* 27:07, which may predispose to a less favorable outcome and severe COVID-19[41].

Preliminary results from China also indicated that the HLA-A*11:01, -B*51:01, and -C*14:02 alleles predispose patients to the worst clinical outcome[42]. Much more studies are needed to understand fully the role of single HLA alleles in COVID-19 severity.

Recently, the HLA system has been under thorough investigation for its crucial role in autoimmunity and infectious disease susceptibility[10,40]. A strong association has been established between the HLA region and autoimmune diseases such as type 1 diabetes (T1D – DR3; DR4; DQB1), multiple sclerosis (MS–DR3), rheumatoid arthritis (RA–DRB1; DR4), Graves' disease (GD–DR3; DRB1*08; B*08; C*07), ankylosing spondylitis (AS–B27;), systemic lupus erythematosus (SLE–DR3; DR8; DR15), Hashimoto's thyroiditis (HT–DR3; DR4), narcolepsy (DQ6), Addison's disease (DR3), and multiple sclerosis (MS-DR15)[43-45]. Nevertheless, a comprehensive explanation of the link between autoimmune diseases and infection susceptibility is yet to be given.

TMEM189-UBE2V1

GWAS in China analyzed 22.2 million genetic variants in 332 COVID-19 patients from the Shenzhen Third People's Hospital. During hospitalization, 64 laboratory analyses were performed for each of the patients to classify their severity condition based on the demographic features age and gender as well as medical comorbidities and treatments[42]. The features of greatest importance that contribute to more severe disease outcomes included decreased lymphocyte and platelet counts, increased C-reactive protein, D-dimer, IL-6, age, and concomitant diseases[29,46]. Obviously, the genes that encode proteins of the immune system are responsible for the disease severity.

The most significant SNP, rs6020298, is located in the intron of the transcript TMEM189–UBE2V1 in the 20q13.13 region. This SNP also affects the genes *UBE2V1* and *TMEM189*. TMEM189–UBE2V1 has been involved in the IL-1 signaling pathway [47]. In COVID-19 patients, IL-1 is elevated, especially in the critically-ill ones who suffer from the cytokine storm[48]. TMEM189-UBE2V1 has a lot of functional associations with the biological processes in different cell types and tissue, but the main function of its protein product has not yet been determined.

ACE2 and TMPRSS2

Depending on virus strains and cell types, coronavirus spike proteins may be cleaved by one or several host proteases-neutrophil elastase (ELANE), furin, cathepsins, TMPRSS-2, and TMPRSS11A[49-53].

The availability of these proteases on the target cells determines whether the virus particles enter the cells through the plasma membrane or endocytosis. SARS-CoV-2 infection of the host depends on two factors: The ACE2 receptor for the viral entry and the TMPRSS2 for the viral spike protein priming[54]. A recently published comparative genetic analysis in different populations has shown possible associations between the coding region variants of ACE2 and TMPRSS2 with COVID-19 severity and outcomes[30].

The *ACE2* gene, located on chromosome Xp22.2, exhibits a high level of polymorphism. The ACE2 receptor is highly expressed in the alveolar type-2 cells in the lung but also in the proximal kidney tubules, liver cholangiocytes, esophagus keratinocytes, myocardial cells, bladder cells, and gastrointestinal epithelial cells[55, 56].

SARS-CoV-2 enters the cell by binding to the ACE2-an integral membrane protein that catalyzes the production of angiotensin 1–7 from angiotensin II[57]. ACE2 is expressed on the vascular epithelium, renal tubular epithelium, and Leydig cells in the testes. In the respiratory system, ACE2 is mainly expressed on type II pneumocytes [54]. After the viral spike protein binds to the ACE2, the S-protein undergoes structural changes through proteolysis by the receptor TMPRSS2[58]. These changes are essential for the fusion between the cellular and viral membrane and the following viral RNA release. In the host cell, the viral genome uses the cellular machinery for new virions formation[6,59]. In the respiratory system, the pneumocytes type II are the target cells that SARS-CoV-2 attacks. Persistent target cell infection leads to ACE2 downregulation and subsequent ACE2 deficiency[59]. The latter prevents angiotensin II conversion to angiotensin I. Angiotensin II excess activates the angiotensin II type 1 receptor and results in vasoconstriction and various physiological effects that include inflammation, fibrosis, thrombosis, and reactive oxygen species (ROS) production. On the other hand, angiotensin has opposite functions by binding to specific receptors, it causes vasodilation, anti-inflammation, anti-fibrosis, anti-thrombosis, and ROS neutralization. That is why ACE2 is considered to provide protection from ROS production in the inflammatory process. Moreover, ACE2 controls the macrophages'

overexpression of tumor necrosis factor-α and IL-6, both playing an essential role in the inflammation[60,61]. Thus, the ACE2 deficiency leads to an imbalance of the renin-angiotensin system, which appears to be a crucial mechanism in COVID-19 pathogenesis[62].

Owing to the fact that the *ACE2* gene is located on the X chromosome, it has been suggested that the higher mortality rate among males should possibly be related to its lower expression. Furthermore, estrogen increases the ACE2 expression and activity in women[63,64]. Renin-angiotensin system balance is maintained by the ACE and ACE2 function; thus, *ACE2* gene variants or their overexpression lead to renin-angiotensin system imbalance resulting in vasoconstriction, hypercoagulation, fibrosis, alveolar cell apoptosis, increased ROS production, and lung damage overall. Common gene polymorphism might alter both *ACE* and *ACE2* gene expression and have a similar effect. It is possible for ACE/ACE2 balance to be influenced by other gene products, for instance, ABO locus, angiotensinogen (AGT), sex-determining region Y gene, SOX3, A disintegrin and metalloprotease 17, angiotensin II receptor type 1, and angiotensin II receptor type 2[10,62,65,66]. Allele frequency variations of the *ACE2* gene in different populations might be due to SNPs. Compared to a global average, the protective variants were found to be of higher frequency in the Asian population, whereas the risk variants were more frequent among the population of European descent[10,63].

Polymorphisms in ACE2 were found to associate with pulmonary and cardiovascular conditions by altering the AGT-ACE2 interactions, such as p.Arg514-Gly in the African and African-American populations[30].

TMPRSS2 is localized in 21q22.3 and is a key gene in prostate cancer. The product of the gene is plasma membrane-anchored serine protease that participates in proteolytic cascades for the normal physiologic function of the prostate[67,68].

Matsuyama *et al*[69] demonstrated that TMPRSS2-expressing cell lines are highly susceptible to SARS-CoV, Middle East respiratory syndrome coronavirus, and SARS-CoV-2. The susceptibility to COVID-19 could be explained with prevalent polymorphism Val160Met (rs12329760) in TMPRSS2. The harmful effect of the rs12329760 polymorphism in the coding region of the *TMPRSS2* gene has been confirmed by a recent study that used data of the 1000 genome project[70]. The p.Val197Met missense variant that impacts the TMPRSS2 protein stability demonstrated a decreasing allele frequency among the severe patients compared to the higher frequency in the asymptomatic and mild groups. This variant is associated with valine to methionine alteration at the 197th amino acid (p.Val197Met). This results in a decrease in the TMPRSS2 protein stability and ACE2 binding[70]. Moreover, p.Val197Met was previously found to exhibit greater allele frequency in East Asians (0.31–0.41) and Finnish (0.36) but not in South Asians (0.14–0.29) and Europeans (0.17–0.23)[71]. The study of Chinese patients has shown a reduced allele frequency of the p.Val197Met missense variant. That variant affects the stability of the TMPRSS2 protein in the severely infected compared to the mildly infected patients and the general population [42]. The localization of the *TMPRSS2* gene on 21q22.3 suggests that people with Down syndrome are more prone to COVID-19 infection[30].

A recently published study from Italy has identified a number of ACE2 variants with a potential effect on the spike protein stability[72]. Three missense changes may interfere with the protein structure and stabilization, p.(Asn720Asp), p.(Lys26Arg), and p.(Gly211Arg). Two rare variants, p.(Leu351Val) and p.(Pro389His), affect the binding and entry of the spike of SARS-CoV-2[40]. Exome sequencing of COVID-19 patients from Italy for genetic variants of *TMPRSS2, PCSK3, DPP4,* and *BSG* genes identified 17 variants[73].

The X-chromosomal toll-like receptor (TLR7)

TLRs are highly conserved from Drosophila to humans. They mediate the production of cytokines that are necessary for the development of effective immunity. The various TLRs exhibit different patterns of expression, TLR7/8 can identify the single-stranded RNA ssRNA of the virus. The immunoinformatic approach revealed that the SARS-CoV-2 genome has more single-stranded RNA fragments that could be recognized by TLR7/8. These findings suggest the innate immune hyperactivation by SARS-CoV-2 and the possibility to provoke a strong proinflammatory response *via* TLR7/8 recognition and to cause severe lung injury, as well[74].

By whole-exome sequencing of the patients and family members in the Netherlands, there have been identified loss-of-function variants of the *TLR7* gene in X–chromosome (Xp22.2) associated with impaired interferon type I and II responses. The first family possessed a 4-nucleotide deletion [c.2129_2132del; p.(Gln710Argfs*18)], which was maternally inherited; and in the affected members of the second family, a

missense variant [c.2383G>T; p.(Val795Phe)] in TLR7 was observed. Thus, TLR7 seems to be an essential component of the innate immune response against SARS-CoV-2[29,75-77]. The study has also provided an explanation for the higher fatalities from COVID-19 in men than in women. Several immune-related genes have been found in the X chromosome. The males are hemizygotes on the X chromosome that they inherit from their mothers. Therefore, any abnormality in the X chromosome genes is more likely to be expressed phenotypically and have more pronounced immunological consequences. Females carry both a maternal and a paternal X chromosome, and due to X chromosome inactivation, they are functional mosaics for X-linked genes[77-79]. Loss-of-function mutation in the *TLR7* gene gives evidence that genetic errors in interferon (IFN)-I and II pathways contribute to severe COVID-19.

Apolipoprotein E (ApoE)

ApoE is synthesized in brain astrocytes, adipocytes, hepatocytes, and arterial wall macrophages. For their role in lipid transport, ApoE is critical for brain, immune, and vascular functions[80-83].

Dementia, cardiovascular disease, and type 2 diabetes were identified as major risk factors for severe COVID-19 in older individuals in the United Kingdom[84-86].

The *APOE* gene, with its three major isoforms APOE2, APOE3, and APOE4, is encoded by ε2, ε3, and ε4 alleles. The ApoE ε4 genotype is associated with dementia and delirium[85], and the ε4ε4 homozygous genotype are at a 14-fold increased risk of Alzheimer's disease[86].

Using the United Kingdom Biobank data, associations between ApoE ε4 alleles and COVID-19 severity have been found. ApoE homozygotes have a 2.2-fold higher risk for severe COVID-19, independently of major risk factors, and 4.3-fold higher case-fatality after COVID-19 than ApoE ε3 homozygotes[84,85]. The heterozygotes (ε3/ε4) are at lower risk.

If the ApoE ε4 allele has an influence on COVID-19 severity, this may explain the prevalence of severe disease amongst certain ethnicities. According to a study, the allele frequency was 29.5% for homozygous individuals *vs* 12.1% for the Caucasian group[87]. Furthermore, till April 2020, 34% of the COVID-19 deaths in the United States occurred amongst homozygotes, despite the population representing only 13% of all Americans[88]. ApoE ε4 may have multiple effects in COVID-19, which may also be reflected in ethnicity.

Interferon-induced transmembrane protein 3 (IFITM3)

Five IFITM genes (interferon-induced transmembrane proteins) have been identified in humans, *IFITM1*, *IFITM2*, and *IFITM3*, as well as *IFITM5* and *IFITM10* with unknown immunity role[89]. Interferon-induced transmembrane proteins are a family of small proteins that are localized in the plasma and endolysosomal membranes. They inhibit viral entry into the host cells and reduce the production of infectious virions. Many SNPs have been identified in these genes, some of which have been associated with the severity of the viral infection.

IFITM3 gene variants have been related to distinctive clinical responses to viruses like influenza A (H1N1) virus, Marburg virus, Ebola virus, West Nile virus, human immunodeficiency virus type 1, vesicular stomatitis virus, and dengue virus[42-48]. A human IFITM3 SNP rs12252 C/T was associated with the severity of avian influenza and severe illness with influenza H1N1/09. The IFITM3 rs12252 has also been associated with the progression of human immunodeficiency virus type 1 infection [90]. Two polymorphisms have been found to have an association with a severe COVID-19, rs12252-C and rs34481144-A. The SNP rs12252-C/C in the gene *IFITM3* was detected for the first time in a mild-to-moderate COVID-19 patient from Wuhan, China that required hospitalization but eventually recovered[91]. However, this SNP's prevalence was found to be 26.5% in the Chinese population[92]. The results have shown an association between IFTM3 rs12252 polymorphism and the risk of COVID-19 and patient hospitalization[93,94].

Recently, the *IFITM3* gene rs12252 has been associated with the severity of COVID-19 in a cohort of 80 patients admitted to Beijing Youan Hospital[55,56]. Patients were classified as mild and severe, and CC-homozygotes were among the severe cases. The rs12252 C frequency was significantly higher among Chinese compared to individuals of European ancestry. Another study was conducted to determine the link between IFITM3 rs12252 and the risk of developing severe COVID-19 in a Spanish cohort[93].

The significance of the IFITM3 rs12252-C polymorphism for severe COVID-19 seems to be population-dependent. The second IFITM3 SNP, rs34481144-A, was not reported to influence the severity of COVID-19 in humans.

Cathepsin B/Cathepsin L

SARS-CoV-2 uses ACE2 as an entry receptor[95], and TMPRSS2 for the spike protein priming[54]. ARS-CoV-2 could also use cathepsin B (CTSB) or cathepsin L (CTSL) entering TMPRSS2-negative cells[96].

Three variants in the active sites for CTSB (two missense variants and one synonymous variant) and one missense variant for CTSL were found. Although all missense variants on active sites of CTSB/L are associated with severe disease, their allele frequency (AF) was very low (AF < 0.01%). CTSB has 429 nonsynonymous variants including 51 loss-of-function variants (all with AF < 0.01%). CTSL has 211 nonsynonymous variants including 17 loss-of-function variants[97].

Cardiac damage related to SARS-CoV-2 has been attracting more and more attention. The mechanism of cardiovascular injury caused by COVID-19 has not been fully elucidated yet[98].

The increase in the ACE2 and CTSL expression levels creates a favorable condition for the SARS-CoV-2 to invade the heart, and these patients may experience severe cardiac injury. In addition, cytokine storm in severe COVID-19 can aggravate the myocardial damage[99,100].

Piezo-type mechanosensitive ion channel component 1 (PIEZO1)

There is evidence that membrane proteins such as ACE2 and TMPRSS2 are important in SARS-CoV-2 entry[54,101]. It is indisputable that viral entry is affected by other membrane proteins and lipids[102,103].

Membrane proteins are ion channels[104,105] embedded in the membrane. They allow transmembrane flux of ions such as Ca^{2+}, an ion that fulfills regulatory functions in coronaviral mechanisms[106,107].

PIEZO1 gene encodes a non-selective cation channel that mediates endothelial responses to blood flow. It forms Ca^{2+}-permeable non-selective cation channels with the capability to respond to membrane tension caused by fluid flow along the endothelial membrane surface[108]. PIEZO1 indents the membrane in an inverted dome-like fashion and therefore modifies the overall structure of the membrane[109]. There is increasing evidence of its roles in many aspects of endothelial function, such as angiogenesis[100] and pulmonary vascular permeability. It also regulates IL-6, which is a key inflammatory mediator of COVID-19[110].

The genome associate analysis suggests three missense PIEZO1 SNPs (rs7184427, rs6500495, and rs7404939) associated with COVID-19 fatality independently of the risk factors. All of them affect amino acids in the proximal N-terminus of PIEZO1. Human PIEZO1 comprises 2521 amino acids in total, and rs6500495 affects position 83, rs7404939 position 152, and rs7184427 position 250. rs6500495 encodes a switch at position 83 from the reference isoleucine to threonine; rs7404939 encodes the reference proline rather than leucine at position 152, and rs7184427 encodes alanine rather than the reference valine at position 250.

A genome sequence analysis showed that these SNPs vary in prevalence with ethnicity and that the most significant SNP (rs7184427) varies between 65% to 90%. The analysis also suggests that rs7184427 affects a residue that is highly evolutionarily conserved and therefore has functional importance for COVID-19 severity and fatality [101].

Interferon-α/β receptor (IFNAR), tyrosine kinase 2 (TYK2), Oligoadenylate synthetase 1 (OAS1), dipeptidyl peptidase 9 (DPP9), and CC chemokine receptor 2 (CCR2)

Recently, the Genetics of Mortality in Critical Care (GenOMICC, https://genomicc.org/) GWAS, which involved 2244 COVID-19 critically ill patients in the United Kingdom intensive care units, has reported robust genetic predisposition related to essential antiviral host defense and inflammatory mediators, associated with severe COVID-19 inflammatory organ damage[24]. It has shown that the low expression of IFNAR2 or the high expression of TYK2 was related to life-threatening illness. In addition, the high expression of the monocyte-macrophage chemotactic receptor CCR2 correlates with extreme COVID-19 viral spread in the lung tissue.

The GenOMICC study has also revealed that hospitalized COVID-19 patients were affected by alterations in two biological mechanisms: Innate antiviral defenses and host-driven inflammatory lung injury. In the early disease, IFNAR2 and interferon-inducible OAS gene cluster (*OAS1, OAS2, OAS3*) have been considered critical, whereas in the late and life-treating disease, the most important are DPP9, TYK2, and CCR2[24,111].

It is well-established that interferons are essential during viral infection; thus, the increased IFNAR2 interferon expression decreases the chances of serious COVID-19

[111]. Since the *IFNAR2* gene has a protective role for severe COVID-19, it was shown that rare loss-of-function mutations in IFNAR2 were related to severe disease and many other viral diseases[112]. One can speculate that interferon administration may reduce the probability of critical COVID-19. However, this was not confirmed by the studies[113]. Furthermore, IFN deficiency, in particular IFN-I, was documented during SARS-CoV-2 infection. These deficiencies can occur by inherited mutations in the genes encoding key antiviral molecules or by producing antibodies that bind and 'neutralize' IFN-I[114]. The latter is mostly seen in severe COVID-19 patients[115]. Zhang *et al*[116] reported that life-threatening COVID-19 pneumonia was observed in people with mutations in genes previously associated with severe influenza. Mice with defective IFN-I pathway are more likely to die of influenza due to disproportionate inflammasome activation, not just because of high levels of viral replication. Probably, this may explain severe COVID-19 cases if IFN deficiency is presented. These genes that belong to the TLR3 and IFN-I signaling pathways were altered in 3.5% of the tested individuals, resulting in the incapability of producing or responding to IFN-I. Another study by Bastard *et al*[117] showed that a form of autoimmunity may contribute to viral infection susceptibility, such as autoantibodies to IFNs. People with autoimmune polyglandular syndrome type 1 were reported to developed severe COVID-19 pneumonia.

Anti-IFN-I autoantibodies have been found in various diseases. However, the underlying mechanisms for severe COVID-19 include uncontrolled viral replication and spread but also disruption of immune system function as suppression of inflammasome or enhanced cytokines production[118-120]. Regarding the gene cluster encoding antiviral restriction enzyme activators (OAS), they encode enzymes, producing a host antiviral mediator [2′,5′-oligoadenylate (2-5A)]. The latter activates an effector enzyme RNase L which degrades double-stranded RNA[121]. Vietnamese and Chinese studies documented the OAS1 variants role in SARS-CoV susceptibility[122, 123]. Variants in chromosome 19p13.3 (rs2109069) that encodes DPP9 were clinically related to pulmonary fibrosis. DPP9 encodes a serine protease with important immune functions such as antigen presentation and inflammasome activation as well as cleavage of CXCL (a key antiviral signaling mediator)[124].

The association between TYK2, CXCR6, CCR2, and CCR3 expression and severe COVID-19 was also demonstrated[24].

CCR2 for monocyte chemoattractant protein-1 is expressed strongly in the lung tissues, promoting chemotaxis of monocytes and macrophages towards sites of inflammation. In critical COVID-19 patients on mechanical ventilation, CCR2 is overexpressed and detectable in bronchoalveolar lavage fluid samples[125]. Moreover, circulating monocyte chemoattractant protein-1 amounts are related to a more serious disease[126].

Data on the candidate genes associated with severe COVID-19 are summarized in Table 1.

TRENDS IN THERAPEUTIC STRATEGIES AND THE GENETIC FACTORS SIGNIFICANCE

In serious COVID-19, it is the lung inflammation that mainly leads to fatal outcomes. This is why many efforts were given to identify the possible host genetic variants associated with critical illness[127]. Evidence has shown that hospitalized patients differed significantly from those with mild or moderate diseases. Many distinct disorder phenotypes occur with different symptom patterns. Furthermore, they exhibit different responses to immunosuppressive treatment[114].

Some experts suggest that corticosteroid therapy is detrimental in patients with non-respiratory failure, although there are major benefits in patients with critical respiratory failure[113]. Hence, it is considered that different pathophysiologic mechanisms contribute to critical COVID-19 cases with respiratory failure.

Based on the possible genetic alterations harbored by the critically ill COVID-19 patients, some trends were observed regarding the treatment options. For example, individuals with IFN-I genetic mutations would benefit from interferon treatment, but such therapy would not be of any advantage to people who have IFNAR encoding gene mutations. Moreover, whether patients have IFN neutralizing antibodies, therapies such as IFN-β or IFN-α in early infection may also be beneficial[115].

The *OAS* genes are also a potential therapeutic target. Inhibitors of endogenous phosphodiesterase 12 were shown to augment OAS-mediated antiviral activity[128]. In line with this, TYK2 is one of the targets for janus kinase inhibitors (*i.e.*, baricitinib),

Table 1 Summary of reported genome wide association studies between human genes and severe coronavirus disease 2019

Gene(s)	Polymorphism(s) and genotypes	Chromosome location	Reported COVID-19 associations	Ref.
SLC6A20, LZFTL1, CCR9, CXCR6, XCR1, and FYCO1	Rs11385942-GA	3p21.31	Severe disease (respiratory problems)	[28]
ABO	rs657152	9q34.2	Higher risk of infection in blood group A and a protective effect in blood group O as compared with other blood groups	[37, 38]
HLA	a/HLA-B*15:03 and HLA-B*46:01; b/HLA-DBR*15:01 HLA-DQB*06:02 and HLA-B*27:07; c/ HLA-A*11:01, HLA-B*51:01 and HLA-C*14:02	6p21.33	Vulnerable to COVID-19 for HLA-B*46:01 and protective T-cell immunity for HLA-B*15:03 may predispose to a less favorable outcome and severe COVID-19; Preliminary results in the worst clinical outcome in China patients	[41]
TMEM189-UBE2V1	rs6020289-A	20q13.13	Severe disease	[42]
ACE2	p.Arg514-Gly	Xp22.2		[30]
TMPRSS2	p.Val160Met (rs12329760)	21q22.3	Severe disease, vulnerable to COVID-19 with risk factors	[29, 30]
TLR7	g.12905756_12905759del and g.12906010G>T	Xp22.2	Severe disease	[29]
ApoE	rs429358-C-C (e4e4)	19q13.32	Severe disease especially with dementia, cardiovascular disease and type 2 diabetes	[84, 85]
IFITM3	rs12252-C/C	11p15.5	Mild to moderate disease (with hospitalization)	[91, 94]
CTSB, CTSL		8p23.1, 9q21.33	Low frequencies; severe disease with cardiovascular conditions	[97, 100]
PIEZO	rs7184427, rs6500495 and rs7404939	16q24.3	Severe COVID-19 and fatality, independently of the risk factors	[101]
OAS1, OAS2 and *OAS3*	rs10735079	12q24.13	Severe COVID-19 and critical illness	[24]
TYK2	rs2109069	19p13.2	Critical illness	[24]
DPP9	rs2109069	19p13.3	Severe COVID-19; Idiopathic pulmonary fibrosis	[24]
IFNAR2	rs2236757	21q22.1	Severe COVID-19 and other viral diseases	[24]

COVID-19: Coronavirus disease 2019.

and anti-CCR2 has also shown safety for other diseases, such as rheumatoid disease. However, all these therapies could be called only experimental[129].

Immunosuppressive agents prescribed to patients with autoimmune diseases might have a beneficial effect on the COVID-19 course in these patients by reducing the risk of cytokine storm. Although we have made detailed literature research, sufficient evidence was not found.

Notwithstanding, the continuous search for appropriate therapy insists on further studies on the genetic factors, their contribution to severe COVID-19, as well as their potential role in the invention of effective treatment.

CONCLUSION

GWAS contributes to understanding the genetic basis of COVID-19 and potential associations between the virus infection severity and specific gene loci. The global aim is to elucidate the molecular mechanisms and the optimizing of prevention and treatment of SARS-CoV-2 infection. In the last year, research on polymorphic variants or in proximity to the candidate genes has shown a strong, statistically significant association with the severity of the disease. Further study of genes and genetic variants will be of great benefit for the prevention and individual risk assessment and disease severity in different populations. These scientific data will serve as a basis for the development of clinically applicable diagnostic and prognostic tests for patients at

high risk of COVID-19.

However, GWAS has some limitations. The present data may not be fully comprehensive, as well as genotype-phenotype elaboration and corrections cannot be made for all conceivable causes of bias (*e.g.*, cardiovascular and metabolic underlying factors contributing to COVID-19). Further studies regarding the genetic data are warranted, both in terms of their utility for the therapeutic risk profiling of COVID-19 patients and in terms of avoiding the mechanical knowledge of infection pathophysiology.

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MINIREVIEWS

Exploiting epidemiological data to understand the epidemiology and factors that influence COVID-19 pandemic in Libya

Abdusalam S Mahmoud, Abdunaser S Dayhum, Abdunnabi A Rayes, Badereddin B Annajar, Ibrahim M Eldaghayes

ORCID number: Abdusalam S Mahmoud 0000-0002-2182-1647; Abdunaser S Dayhum 0000-0002-3488-5519; Abdunnabi A Rayes 0000-0001-9647-7361; Badereddin B Annajar 0000-0001-5585-7309; Ibrahim M Eldaghayes 0000-0002-1750-1448.

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Abdusalam S Mahmoud, Abdunaser S Dayhum, Department of Preventive Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli 13662, Libya

Abdunnabi A Rayes, Department of Internal Medicine, Faculty of Medicine, University of Tripoli, Tripoli 13662, Libya

Badereddin B Annajar, Department of Public Health, Faculty of Medical Technology, University of Tripoli, Tripoli 13662, Libya

Badereddin B Annajar, National Center for Disease Control, Tripoli 71171, Libya

Ibrahim M Eldaghayes, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli 13662, Libya

Corresponding author: Ibrahim M Eldaghayes, PhD, Professor, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Sidi Almasri Street, Tripoli 13662, Libya. ibrahim.eldaghayes@vetmed.edu.ly

Abstract

There were only 75 confirmed cases of coronavirus disease 2019 (COVID-19) reported in Libya by the National Center for Disease Control during the first two months following the first confirmed case on 24 March 2020. However, there was dramatic increase in positive cases from June to now; as of 19 November 2020, approximately 357940 samples have been tested by reverse transcription polymerase chain reaction, and the results have revealed a total number of 76808 confirmed cases, 47587 recovered cases and 1068 deaths. The case fatality ratio was estimated to be 1.40%, and the mortality rate was estimated to be 15.90 in 100000 people. The epidemiological situation markedly changed from mid-July to the beginning of August, and the country proceeded to the cluster phase. COVID-19 has spread in almost all Libyan cities, and this reflects the high transmission rate of the virus at the regional level with the highest positivity rates, at an average of 14.54%. Apparently, there is an underestimation of the actual number of COVID-19 cases due to the low testing capacity. Consequently, the Libyan health authority needs to initiate a large-scale case-screening process and enforce testing capacities and contact testing within the time frame, which is not an easy task. Advisably, the Libyan health authority should improve the public health capacities and conduct strict hygienic measures among the societies and vaccinate as many people against COVID-19 to minimize both the case fatality ratio and

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socio-economic impacts of the pandemic in Libya.

Key Words: COVID-19; Pandemic; Epidemiological patterns; Potential factors; Prevalence; Libya

Core Tip: This review is aimed to explain and show potential reasons for having only 75 confirmed cases of coronavirus disease 2019 (COVID-19) reported in Libya during the first two months following the first confirmed case till hundreds of positive cases everyday in the following months. The epidemiological situation markedly changed from mid-July to the beginning of August as the country proceeded to the cluster phase and COVID-19 has spread in almost all Libyan cities. The Libyan health authority needs to improve its service in order to do better job to control the pandemic and reduce the virus spread within the country.

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INTRODUCTION

In late December 2019, the etiologic agent responsible for the epidemic outbreak emerged in Wuhan, China, where about 27 cases of acute respiratory pneumonia was reported by the Wuhan Municipal Health Commission[1,2]. The first spread was reported in the Huanan Seafood Wholesale Market, an area that is well known for selling live animals[3]. On 9 January 2020, Chinese investigators were able to isolate and obtain the genetic sequence of the virus in a short period of time, which led to the preliminary identification of this novel virus[3,4]. Later, the disease was diagnosed as coronavirus and named 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2). According to serological and phylogenetic analyses, coronaviruses are divided into four genera, named Alpha-, Beta-, Gamma- and Delta-coronavirus[5]. SARS-CoV-2 is a β coronavirus of group 2B with at least 70% similarity in its genetic sequence to SARS-CoV[6-8]. SARS-CoV-2 is the seventh member of the family of coronaviruses that infect humans[7]. Despite the coronavirus having been reported in China in late December 2019 and the first sporadic case reported outside China on 13 January 2020, it was not until 30 January 2020 that the World Health Organization (WHO) declared the outbreak as a public health emergency of international concern. In fact, it took a long time for WHO to then announce the coronavirus outbreak as a pandemic on 11 March 2020[9]. Since then, the coronavirus disease 2019 (COVID-19) has spread and struck many countries with a high case fatality rate while others with a moderate to low case fatality rate. Significantly, variations were highly considered and needed to be explained further and clarified. The epidemiological patterns of COVID-19 are unique all over the world, characterized by a highly pathogenic index and strong socio-economic impacts. However, there is a clear variation with respect to the temporal and spatial distribution of COVID-19 among different countries at the same regional level. These variations may be due to factors that influence the distribution of the disease in the populations, many of which remain unknown[10].

Still, there is a little knowledge about the epidemiology and course of COVID-19 in Libya. Therefore, in this review, we have explored the relevant data to understand the epidemiological patterns of COVID-19 in Libya.

EPIDEMIOLOGICAL PATTERNS IN LIBYA

The first confirmed case of COVID-19 in Libya was reported on 24 March 2020; the affected was a man in Tripoli who had a history of travelling to Saudi Arabia[11]. Since

then, many infected cases were reported with mild clinical signs. It is well known that COVID-19 seems to affect some people more critically than others — some people experience only mild symptoms while others end up hospitalized, requiring intensive care and ventilation[12-14]. During the first two months following the first confirmed case, the epidemic curve was flattened with only 75 confirmed cases[15]. In fact, the low number of reported cases during the months of March, April and May could be attributed to the various reasons provided in the study of Rayes *et al*[15].

The first confirmed case in Libya was on 24 March 2020. The next day, there were huge differences between the total number of confirmed COVID-19 cases reported in Libya and those of the neighbouring Arab countries (Figure 1).

The epidemic curve of COVID-19 in Libya could have been influenced by the travellers returning from different countries (Figure 2).

The distribution of the number of daily cases, total cases and deaths of SARS-CoV-2 reported between 24 March and 22 April 2020, *i.e.*, over a 30-d period, with about 1181 samples screened by reverse transcription polymerase chain reaction, revealed 59, 1 and 15 confirmed cases, deaths, and recovered cases respectively (Figure 3). However, the epidemiological situations of the neighbouring countries were highly variable and significant in comparison to the confirmed cases reported in Libya (Figure 1).

The first confirmed case of COVID-19 in Egypt was on 14 February 2020, in Algeria on 25 February 2020, in Morocco on 2 March 2020, in Tunisia on 4 March 2020 and in Libya on 24 March 2020; however, the disease pattern was different for each country [16].

In Libya, there were 9 and 13 confirmed cases of COVID-19 reported on 14 and 15 April respectively, clearly indicating the presence of asymptomatically underestimated active cases before this time[17,18]. Consequently, on 16 April 2020, following the recommendation of the Scientific Advisory Committee (SAC), the Libyan authorities imposed a complete lockdown for one week, starting from 17 April 2020. Indeed, the early lockdown and various precautionary measures that were taken by Libyan authorities were highly significant in preventing the transmission of the virus among the populations.

Several precautionary measures have been implemented by Libyan authorities following the recommendation of SAC, including the closing of schools, cancelling of all festivals, closing of airports, and lockdown of most commercial private industrial units. These precautionary measures were taken for COVID-19 control and prevention, as recommended by WHO, to reduce the exposure and transmission of virus infection among the population.

Thus, the country attempted to prevent the spread of the infection and minimize the risk of virus transmission. Further, the political instability of the country and civilian war indirectly impacted the prevention of virus transmission at the beginning of COVID-19 in Libya.

Despite the precautionary measures taken to minimize the possibility of transmission of the virus from travellers coming from infected countries, there were many confirmed cases of SARS-CoV-2 among those who returned to Libya. These travellers belong to different regions of the country, which resulted in a change in the epidemiological situation of the disease and led to an increase in the number of cases recorded in different cities.

The first batch of returning flights to Libya was on 5 May 2020, during which time the epidemiological situation of COVID-19 in Libya was stable, and the number of confirmed cases started to increase by the end of May (Figure 4).

COVID-19 IN THE SOUTH OF LIBYA

On 26 May 2020, two cases (36-year-old male and 55-year-old female) of COVID-19 were reported for the first time in the southern region of the country (Sabha province). These cases might be linked with the history of the travellers returning from countries highly affected by COVID-19. However, according to the National Center for Disease Control (NCDC), the COVID-19 cases that were reported in Sabha were found to have resulted from contact with a woman who had been suffering from respiratory symptoms and died on 26 May 2020. In fact, there are two potential pathways for the entrance of disease to the south of Libya: first, the return of travellers from infected countries; and second, from asymptomatically infected immigrants crossing the southern Libyan border from the neighbouring countries. It took only one week, *i.e.*, from the end of May to the beginning of June, for southern Libya to report 80 new confirmed cases of COVID-19. Therefore, the epidemiological patterns of COVID-19 in

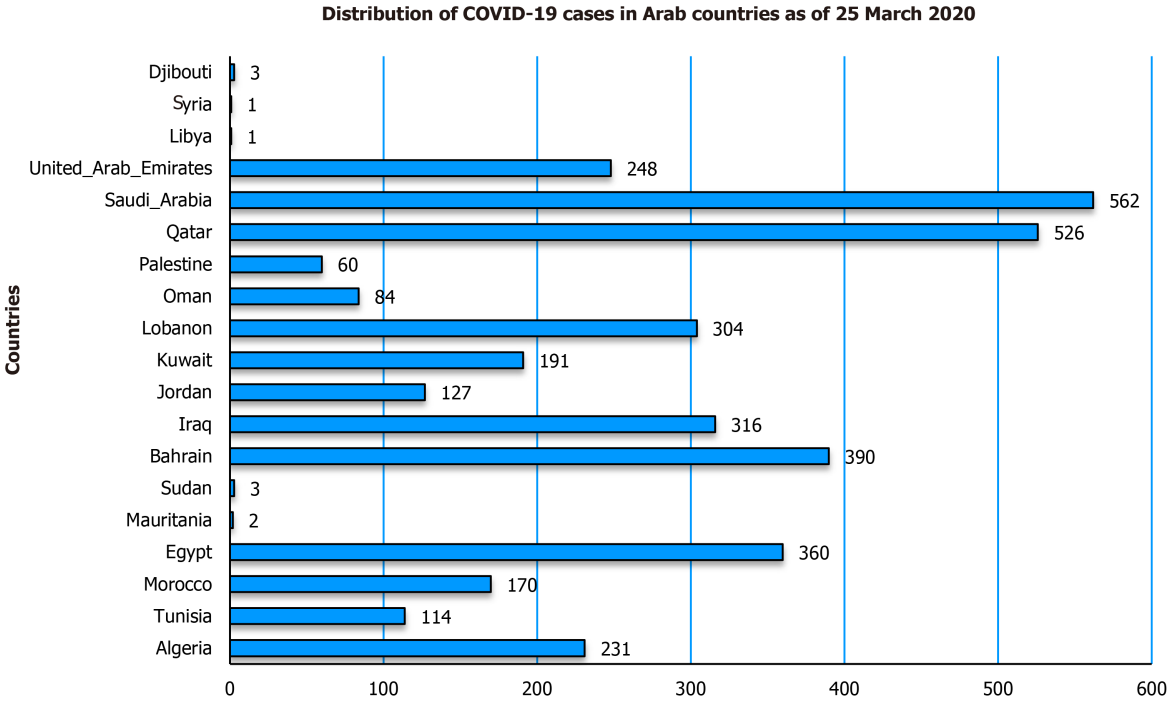

Figure 1 Distribution of coronavirus disease 2019 cases in Arab Countries as of 25 March 2020. COVID-19: Coronavirus disease 2019.

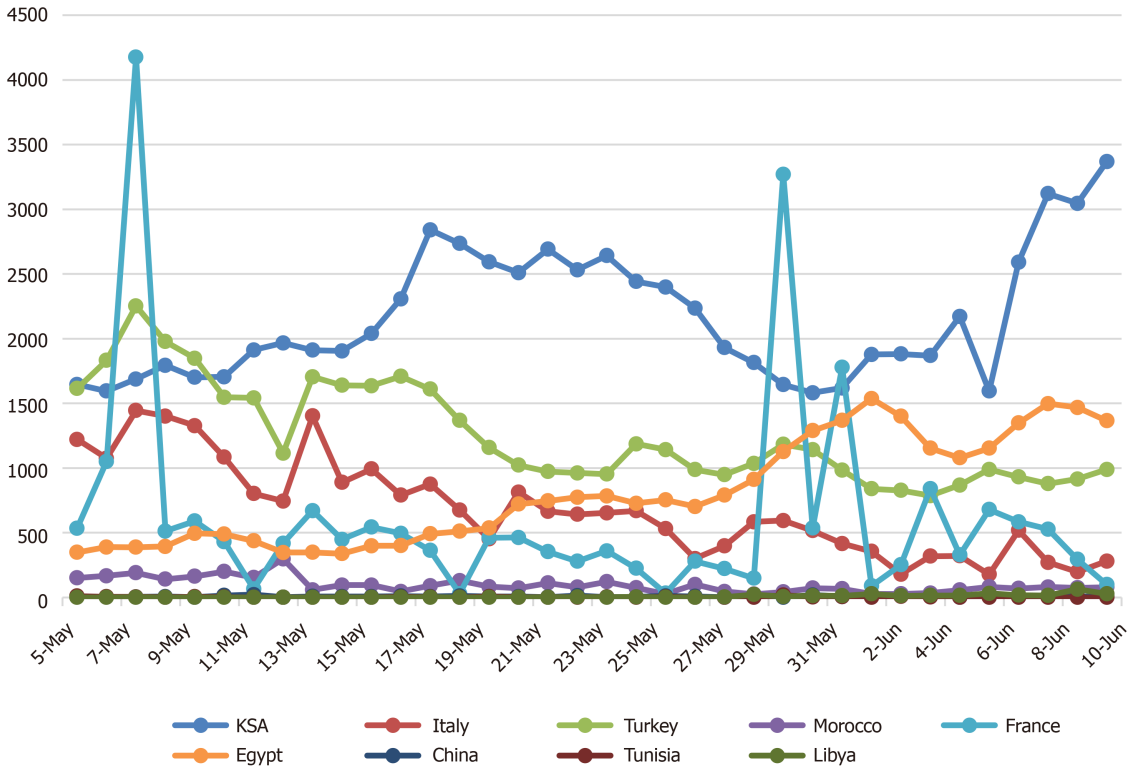

Figure 2 Distribution of coronavirus disease 2019 cases as of 5 May 2020 (in some countries with a history of being linked with Libyan travellers).

southern Libya, especially in the Sabha province, were totally different from the Tripoli area and the rest of the Libyan region. For the given period, the WHO published an estimation of R0 to be 1.4-2.5 in the southern region (Sabha), which was higher than that of Tripoli. Expectedly, a high number of COVID-19 confirmed cases reported in the southern region were attributed to multifactorial determinant causes correlated to the social lifestyle of the people in the area, which included unrestricted

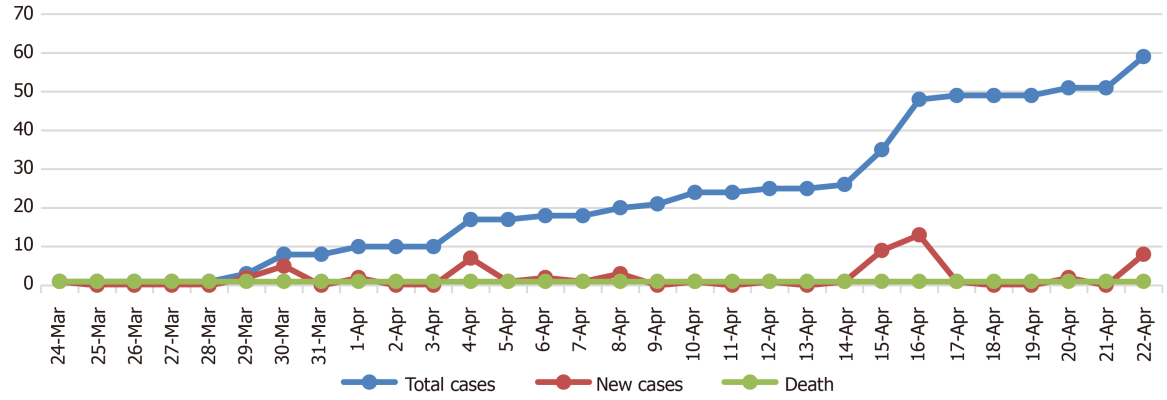

Figure 3 Distribution of coronavirus disease 2019 total cases, new cases and deaths from 24 March to April 2020.

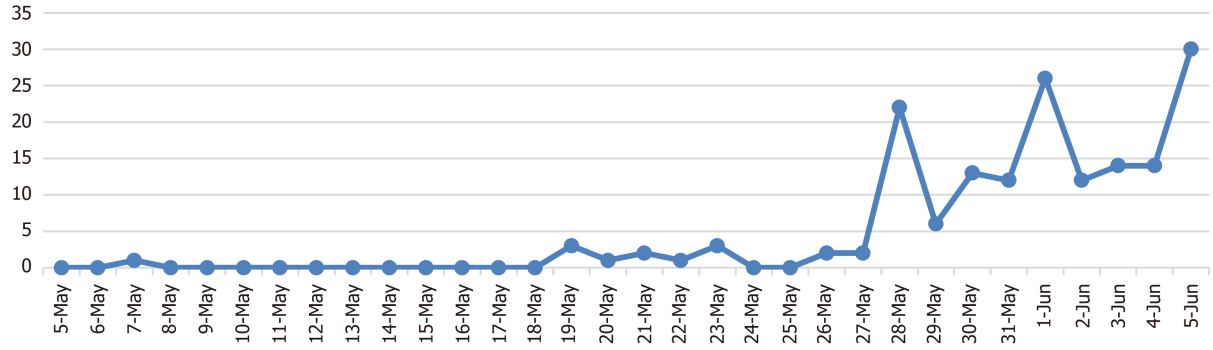

Figure 4 Daily reported positive cases of coronavirus disease 2019 in Libya as of 05 May 2020.

transitional movements between small villages and cities of the south. Additionally, weak quarantine measures that led to the easy movement of people from known infected areas to others without control from the authorities was another contributing factor. Consequently, on 28 May 2020, the Libyan authorities decided to block all the administrative borders of Sabha city and impose a lockdown and curfew within the city for seven days.

The return of Libyan travellers from high-risk areas was considered the principal factor for the entrance of COVID-19 into the southern region, despite the measures taken by the Libyan authorities to minimize the likelihood of the virus' entrance into the country. Over the previous years, various transboundary viral diseases of public health and socio-economic importance, including the rift valley fever, were reported in the southern region of the country[19,20]. Further, despite the period of pre-quarantine measures and the quarantine throughout the pandemic, there was uncontrolled transportation between the cities and within the cities of the country, which potentially influenced the positive test rate of COVID-19. According to the CDC, Libya, the individuals who were COVID-19 positive, as reported in different Libyan cities, had a history of traveling to the southern region. At the beginning of June 2020, 30 of the 62 confirmed cases of COVID-19 were linked to people with a history of traveling to infected countries.

CLUSTER PHASE

Predictably, the epidemiological situation markedly changed from mid-July to the beginning of August, and the country proceeded to the cluster phase. There was an increase in the testing capacities by mid-July; consequently, the positive test rate increased as well. The average daily positivity rate from mid-July to August was estimated to be 14.54% (Figure 5A and B), while throughout September this rate was

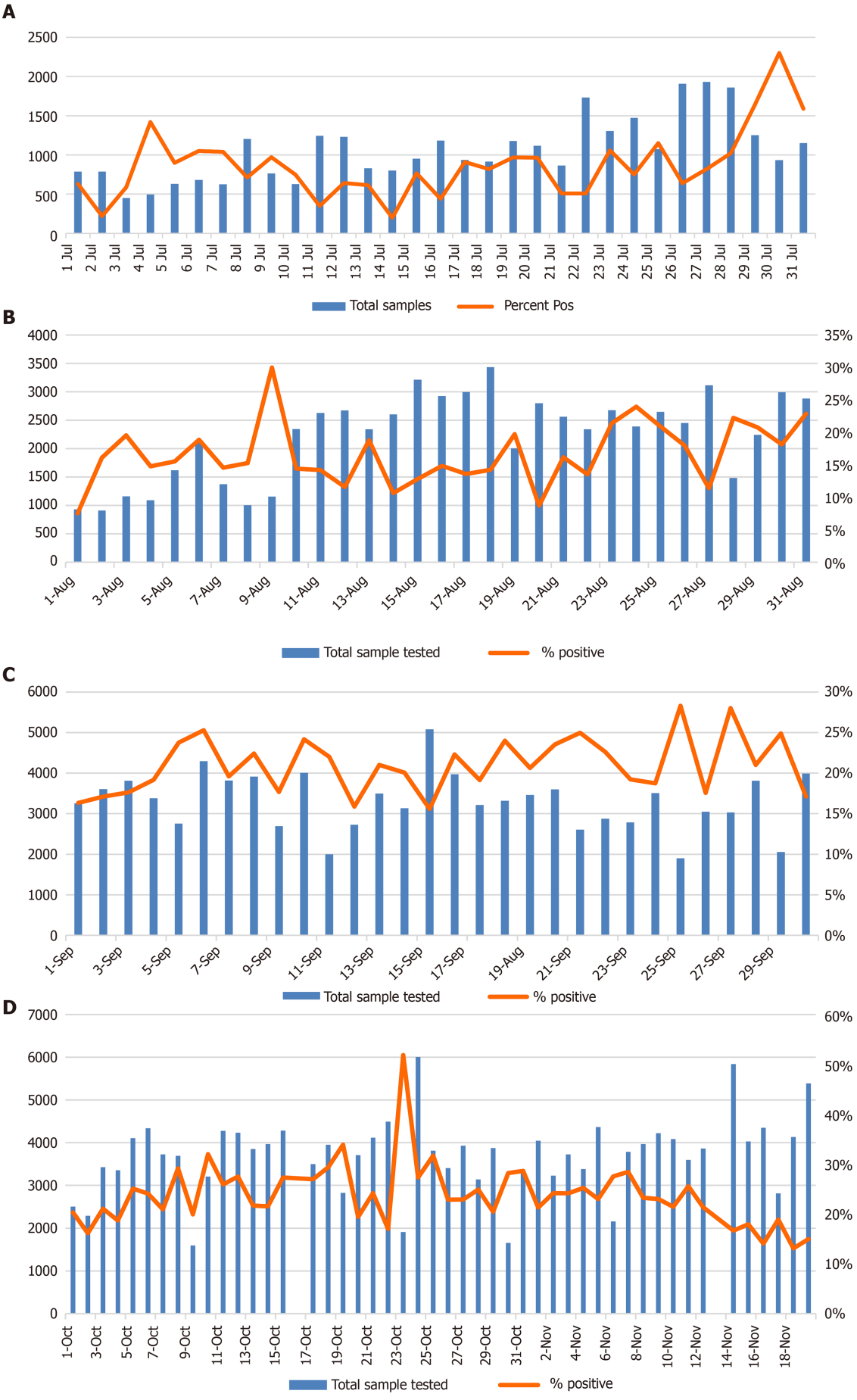

Figure 5 Percent of positive coronavirus disease 2019 cases correlated to the total of samples tested in Libya. A: During July 2020; B: During

August 2020; C: During September 2020; D: During October and until 19 November 2020.

estimated to be 21% (Figure 5C). The average positivity rate from October to 19 November 2020 was estimated to be 23.84% (Figure 5D).

COVID-19 SITUATION IN CITIES

COVID-19 prevalence has been reported in many cities of the country, and this reflects the high transmission rate of the virus at the regional level (Figure 6). However, a significant difference in the prevalence rate of COVID-19 has been found between the cities. This variation might be attributable to the following factors: (1) The number of samples tested per day; (2) Population density in each city; (3) Different activities for different cities; and (4) Different cultural and social lifestyles in each city. The highest positivity rates were estimated to be 44.09%, 36.56%, 23.05%, 22.23%, 18.50% and 16.14% in the cities of Surman, Alzintan, Sabratha, Zliten, Sabha and Misrata respectively, while the lowest positivity rates were estimated to be 11.05%, 11.39%, 7.90% and 4.90% in Zawiya, Nalut, Tripoli and Benghazi respectively. The aforementioned rates in Libyan cities have not been constant and have changed every month. Indeed, the high average positivity rates in Libya from September to mid-November (21%; 23.84%) constitute another indicator of the high transmission rate among the population (Figure 5C and D). According to the WHO's recommendation, the capacity for testing should be increased, and the positivity rate should remain below 10%. A positivity rate of less than 5% is recommended before the reopening of schools and businesses. According to the CDC, Libya, 76808 confirmed cases, 28153 active cases, 47587 recovered cases and 1068 deaths have been announced as of 19 November 2020, while the case fatality ratio (CFR) was estimated to be 1.40%; and as of 19 November 2020, Libya has a COVID-19 mortality rate of 15.90 deaths/100000 people.

THE POTENTIAL FACTORS THAT INFLUENCE THE COVID-19 COURSE IN LIBYA

The epidemiological situation of COVID-19 in Libya may be influenced by the following potential risk factors: the government's level of transparency, prevention and control measures, population density, susceptibility of the population, age structure, *etc.* These factors contribute to and potentially influence the course of the disease in the country, and they might be variable in different environments.

In general, if any government has low or a complete lack of transparency, it will have a negative impact on the success of any strategy to combat or confront the pandemic. However, if the government prioritizes transparency, it would prompt trust and sentiments of solidarity and belief among the citizens. It is difficult to build up trust between the government and citizens. Therefore, all the governmental authorities must work hard in collaboration to improve their communications and make all the relevant information available. It is clear that transparency and the sharing of information among the authorities are of great importance to the success of the prevention and control strategies of COVID-19. Indeed, misleading and false information as well as a shortage of data about the epidemiological situation in the country could lead to the wrong decision by the government with regard to the implementation of strategies for the prevention and control of COVID-19.

THE NATIONAL STRATEGY FOR THE PREVENTION AND CONTROL OF THE COVID-19 EPIDEMIC

The strategy for the prevention and control of the epidemic must be well designed and established according to the situation of the country and epidemiological patterns of the disease in the country so that it can be linked to and complemented by the data collected; therefore, this strategy reflects the real situation of the epidemic. The Libyan strategy for prevention and control of COVID-19 was performed and implemented according to the WHO recommendation criteria[21]. The Libyan Ministry of Health

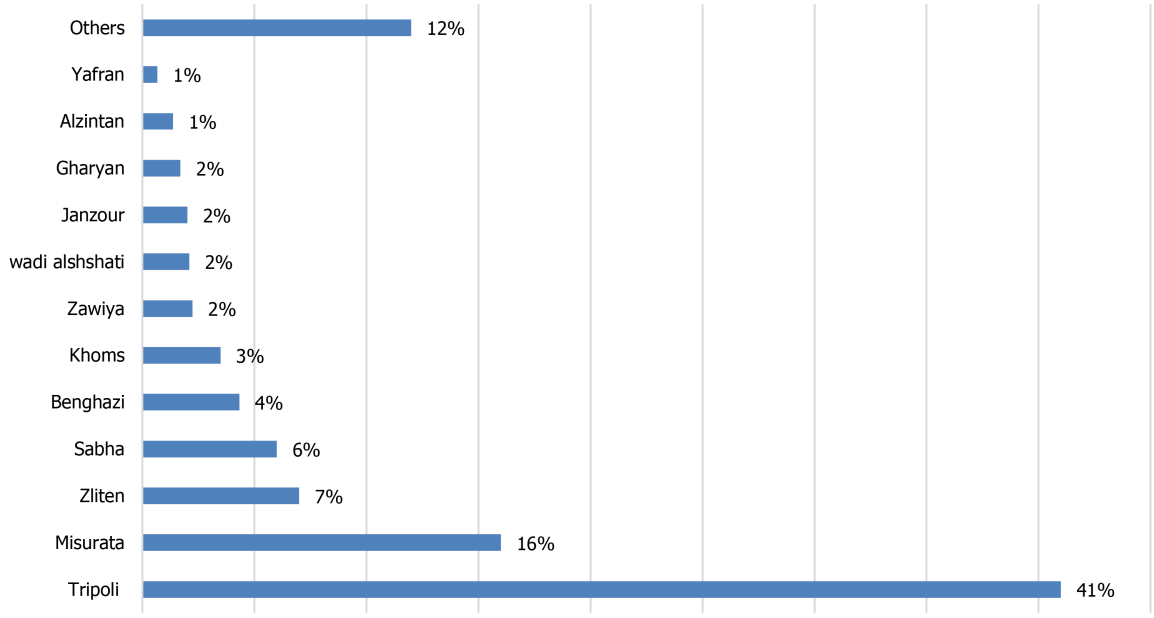

Figure 6 The percent of severe acute respiratory syndrome coronavirus 2 infection in different Libyan Municipalities as of 30 August 2020.

and CDC of Libya formulated response plans and alertness measures and issued early announcements, recommending the government authorities to prepare for combating the novel pandemic. As a result, the Libyan government designated members for the SAC of COVID-19. The Libyan SAC of COVID-19 has issued many sanctions, which include recommendations related to the strategy for combating the disease all over the country. Since the first case of COVID-19 was reported in Libya, the government authorities have followed these recommendations and have taken early action responses, as mentioned previously.

Consequently, at the beginning of the pandemic, Libyan citizens were strictly following all those recommendations related to the basic principles and precaution measures for preparedness and prevention from the infection and transmission of the virus. During the first two months following the first reported case, the number of confirmed cases were low as compared to other countries.

Currently, the epidemiological situation of COVID-19 has changed and worsened; there are many reasons for this, such as people losing their trust in the government and several people not following the health instructions. As a consequence, it was considerably difficult to implement the Libyan national strategy for the prevention and control of COVID-19.

THE LIBYAN PUBLIC HEALTH CAPACITIES

The countries with the weakest and lowest strength of the public health system face the most challenges in the control and prevention of COVID-19. The public health system capacities play a crucial role in the control of the infection, and any weakness affects the strategy for the control and prevention of COVID-19. The Libyan authorities did not sufficiently prepare to improve their health capacities to face the pandemic. The diagnostic capacity and the tracing of contacts or suspected cases are crucial factors in combatting and minimizing the virus infection among the populations. Notwithstanding the high financial support extended by the Libyan authorities, the medical capacities are still lacking to address the minimum healthcare priorities. Most of the healthcare workers (HCWs) at the beginning of the pandemic were afraid because they did not have proper preventive measures in place; moreover, there was a deficiency in the availability of personal protective equipment (PPE). In the healthcare units, there is a shortage in the medical supplements and most of the hospitals do not provide triages or filter rooms. In fact, the challenge is that when a country faces a rise in the COVID-19 cases above their public health capacity, they will not be able to

mitigate deaths from the viral spread within the community or among their HCWs. The efficiency of health services in isolation centres and hospitals is a significant factor that contributes to reducing the impact of the viral spread and improving the recovery of infected patients. The health sector, including HCWs, laboratory technicians and groups of high-risk professionals, are considered the first line of defence during the COVID-19 pandemic in all medical care units; therefore, those in the frontline during an infectious disease's outbreak must especially be well trained. Healthcare units must meet the standard level and follow the criteria as required by the WHO and Libyan CDC to prevent the medical staff from exposure to the viral infection. Many HCWs have been infected by SARS-CoV-2 and sacrificed their lives to save their patients during the COVID-19 pandemic; according to data published on 23 July 2020 by the WHO, approximately over 10000 HCWs in Africa were infected with COVID-19. Many countries had low levels of medical service and a lack of PPE at the beginning of the pandemic. In contrast, the scenario in China has indicated that the Chinese health authorities were well prepared to combat the outbreak of any epidemic disease, having learnt from previous outbreaks such SARS 2003, HIV and human avian flu; accordingly, China was able to implement a consolidated and comprehensive blended strategy for the prevention and control of COVID-19 and also strengthen the public health capacity, which is one of the key factors for the effective combating of COVID-19. The strict quarantine measures constituted another key factor for success of the Chinese strategy. The Chinese health authorities were further able to isolate the virus and perform the genetic sequence of SARS-CoV-2 in a short time[22].

POPULATION DENSITY

Libya is situated on the coast of North Africa, and it belongs to the Maghreb region in North Africa, bordered by the Mediterranean Sea to the north, Egypt to the east, Sudan to the southeast, Chad to the south, Niger to the southwest, Algeria to the west and Tunisia to the northwest. It is a large country with a relatively small population density of about 50 persons per km² (130/sq. mi.). 90% of the people live in less than 10% of the area, primarily along the coast. About 88% of the population is urban, mostly concentrated in the largest cities such as Tripoli (1150989), Benghazi (650629), Misrata (386120), Tarhuna (210697) and Al Khums (201943). Libya has a population of about 6.7 million, and about four people per km² (10 people/ sq. mi), calculated on a total land area of 1759540 km² (679362 sq. miles)[23,24]. The population density is one of the potential factors that increases community spread and individual risk of COVID-19. Consequently, the epidemiological patterns of COVID-19 in Libya could also be greatly influenced by the crowded situation due to the high population density; however, in contrast, most of the Libyan population live in independent department with low crowded. Therefore, the risk of exposure to SARS-CoV-2 infection among the Libyan community is limited as compared to other international societies characterized by crowded situations and of high public traffic within cities. According to the population data, it was suggested that the population density might be linked with the COVID-19 pandemic, especially in the urban areas and big cities around the world that are characterized by intense crowds, which could lead to the virus spreading within and outside those cities[25,26]. In contrast to study led by Johns Hopkins University, the study revealed that urban density is not linked to higher COVID-19 infection and death[27].

DISPLACED POPULATIONS

Libya is a country with a moderate level of population displacement. According to the data published by the UNHCR and the International Organization for Migration (IOM), the country had the worst displacement scenario for a period of time since 2014, with approximately 217002 people being displaced inside the country and 348372 internally displaced persons[28]. The instability of the country and the fragility of the quarantine measures in Libyan borders made it easy for refugees to travel alongside migrants through dangerous routes towards Europe. In Libya, about 43113 refugees and asylum-seekers are registered with UNHCR. However, since 2016, the IOM and Displacement Tracking Matrix identified and located 276957 migrants out of around 700000 to 1 million migrants expected to be within the country[28].

COVID-19 VACCINATION IN LIBYA

Libya is one of the self-financing participants under the COVAX facility. Total of 9.7 million US dollars has been transferred to the COVAX Facility to secure 2.8 million doses of COVID-19 vaccines. This amount of vaccine doses will be enough to vaccinate around 1.25 million people as two doses per person in addition to 10% as vaccine wastage. However, the country is hosting over 574 000 migrants and refugees who have not been included in Libya's national vaccination plan for COVID-19. The government is revising the plan to add a component addressing those vulnerable group. Once the revised plan is endorsed, WHO will ask the Global Vaccine Alliance to consider making vaccines available for around 16200 high-risk migrants and refugees under its Humanitarian Buffer fund.

The Libyan Ministry of Health has secured enough vaccines from the COVAX Facility to immunize approximately 20% of the Libyan population. Priority will be given to frontline health care workers, adults over 60 years of age, and patients with chronic underlying health conditions in all areas of the country[29]. Online registration for COVID-19 vaccination has already started in Libya on the first of March 2021 using the following link: https://www.eservices.ly.

The Libyan NCDC is responsible for coordinating vaccination throughout the country.

The Libyan Government of National Unity has received the first shipment of 101250 doses of Sputnik V vaccines on the April 4, 2021, and the second shipment with 100000 doses of Sputnik V vaccines was received on the 9th of April.

The only vaccine that was sent to Libya through COVAX Facility was AstraZeneca vaccine on the 8th of April with a total of 57600 doses.

A shipment of a total of 150000 doses of Sinovac vaccine was received as a gift to Libya from the Turkish government on the 14th of April. The vaccination campaign has started on the 10th of April and up to the 10th of May a total of about 100000 people have been vaccinated with a single dose.

CONCLUSION

The information and data across the country regarding COVID-19 still remain unclear; consequently, the Libyan authorities need to initiate large-scale case screening, improve testing capacities and enforce contact tracing within the time frame, which are not easy tasks to perform in a country facing troubles, conflicts and instability. Currently, neither an increase in the testing capacities nor quarantine or lockdown of the cities would be a unique solution or strategy for the control and prevention of COVID-19. Advisably, the Libyan health authority should improve the public health capacities and enforce strict hygiene measures within the societies to minimize both the CFR and socio-economic impacts of the SARS-CoV-2. Most important that Ministry of health and NCDC should focus and do all possible efforts in order to get as many people vaccinated within a short period of time.

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ORIGINAL ARTICLE

Retrospective Study

Bacterial co-infection in patients with SARS-CoV-2 in the Kingdom of Bahrain

Nermin Kamal Saeed, Safaa Al-Khawaja, Jameela Alsalman, Safiya Almusawi, Noor Ahmed Albalooshi, Mohammed Al-Biltagi

ORCID number: Nermin Kamal Saeed 0000-0001-7875-8207; Safaa Al-Khawaja 0000-0003-1424-3348; Jameela Alsalman 0000-0002-5500-9905; Safiya Almusawi 0000-0003-0884-9907; Noor Ahmed Albalooshi 0000-0003-3777-2613; Mohammed Al-Biltagi 0000-0002-7761-9536.

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Nermin Kamal Saeed, Safiya Almusawi, Noor Ahmed Albalooshi, Medical Microbiology Section, Pathology Department, Salmaniya Medical Complex, Manama 00000, Bahrain

Nermin Kamal Saeed, Safiya Almusawi, Microbiology Department, Royal College of Surgeons in Ireland - Bahrain, Manama 00000, Bahrain

Safaa Al-Khawaja, Jameela Alsalman, Infection Disease Unit, Department of Internal Medicine, Salmaniya Medical Complex, Ministry of Health, Kingdom of Bahrain, Manama 00000, Bahrain

Safaa Al-Khawaja, Jameela Alsalman, Department of Infectious Disease, Arabian Gulf University, Manama 00000, Bahrain

Mohammed Al-Biltagi, Department of Pediatrics, University Medical Center, King Abdulla Medical City, Arabian Gulf University, Manama 00000, Bahrain

Mohammed Al-Biltagi, Department of Pediatrics, Faculty of Medicine, Tanta University, Tanta 000000, Al Gharbia, Egypt

Corresponding author: Mohammed Al-Biltagi, MD, PhD, Chairman, Professor, Department of Pediatrics, University Medical Center, King Abdulla Medical City, Arabian Gulf University, Adliya, Block 328, Bldg 61, King abdulaziz Avenu, Manama 00000, Bahrain.
mbelrem@hotmail.com

Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic presents a significant challenge to the medical profession, increasing in the presence of microbial co-infection. Bacterial and Fungal co-infections increase the risk of morbidity and mortality in patients with COVID-19.

AIM

To study the bacterial profile in patients with COVID-19 who needed admission to receive treatment in the main centres concerned with managing COVID-19 disease in the Kingdom of Bahrain.

METHODS

Data sharing statement: The data that support the findings of this study are available from the corresponding author, [Al-Biltagi M], upon reasonable request.

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The study was a retrospective observational analysis of the bacterial profile and the bacterial resistance in patients with confirmed COVID-19 disease who needed admission to receive treatment in the main centres assigned to manage patients with COVID-19 disease in the Kingdom of Bahrain from February to October 2020. We used the electronic patients' records and the microbiology laboratory data to identify patients' demographics, clinical data, microbial profile, hospital or community-acquired, and the outcomes.

RESULTS
The study included 1380 patients admitted with confirmed COVID-19 disease during the study period. 51% were admitted from February to June, and 49% were admitted from July to October 2020, with a recurrence rate was 0.36%. There was a significant increase in bacterial and fungal co-infection in the second period compared to the first period. The most common isolated organisms were the gram-negative bacteria (mainly *Klebsiella pneumoniae, Pseudomonas aeruginosa,* multi-drug resistant *Acinetobacter baumannii*, and *Escherichia coli*), the gram-positive bacteria (mainly coagulase negative *Staphylococci, Enterococcus faecium, Enterococcus faecalis, Staphylococcus aureus)* and fungaemia (*Candida galabrata, Candida tropicalis, Candida albicans, Aspergillus fumigatus, Candida parapsilosis, Aspergillus niger*). The hospital-acquired infection formed 73.8%, 61.6%, 100% gram-negative, gram-positive and fungaemia. Most of the hospital-acquired infection occurred in the second period with a higher death rate than community-acquired infections.

CONCLUSION
Bacterial and fungal co-infections in patients admitted with confirmed COVID-19 disease pose higher morbidity and mortality risks than those without co-infections. We should perform every effort to minimize these risks.

Key Words: COVID-19; Bacterial co-infection; Fungi; Hospital-acquired infection; Kingdom of Bahrain

Core Tip: Coronavirus pandemic presents a significant challenge to the medical profession. Bacterial and fungal co-infections are common complications of viral infections with increasing morbidity and mortality. We observed a significant increase in the number of bacterial and fungal co-infection over the study period. In addition, gram-negative infections carry a higher risk of morbidity and mortality.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, which began with the first reported case in December 2019 in China, led to a Public Health Emergency worldwide, including in Bahrain. This pandemic presents a significant challenge to the medical profession, especially with the contradicting data about the origin of the virus [1-3].

Bacterial co-infection is a common complication of viral infections with increasing morbidity and mortality in conjunction with more burden on healthcare resources. Serious bacterial infections may be missed when all attention focuses on COVID-19. Therefore, recognition of co-infection in patients with COVID-19 is of utmost importance. It enables us to implement the appropriate management and proper control of antibiotic use, with effective delivery of antimicrobial stewardship[4]. There

are different reports about the prevalence of bacterial co-infection with COVID-19 assuming less bacterial co-infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) than influenza and other viral diseases[5]. On the other hand, some opinions based on the previous experience with the severe acute respiratory syndrome (SARS) outbreak in 2003 and the Middle East Respiratory Syndrome outbreak in 2012 suggest underestimation of bacterial co-infections in COVID-19 because of non-discriminatory use of antibiotics or the limitation of the overwhelmed clinical examinations in healthcare systems during the pandemic[6]. Bacteria can promote viral capability by augmenting virion stability, promoting viral infection of eukaryotic cells, and increasing co-infection rates. At the same time, virus binding of bacteria can also impact bacterial biology, including bacterial adherence to eukaryotic cells[7].

Bacterial co-infections in patients with COVID-19 are especially important when they require intensive care, including invasive mechanical ventilation support. For example, bacterial co-infections occurred in more than a third of children requiring invasive ventilation for bronchiolitis and were associated with more extended pediatric intensive care unit stay and mechanical ventilation[8]. Furthermore, patients admitted to intensive care unit (ICU) with prolonged illness/intubation have more frequent detection of multidrug-resistant gram-negative pathogens, likely reflecting hospital-acquired infection[9]. Therefore, it is vital to consider (investigate and empirically treat) bacterial co-infection when assessing these patients. Unfortunately, there is no consensus about treating patients with COVID-19 disease, which differs from one setting to another and from one country to another. Therefore, experts suggest not to use prophylactic antibiotics as a routine in patients with COVID-19, especially at the early stage or for non-intubated patients and recommend close monitoring of the signs of secondary infection, especially in critically ill patients who have been admitted to ICU for more than 48 h[10]. Furthermore, considering the long-term impact of the antimicrobial resistance development due to the unnecessary usage of antimicrobial agents, we should know the common bacterial and fungal infections that could complicate COVID-19, and know their expected antibiogram, and strictly monitor the rate of development of resistant bacterial strains[11]. Unfortunately, there are not enough data about the bacterial co-infections in patients admitted with COVID-19 disease. Therefore, we aimed to study the microbiological profile and the bacterial antibiogram in patients with COVID-19 who needed admission to receive treatment in the main centres concerned with managing COVID-19 disease in the Kingdom of Bahrain.

MATERIALS AND METHODS

Study design and setting

The study was a retrospective observational analysis of the microbiological profile of the patients admitted with confirmed COVID-19 disease to the different Ministry of Health (MOH) COVID isolation and treatment centres in the Kingdom of Bahrain for nine months period from February 2020 to October 2020. Inpatients with confirmed SARS-CoV-2 infection who had clinical suspicion of sepsis and/or bacterial co-infection were included in the study. Data were extracted and reviewed from the inpatients' electronic health medical records from all MOH inpatients. The demographics, clinical data, microbiological profile, and outcomes of included patients were extracted, and the data were tabulated using the Microsoft Excel database.

Definitions

According to the national guidelines, the patients were stratified and allocated to specific COVID-19 Care centres into mild, moderate, and severe. The severe cases were assigned to the tertiary care centres with advanced care facilities. The medications differed according to the severity of the case and the presence of criteria of suspected sepsis.

Inpatients with the clinical suspension of sepsis/bacterial co-infection: COVID inpatients suspected clinically to have bacterial co-infection as decided by their treating physician during their clinical care, and septic workup were collected and sent to the microbiology laboratory.

Community-acquired infection: When clinical suspicion of sepsis/bacterial co-infection and the clinical samples for microbiology testing were collected from patients

at the time of admission or within the initial 48 h from admission to COVID-19 facility.

Hospital-acquired infection: When clinical suspicion of sepsis/bacterial co-infection and the clinical samples for microbiology testing were collected after 48 h from the time of admission to COVID-19 facility.

Clinical isolates: The first bacterial pathogen growth for each patient from any clinical specimen was counted as a clinical isolate. Isolates were considered duplicate and not considered if identified from the same patient with the same organism and antimicrobial profile.

Laboratory technique

All the patients confirmed to have COVID-19 disease by positive testing using real-time reverse transcriptase-polymerase chain reaction for nasopharyngeal, sputum, endotracheal aspiration, or bronchoalveolar lavage samples. Clinical samples such as blood culture, sputum culture, stool culture, endotracheal aspirate or bronchoalveolar lavage culture were ordered according to the clinical indications when bacterial co-infection was suspected. These samples were cultured with the relevant media (nutritive, differential and/or selective), atmospheres and duration. The phenotypic detection was done using MALDI-TOF MS (Bruker Daltonics, Germany). Antimicrobial Susceptibility Testing was performed using BD Phoenix (BD Diagnostics, Baltimore, MD, United States) and interpreted according to the Clinical Laboratory Standards Institute[12]. We followed the trend of antibacterial sensitivity to evaluate the antimicrobial resistance.

Data analysis

All data were anonymized and collated on Excel 2017 (Microsoft, Redmond, WA, United States). We used TexaSoft, WINKS SDA Software 2011 (Sixth Edition, Cedar Hill, TX, United States) to perform the statistical analysis. We computed the percentages and frequencies for different categorical variables, and a cross-tabulation was computed between every two categorical variables. Finally, the Chi-Squared test determined whether there were significant relationships between every two categorical variables. We considered a P value of less than 0.05 as statistically significant. A biomedical statistician performed the statistical review of the study.

Ethical approval

The study was approved by the National COVID-19 Research Team and Secondary Care Research Committee of Salmaniya Medical Complex, Ministry of Health, the Kingdom of Bahrain. However, the study had no ethical consideration as it was a retrospective non-interventional study with no exposure to any patient data.

RESULTS

Table 1 showed the demographics of the included inpatients. The study included 1380 patients admitted with confirmed COVID-19 disease and had clinical suspicion of sepsis during the study period from February to October 2020, with a Male: Female ratio of 0.9, mean age of 50.2 ± 18.1 years, and 73% of them were Bahraini. The death rate was 11.5% for all the admitted patients during the study period. 51% of inpatients with clinical suspicion of sepsis were admitted from February to June, and 49% were admitted from July to October 2020. Five patients had confirmed recurrences (0.36%), all five patients recovered. From those admitted patients with confirmed COVID-19 diseases and clinical suspicion of sepsis, 261 patients (19%) had confirmed bacterial and fungal co-infections, 75% of them were Bahraini with a mean age of 58.5 ± 18.7 years, Male: Female ratio of 0.8, and a death rate of 42.5%. Two of these patients had a recurrence, and both survived. The remaining 1119 admitted patients (81%) had negative bacterial and fungal culture. Their mean age was 48.4 ± 17.6 years, with a male: female ratio of 0.9; 73% of them were Bahraini with a death rate of 4.3%. The group with confirmed bacterial and fungal co-infections had a significantly higher age ($P < 0.0001$) and rate of death ($P < 0.0001$) than the group without confirmed bacterial or fungal co-infection.

Table 2 showed the demographics of the patients with gram-positive, gram-negative bacteria, fungal and mixed infections. There were no significant differences between the number, age, gender, and nationality between the gram-positive and gram-negative bacteria. However, gram-negative infection occurred in older age and has a

Table 1 Comparison patients' demographic for total admitted patients with/without Bacterial or fungal coinfections

	Total admitted patients (COVID with clinical suspicion of sepsis)	Patients without coinfection (negative bacterial culture)	Patients with coinfection (positive bacterial culture)	*P* value
n (%)	1380	1119 (81.1)	261 (18.9)	< 0.0001
Male/female	0.92	0.87	1.13	> 0.05
Bahraini/non-Bahraini	2.80	2.70	3.10	> 0.05
Mean age (yr) ± SD	50.2 ± 18.1	48.4 ± 17.6	58.5 ± 18.7	< 0.0001
Death	159 (11.5%)	48 (4.30%)	111 (42.5%)	< 0.0001
Recurrences	5 (0.36%)	3 (0.27%)	2 (0.77%)	> 0.05

COVID: Corona virus disease; SD: Standard deviation.

Table 2 Comparison patients' demographics and microbial profile for patients with gram-positive and gram-negative Bacteria and mixed infections

	Gram + ve coinfection	Gram-ve coinfection	Mixed coinfection	Candida	*P* value[1]	*P* value[2]	*P* value[3]
n (%)	136 (54)	115 (46)	82 (23.8)	115 (46)	> 0.05	< 0.0001	< 0.0001
Male/female	0.82	0.67	0.74	0.88	> 0.05	> 0.05	> 0.05
Bahraini/non-Bahraini	2.50	3.10	1.90	2.10	> 0.05	> 0.05	> 0.05
Mean age (yr) ± SD	57.7 ± 18.2	60 ± 18.2	64.3 ± 14.3	63.4 ± 16.4	> 0.05	< 0.01[a]	< 0.05
Death	39 (28.7%)	61 (53%)	62 (75.6%)	81 (70.4%)	< 0.0001	< 0.0001	< 0.01
HA infection	78 (57.3%)	86 (75%)	79 (96%)	97 (84.3%)	< 0.001	< 0.0001	< 0.0001

[1]Comparison between gram + ve and gram-ve coinfection.
[2]Comparison between gram + ve and mixed coinfections.
[3]Comparison between gram-ve and mixed coinfections. HA: Hospital acquired; SD: Standard deviation.

significantly higher death rate and more hospital-acquired infection rates than gram-positive bacteria. All the gram-negative isolates were detected from the centres allocated for the severe cases. Moreover, mixed infections occurred in less than a quarter of cases, with significantly higher age and death rate than other types of co-infections. All cases of mixed infections were hospital-acquired. We also observed that the number of patients with bacterial or fungal infection was significantly higher in the July-to-October period ($P < 0.0001$) with higher mean age ($P < 0.01$) compared to the first period of the study between February to June. In addition, the number of co-infections with gram-negative bacteria was significantly higher ($P < 0.0001$) in the July to October period than that of the February-to- June. The same also was observed in fungal co-infections. The number of mixed co-infections was also significantly higher in the July-to-October period ($P < 0.01$).

Table 3 showed the microbiological profile in patients with confirmed COVID-19 disease in the whole study period with a total of 472 isolates from 261 admitted patients. The gram-negative bacteria were isolated from 34.7% [59% showed Multidrug-resistant (MDR) strains], and gram-positive isolates were isolated from 34.7% of the patients (53% showed MDR strains). In comparison, fungal infections were isolated from 32% of the patient, 25% were isolated from the blood (Fungaemia). There was no significant difference in the isolates number in the two study periods, from February to June and July to October. However, the percentage of gram-negative isolates increased from 26.8% in the first period to 73% in the second period ($P < 0.0001$) and the percentage of MDR among gram-negative strains increased from 41% in the first period to 65.8% in the second period ($P < 0.01$). Thus, the MDR gram-negative strains isolated in the second period formed 81.4% of the total MDR strains isolated throughout the study ($P < 0.0001$). The most common gram-negative strains

Table 3 Microbiological profile in the admitted patients with confirmed coronavirus disease 2019 during the study period (472 isolates)

Type of the organism				Number	% of MDR
Gram Negative Isolates (164)	*Klebsiella pneumoniae*		Total	39	97.4
			ESBL	11	
			CRE	27	
	Pseudomonas aeruginosa		Total	38	26.3
			CRP	8	
			MDR	2	
	Acinetobacter baumannii (MDR)			36	100
	Escherichia coli		Total	28	68
			ESBL	11	
			CRE	8	
	Stenotrophomonas maltophilia			12	0
	Enterobacter cloacae		Total	2	100
			CRE	2	
	Other			9	0
	Total G-ve isolates			164	59
	Total G-ve MDR strains			97	
Gram positive isolates (164)	Coagulase negative *Staphylococci* (CoNS)	*Staphylococcus hominis*	Total	31	58
			MRCoNS	18	
		Staphylococcus epidermidis	Total	25	78.6
			MRCoNS	22	
		Staphylococcus heemolyticus	MRCoNS	18	100
		Staphylococcus capitis	Total	10	50
			MRCoNS	5	
		Staphylococcus pettenkoferi	MRCoNS	1	100
		Total CoNS	Total	85	75.3
			MRCoNS	64	
	Enterococcus faecium		Total	24	16.6
			VRE	3	
			HLGR	1	
	Enterococcus faecalis		Total	20	5.0
			HLGR	1	
	Staphylococcus aureus		Total	15	53.3
			MRSA	8	
	Others			20	
	Total G + ve isolates			164	47
	Total G + ve MDR Strains			77	
Fungal isolates(144)	Fungaemia		*Candida galabrata*	11	
			Candida tropicalis	9	
			Candida albicans	7	
			Aspergillus fumigatus	3	
			Candida parapsilosis	3	

	Aspergillus niger	3	
	Total	36	
Candida species		108	
Total fungal isolates		144	
Total number of the microbial isolates		472	36.9
Total number of mdr bacterial strains		174	

CRE: Carbapenem-resistant Enterobacteriaceae; ESBL: Extended spectrum beta-lactamase; HLGR: High level aminoglycoside resistance; MDR: Multidrug-resistant; MRCoNS: Methicillin-resistant coagulase-negative *Staphylococci*; MRSA: Methicillin-resistant *Staphylococcus aureus*; VRE: *Vancomycin-resistant enterococci*; Other gram-negative bacteria: *Citrobacter freundii, Salmonella* species, *Pantoea* species, *Proteus mirabilis*, *Serratia marcescens*, *Elizabethkingia* meningoseptica; Other gram-positive bacteria: *Streptococcus agalactiae* (Strep. Group B), *Corynebacterium afermentans, Bacillus licheniformis, Leuconostoc mesenteroides, Staphylococcus caprae, Staphylococcus lugdunensis, Staphylococcus warneri, Streptococcus parasanguinis, Gemella sanguinis, Micrococcus luteus, Propionibacterium acnes, Rhodococcus erythropolis, Aerococcus viridans, Staphylococcus gallinarum.*

isolated through the study were *Klebsiella pneumoniae* (*K. pneumoniae*) followed by *Pseudomonas aeruginosa* (*P. aeruginosa*), then MDR *Acinetobacter baumannii* (*A. baumannii*), *Escherichia coli* (*E. coli*), *Stenotrophomonas maltophilia* (*S. maltophilia*), and *Enterobacter cloacae* (*E. cloacae*).

On the other hand, the gram-positive bacteria showed a significant increase in the total number of isolates in the second period but no significant difference in the number of total MDR strains or the number of coagulase-negative *Staphylococci* in the two study periods. Moreover, there was a significant increase in the number of methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) in the second period compared with the first periods. The most common gram-positive strains isolated throughout the study were *Staphylococcus hominis* (*S. hominis*) (MRCoNS), followed by *Staphylococcus epidermidis* (*S. epidermidis*) (CoNS), *Enterococcus faecium* (*E. faecium*), *Enterococcus faecalis* (*E. faecalis*), and *Staphylococcus aureus* (*S. aureus*). In addition, the rate of fungaemia was significantly higher in the second period (6-fold increase) compared to the first period ($P < 0.0001$).

Table 4 and Figure 1 showed a comparison between the community and hospital-acquired infections (HAI) and their microbiologic profile in patients with confirmed COVID-19 disease with a total of 472 isolates during the whole study periods. Hospital-acquired infections formed 70% of the total infections. Those patients with HAI had a significantly higher mean of age ($P < 0.01$) than those of CAI. In addition, the percentage of the gram-negative isolates, including the MDR strains, were significantly higher in the HAI than CAI. The most common gram-negative strains were *K. pneumoniae,* followed by MDR *A. baumannii*, *P. aeruginosa, E. coli,* and *S. maltophilia.* At the same time, the total number of gram-positive isolates, including the MDR strains, were significantly higher in patients with HAI compared to patients with CAI ($P < 0.0001$). The most common gram-positive strains were *S. epidermidis* (CoNS), followed by *E. faecium*, *E. faecalis*, *Staphylococcus haemolyticus* (CoNS), *S. hominis* (CoNS), and *S. aureus,* as shown in the table. All isolates with fungaemia were obtained from patients with HAI. No cases with fungaemia were recorded from CAI.

DISCUSSION

Microbial co-infections are commonly identified in viral respiratory infections. They are key reasons for difficult diagnosis, poor prognosis, increased morbidity and mortality, and greater use of healthcare resources. The prevalence and characteristic of bacterial co-infection in patients with confirmed COVID-19 disease are not well studied, especially in the Kingdom of Bahrain, with a broad knowledge gap. Bacterial co-infection could occur before admission of the patient to the hospital (Community-acquired) or could complicate the course of the illness as a secondary infection (Hospital-acquired). Our observational study identified a rate of 19% of bacterial co-infection through the study with increased rates of laboratory-confirmed bacterial and fungal co-infections in patients admitted with confirmed COVID-19 disease during the second period compared to the first period of the study despite that the total number of the admitted patients remained nearly the same. A study by Garcia-Vidal *et al*[13] had similar results to our results in the first period. They found an incidence of 7.2% of bacterial co-infection in their study, which conducted between February and April

Table 4 Comparison between the community and hospital acquired infections and their microbiologic profile from coronavirus disease 2019 confirmed patients (total isolates 472)

Character				HA infection	CA infection	*P* value
Patient number (total 261) 22 patients has both HA and CA				185 (70.8%)	98 (37.5%)	< 0.0001
Age				60.8 ± 16.8	54. ± 20.6	< 0.01
Male: Female				0.85	0.80	> 0.05
Bharani				137 (74%)	71 (72.4%)	> 0.05
Death				102 (55%)	23 (23.5%)	< 0.0001
Gram negative isolates (164)	*Klebsiella pneumoniae*		Total	30	9	
			ESBL	11	0	
			CRE	15	2	
	Acinetobacter baumannii (MDR)			29	7	
	Pseudomonas aeruginosa		Total	28	10	
			CRP	6	2	
			MDR	2	2	
	Escherichia coli		Total	13	15	
			ESBL	6	6	
			CRE	5	2	
	Stenotrophomonas maltophilia			11	1	
	Enterobacter cloacae		Total	2	0	
			CRE	1	0	
	Others			8	1	
	Total number of the G-ve isolates (164)			121 (73.8%)	43 (26.2%)	< 0.0001
	Number of G-ve resistant Strains			75 (62%)	21(48.8%)	> 0.05
	% from total Resistant strains (96)			78.1%	21.9%	< 0.0001
Gram positive isolates (164)	Coagulase negative *Staphylococci (CoNS)*	*Staphylococcus epidermidis*	Total	19	6	
			MRCoNS	18	4	
		Staphylococcus haemolyticus	MRCoNS	14	4	
		Staphylococcus hominis	Total	12	19	
			MRCoNS	6	13	
		Staphylococcus capitis	Total	5	5	
			MRCoNS	5	0	
		Staphylococcus pettenkoferi	MRCoNS	1	0	
		Total CoNS	Total	41 (40.6%)	31 (49.2%)	> 0.05
			MRCoNS	37 (90%)	21 (67.7%)	< 0.05
	Enterococcus faecium		Total	17	7	
			VRE	2	1	
			HLGR	1	0	
	Enterococcus faecalis		Total	16	4	
			HLGR	1	0	
	Staphylococcus aureus		Total	8	8	
			MRSA	3	5	
	Others			9	10	

	Total number of the G + ve isolates (164)		101 (61.6%)	63 (38.4%)	< 0.0001
	Number of G + ve resistant strains		51 (50.5%)	27 (42.9%)	> 0.05
	% from total Resistant strains (78)		65.4%	34.6%	< 0.0001
Fungal isolates (144)		*Candida galabrata*	11	0	
		Candida albicans	7	0	
		Candida tropicalis	9	0	
		Candida parapsilosis	3	0	
		Aspergillus fumigatus	3	0	
		Aspergillus niger	3	0	
		Total	36	0	
	Candida species		95	13	
	Total fungal isolates (144)		131 (91%)	13 (9%)	< 0.0001
Total microbial isolates (472)			353 (74.8%)	119 (25.2%)	< 0.0001
Total number of resistant bacterial strains			126 (35.7%)	48 (40.3%)	> 0.05
Percentage from total resistant bacterial strains (174)			72.4%	27.6%	< 0.0001

CA: Community Acquired; CRE: Carbapenem-resistant Enterobacteriaceae; ESBL: Extended spectrum beta-lactamase; HA: Hospital acquired; HLGR: High level aminoglycoside resistance; MDR: Multidrug-resistant; MRCoNS: Methicillin-resistant coagulase-negative *Staphylococci*; MRSA: Methicillin-resistant *Staphylococcus aureus*; VRE: *Vancomycin-resistant enterococci*; Other gram-negative bacteria: *Citrobacter freundii, Salmonella species, Pantoea* species, *Proteus mirabilis*, *Serratia marcescens*, *Elizabethkingia* meningoseptica; Other gram-positive bacteria: *Streptococcus agalactiae* (Strep. Group B), *Corynebacterium afermentans, Bacillus licheniformis, Leuconostoc mesenteroides, Staphylococcus caprae, Staphylococcus lugdunensis, Staphylococcus warneri, Streptococcus parasanguinis, Gemella sanguinis, Micrococcus luteus, Propionibacterium acnes, Rhodococcus erythropolis, Aerococcus viridans, Staphylococcus gallinarum.*

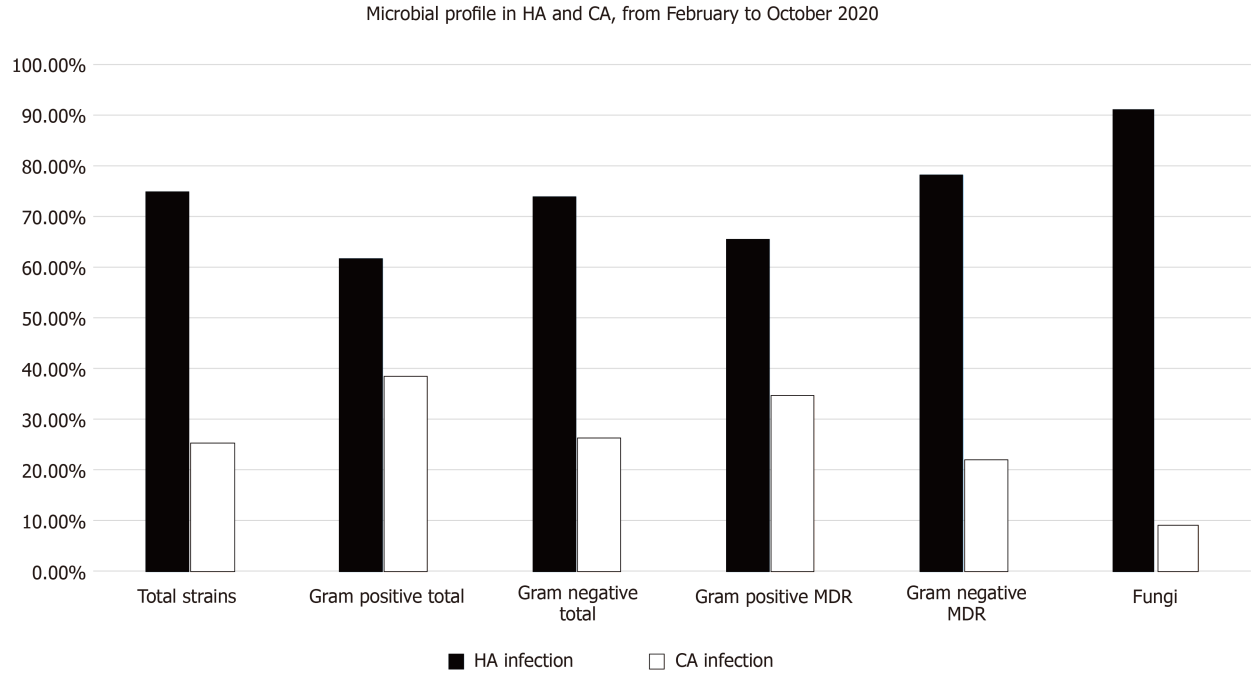

Figure 1 Microbial profile in hospital-acquired infections and community-acquired infections from February to October 2020. MDR: Multidrug-resistant; HA: Hospital-acquired; CA: Community-acquired.

2020[13]. Zhang *et al*[14] showed that the severely affected patients with COVID-19 disease had a significantly higher rate of bacterial (25.5%) and fungal (10.9%) co-infections. At the same time, a meta-analysis by Lansbury *et al*[5] indicated that about 7% of hospitalized patients with COVID-19 disease had bacterial co-infections, which increased to 14% in studies that only included ICU patients.

Nevertheless, this meta-analysis had a lower rate than that observed in our study, as it analyzed data from the earliest cases of the SARS-CoV-2 pandemic, which could differ from the current situation. Another metanalysis by Langford *et al*[15] showed that the overall proportion of COVID-19 patients with bacterial infection was 6.9% and increased to 8.1% of critically ill patients. The increased rate of bacterial co-infection in the second period in our study is related to the change in the admission criteria in the second period of the study to be more selective for the sick patients with medical comorbidities that need hospital management and allowing asymptomatic and mildly symptomatic patients to be managed at home. The death rate reached 42.5% in patients with bacterial co-infection than the patients without (4.3%). This high rate of death in the presence of microbial co-infection was also reported in a previous study in China which showed that 96% of patients with confirmed COVID-19 disease and secondary bacterial infections died. About half of the non-survivors experienced a secondary infection[16].

In the current study, there was a high incidence of gram-negative bacteria in patients who need hospitalization with increased mortality rates. Most of the gram-negative bacterial co-infections were hospital-acquired (75%). Consequently, every effort should be made to minimize this risk. Multi-drug resistant strains were present in more than half of the gram-negative bacterial isolates. This point should be considered during the management till the results of the antibiotic sensitivity are achieved. Being male and older than 60 years carries a higher risk for gram-negative as well as mixed co-infections. There was also a marked increase in the rate of gram-negative bacteria in the second period of the study, notably *K. pneumoniae,* followed by *P. aeruginosa*, MDR *A. baumannii, E. coli, S. maltophilia*, and *E. cloacae, K. pneumoniae* and *P. aeruginosa* were attributed to respiratory, then blood and urine-sourced infections. The MDR rate among the gram-negative bacteria was 65.8% in the second period and 41% in the first period of the study. This agreed with the work of Kokkoris *et al*[17], who reported an increase in the gram-negative blood-stream infections identified in ICU-admitted patients with confirmed COVID-19 disease, primarily due to MDR pathogens. A similar study in Egypt showed that MDR *K. pneumoniae* and *A. baumannii* were the predominant gram-negative bacteria that carried different resistance-associated genes[18]. The improper use of antibiotics could be implicated in increasing the resistance frequency. Many studies showed that antimicrobials were being administered at a high rate in patients with COVID-19 disease even in the presence of a low number of confirmed bacterial infection[19].

In the present study, the rate of co-infection with gram-positive bacteria in admitted patients was 11.8%. The most common isolated organisms were coagulase-negative *Staphylococci* (*S. hominis, S. epidermidis, Staphylococcus heemolyticus,* and others), forming 52.5% of total gram-positive isolates, followed by *E. faecium, E. faecalis,* and *S. aureus* with 47% of them were MDR strains. There was a significant increase in gram-positive bacteria in the second period than the first period of the study ($P < 0.05$). However, the resistance rate non-significantly decreased in the second period compared with the first period ($P > 0.05$). This observation agreed with the work of Sepulveda *et al*[20], who found that coagulase-negative *staphylococcus* species accounted for 59.7% of all positive cultures among patients with COVID-19 disease in New York City. Hughes *et al*[21] also found that coagulase-negative *Staphylococcus* species were the most common organisms isolated from the blood culture, followed by *Acinetobacter species*. Thus, infection with SARS-CoV-2 may reduce the patient's immunity and increase the risk of bacterial infections. In a retrospective study in Wuhan, China, 19 patients in the ICU with confirmed COVID-19 disease had markedly reduced CD4 and CD8 T-cells[22]. This immune compromise increases the risk of co-infection with both viruses and bacteria, increases the risk of bacterial resistance, and the requirements of the patients to extended courses of IV antibiotic therapy[23].

In the current study, we observed the presence of fungaemia in about 10% of microbial co-infection. The most common fungi isolated were *Candida galabrata, Candida tropicalis, Candida albicans,* and *Aspergillus fumigatus*. The death rate in our patients who had fungal co-infection was very high (70.4%). This finding agreed with the study done in Upper Egypt by Ramadan *et al*[18], who found that *Candida albicans* and *Candida glabrata* were the most common fungal isolates. Patients hospitalised for COVID-19 are at risk for HAIs, with fungaemia; bloodstream infections caused by *Candida* or *aspergillus*. Invasive fungal infections add more prudent to the already immune-compromised patients with COVID-19 disease, especially diagnostic tools' limitations and the critical clinical settings that put these patients at additional risk. Fungal infections resistant to antifungal treatment have also been described in patients with severe COVID-19. Early diagnosis and monitoring for *Candida* infections and antifungal resistant infections are essential to reduce death from COVID-19 in patients

with severe COVID-19[24,25]. Mixed infections in the current study had a very high death rate, representing a significant threat to the patients with COVID-19 and necessitate aggressive treatment. To avoid missing these types of severe infection, patients should be recruited on admission to intensive care units and sampled longitudinally throughout the disease course using culture-independent techniques capable of identifying complex mixed infections[26].

In the current study, the HAI was about 71% of the total bacterial, and fungal infections in patients admitted with COVID-19 disease. The death rate in HAI was 55% compared to 23.5% in community-acquired infection. The age in HAI was also higher than in CAI. Older age is a significant risk factor to have HAI in patients with COVID-19[27]. Intrahospital and interhospital clonal transmission of bacteria could be a factor for HAI. Rational utilization of antibiotics and steroids to treat patients with COVID-19 is essential in preventing nosocomial infection. We should give particular attention to diabetic patients and patients with invasive devices[28]. HAI is a risk factor to have resistant strains. The percentage of resistant strains in HAI reached 62% in gram-negative and 50.5% in gram-positive isolates in the current study.

Antimicrobial resistance is a global problem, especially among gram-negative pathogens. The current study showed a high resistance pattern in bacterial co-infection in patients with COVID-19. In the gram-negative bacteria, about 28% of *K. pneumoniae* were extended spectrum beta-lactamase (ESBL), and 69% were CRE. All *A. baumannii* strains were MDR. About 39% of *E. coli* were ESBL, and 22% were CRE. In *P. aeruginosa,* 21% were CRP, while 8% were MDR. In gram-positive isolates, 75% of coagulase-negative *Staphylococci* and 53% of *S. aureus* were Methicillin-resistant. Antibiotic resistance is a critical reason for the failure of antibiotic therapy. At the same time, COVID-19 disease can exacerbate antibiotic resistance[29]. This increased resistance results from the interplay of different factors, including the micro-organisms, patients, and hospital environment, including the antibiotic use and the infection control practices. Increasing antibiotic resistance is also caused by improper antibiotic prescription and transmission of resistant bacterial strains within the hospitals by cross colonisation of patients *via* the hands of healthcare staff and subsequent spread between hospitals by transfer of the colonised patients[30]. Strategies to control antibiotic resistance in hospitals include multidisciplinary cooperation in implementing local policies on the use of antibiotics and infection control measures, timely detection with adequate microbiology laboratory standards and reporting of the antibiotic-resistant strains, improved surveillance, and aggressive control of transmission of epidemic resistant bacteria. We should integrate the antimicrobial stewardship activities into the pandemic response across the broader health system[31].

Limitation of the study

Despite being a multicentre study, it had some limitations. Being a retrospective study reduces control over multiple confounders and data collection. We did not study the mechanism of bacterial resistance due to lack of time and the workload during the pandemic. We also included only the infections that were documented by culture and, therefore, some episodes may be missing, and viral co-infection was not included. Finally, this study was done in the Kingdom of Bahrain, with its own unique local epidemiologic effects on antimicrobial resistance, limiting the generalisability of the findings.

CONCLUSION

Bacterial and fungal co-infections are common and place a significant threat to the patient with COVID-19 disease. At the same time, COVID-19 disease increases the risk of bacterial and fungal co-infections. We observed a high death rate in patients with hospital-acquired gram-negative co-infections. At the same time, older age was noted, especially in HAI. In addition, bacterial resistance was a significant problem in bacterial co-infection. Therefore, we should perform every effort to prevent microbial co-infections to minimize both morbidity and mortality.

ARTICLE HIGHLIGHTS

Research background

The coronavirus (COVID-19) pandemic presents a significant challenge to health worldwide. Bacterial and Fungal co-infections increase the risk of morbidity and mortality in patients with COVID-19, in conjunction with more burden on healthcare resources.

Research motivation

With the increasing risk of mortality among patients with COVID-19, there is a solid need to study the different factors that could increase or decrease this risk. Therefore, recognition of co-infection in patients with COVID-19 is of utmost importance. It enables us to implement the appropriate management and proper control of antibiotic use, with effective delivery of antimicrobial stewardship. Therefore, the centres that provide care for patients with COVID-19 in the kingdom of Bahrain participated in the current research.

Research objectives

We aimed to study the microbiological profile and the bacterial antibiogram in patients with COVID-19 who needed admission to receive treatment in the main centres concerned with managing COVID-19 disease in the Kingdom of Bahrain.

Research methods

The study was a retrospective observational analysis of the microbiological profile of the patients admitted with confirmed COVID-19 disease to the different Ministry of Health COVID isolation and treatment centres in the Kingdom of Bahrain for nine months period from February 2020 to October 2020.

Research results

There was a significant increase in the number of bacterial and fungal co-infection over the study period. The most common isolated organisms were the gram-negative bacteria (mainly *Klebsiella pneumoniae, Pseudomonas aeruginosa,* multi-drug resistant *Acinetobacter baumannii*, and *Escherichia coli*), the gram-positive bacteria (mainly coagulase negative *Staphylococci, Enterococcus faecium, Enterococcus faecalis, Staphylococcus aureus)* and fungaemia (*Candida galabrata, Candida tropicalis, Candida albicans, Aspergillus fumigatus, Candida parapsilosis, Aspergillus niger*). The hospital-acquired infection formed 73.8%, 61.6%, 100% gram-negative, gram-positive, and fungaemia. Most of the hospital-acquired infection occurred in the second period with a higher death rate than community-acquired infections.

Research conclusions

Bacterial and fungal co-infections in patients admitted with confirmed COVID-19 disease pose higher morbidity and mortality risks than those without co-infections. Therefore, we should perform every effort to minimize these risks.

Research perspectives

We need to study bacterial resistance mechanisms among the patients infected with COVID-19 and have co-infection with resistant bacterial strains. We also need to study viral co-infection and its effects on morbidity and mortality. Finally, we should compare our data with the data from other countries to generalize the obtained results.

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SYSTEMATIC REVIEWS

Current systematic reviews and meta-analyses of COVID-19

Mahmoud Nassar, Nso Nso, Mostafa Alfishawy, Anastasia Novikov, Salim Yaghi, Luis Medina, Bahtiyar Toz, Sofia Lakhdar, Zarwa Idrees, Yungmin Kim, Dawa Ongyal Gurung, Raheel S Siddiqui, David Zheng, Mariam Agladze, Vikram Sumbly, Jasmine Sandhu, Francisco Cuevas Castillo, Nadya Chowdhury, Ravali Kondaveeti, Sakil Bhuiyan, Laura Guzman Perez, Riki Ranat, Carlos Gonzalez, Harangad Bhangoo, John Williams, Alaa Eldin Osman, Joyce Kong, Jonathan Ariyaratnam, Mahmoud Mohamed, Ismail Omran, Mariely Lopez, Akwe Nyabera, Ian Landry, Saba Iqbal, Anoosh Zafar Gondal, Sameen Hassan, Ahmed Daoud, Bahaaeldin Baraka, Theo Trandafirescu, Vincent Rizzo

ORCID number: Mahmoud Nassar 0000-0002-5401-9562; Nso Nso 0000-0002-0340-169X; Mostafa Alfishawy 0000-0002-9153-3237; Anastasia Novikov 0000-0001-5260-7101; Salim Yagi 0000-0002-6642-0521; Luis Medina 0000-0001-9518-1470; Bahtiyar Toz 0000-0001-7866-2977; Sofia Lakhdar 0000-0001-5320-2990; Zarwa Idrees 0000-0001-6494-4754; Yungmin Kim 0000-0002-3562-3510; Dawa Ongyal Gurung 0000-0001-5678-122X; Raheel S Siddiqui 0000-0002-7284-4435; David Zheng 0000-0002-4478-5052; Mariam Agladze 0000-0001-7494-1899; Vikram Sumbly 0000-0003-3891-6826; Jasmine Sandhu 0000-0001-9817-7936; Francisco Cuevas Castillo 0000-0001-7727-709X; Nadya Chowdhury 0000-0001-9181-1885; Ravali Kondaveeti 0000-0003-2335-5296; Sakil Bhuiyan 0000-0002-6077-9103; Laura Guzman Perez 0000-0001-7344-8445; Riki Ranat 0000-0001-6166-8168; Carlos Gonzalez 0000-0001-9301-6455; Harangad Bhangoo 0000-0001-8893-3005; John Williams 0000-0001-9074-3622; Alaa Eldin Osman 0000-0002-6336-4923; Joyce Kong 0000-0002-6680-9975; Jonathan Ariyaratnam 0000-0002-3591-5505; Mahmoud Mohamed 0000-0002-6246-229X; Ismail Omran 0000-0001-8632-2104; Mariely Lopez 0000-0002-3543-4269; Akwe Nyabera 0000-0002-2208-9531; Ian Landry 0000-0002-

Mahmoud Nassar, Nso Nso, Anastasia Novikov, Salim Yaghi, Luis Medina, Bahtiyar Toz, Sofia Lakhdar, Zarwa Idrees, Yungmin Kim, Dawa Ongyal Gurung, Raheel S Siddiqui, David Zheng, Mariam Agladze, Vikram Sumbly, Jasmine Sandhu, Francisco Cuevas Castillo, Nadya Chowdhury, Ravali Kondaveeti, Sakil Bhuiyan, Laura Guzman Perez, Riki Ranat, Carlos Gonzalez, Harangad Bhangoo, John Williams, Alaa Eldin Osman, Joyce Kong, Jonathan Ariyaratnam, Ismail Omran, Akwe Nyabera, Ian Landry, Saba Iqbal, Anoosh Zafar Gondal, Sameen Hassan, Vincent Rizzo, Department of Internal Medicine, Icahn School of Medicine at Mount Sinai/NYC H&H Queens, New York, NY 11432, United States

Mostafa Alfishawy, Department of Infectious Diseases, Infectious Diseases Consultants and Academic Researchers of Egypt (IDCARE), Cairo 11221, Outside of the US, Egypt

Mahmoud Mohamed, Department of Medicine, Division of Nephrology, University of Tennessee Health Science Center, Knoxville City, TN 38103, United States

Mariely Lopez, Department of Medical, St. George's University, West Indies 38901, Grenada

Ahmed Daoud, Department of Internal Medicine, Faculty of Medicine, Cairo University, Cairo 11221, Egypt

Bahaaeldin Baraka, Department of Oncology, Broomfiled Hospital, Mid and South Essex NHS Foundation Trust, ESSEX, Chelmsford 12422, United Kingdom

Theo Trandafirescu, Department of Critical Care Unit, Icahn School of Medicine at Mount Sinai/NYC H&H Queens, New York, NY 11432, United States

Corresponding author: Mahmoud Nassar, MD, MSc, PhD, Doctor, Department of Internal Medicine, Icahn School of Medicine at Mount Sinai/NYC H&H Queens, 82-68 164th Street Jamaica, New York, NY 11432, United States. dr.nassar@aucegypt.edu

Abstract

BACKGROUND

Coronavirus disease 2019 (COVID-19) has left a significant impact on the world's health, economic and political systems; as of November 20, 2020, more than 57

9757-4064; Saba Iqbal 0000-0001-5308-2685; Anoosh Zafar Gondal 0000-0002-7991-9700; Sameen Hassan 0000-0002-4047-1742; Ahmed Daoud 0000-0001-6311-3887; Bahaaeldin Baraka 0000-0002-7133-5209; Theo Trandafirescu 0000-0002-5953-9516; Vincent Rizzo 0000-0002-5530-447X.

Author contributions: Nassar M, Nso N, Alfishawy M, Baraka B screened the articles; Nassar M, Nso N, Alfishawy M, Novikov A, Yaghi S, Medina L, Toz B, Lakhdar S, Idrees Z, Kim Y, Gurung DO, Siddiqui RS, Zheng D, Agladze M, Sumbly V, Sandhu J, Castillo FC, Chowdhury N, Kondaveeti R, Bhuiyan S, PerezLG, Ranat R, Gonzalez C, Bhangoo H, Williams J, Osman AE, Baraka B, Ariyaratnam J, Mohamed M, Omran I, Lopez M, Nyabera A, Landry I, Iqbal S, Kong J, Gondal AZ, Hassan S, Daoud A reviewed the included studies and wrote the manuscripts; Trandafirescu T and Rizzo V reviewed and edited the manuscript.

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million people have been infected worldwide, with over 1.3 million deaths. While the global spotlight is currently focused on combating this pandemic through means ranging from finding a treatment among existing therapeutic agents to inventing a vaccine that can aid in halting the further loss of life.

AIM

To collect all systematic reviews and meta-analyses published related to COVID-19 to better identify available evidence, highlight gaps in knowledge, and elucidate further meta-analyses and umbrella reviews that are yet to be performed.

METHODS

We explored studies based on systematic reviews and meta-analyses with the key-terms, including severe acute respiratory syndrome (SARS), SARS virus, coronavirus disease, COVID-19, and SARS coronavirus-2. The included studies were extracted from Embase, Medline, and Cochrane databases. The publication timeframe of included studies ranged between January 01, 2020, to October 30, 2020. Studies that were published in languages other than English were not considered for this systematic review. The finalized full-text articles are freely accessible in the public domain.

RESULTS

Searching Embase, Medline, and Cochrane databases resulted in 1906, 669, and 19 results, respectively, that comprised 2594 studies. 515 duplicates were subsequently removed, leaving 2079 studies. The inclusion criteria were systematic reviews or meta-analyses. 860 results were excluded for being a review article, scope review, rapid review, panel review, or guideline that produced a total of 1219 studies. After screening articles were categorized, the included articles were put into main groups of clinical presentation, epi-demiology, screening and diagnosis, severity assessment, special populations, and treatment. Subsequently, there was a second subclassification into the following groups: gastrointestinal, cardiovascular, neurological, stroke, thrombosis, anosmia and dysgeusia, ocular manifestations, nephrology, cutaneous manifestations, D-dimer, lymphocyte, anticoagulation, antivirals, convalescent plasma, immunosuppressants, corticosteroids, hydroxychloroquine, renin-angiotensin-aldosterone system, technology, diabetes mellitus, obesity, pregnancy, children, mental health, smoking, cancer, and transplant.

CONCLUSION

Among the included articles, it is clear that further research is needed regarding treatment options and vaccines. With more studies, data will be less heterogeneous, and statistical analysis can be better applied to provide more robust clinical evidence. This study was not designed to give recommendations regarding the management of COVID-19.

Key Words: Systematic review; Meta-analyses; COVID-19; Review; Coronavirinae

Core Tip: Coronavirus disease 2019 (COVID-19) has left a significant impact on the world's health, economic and political systems. This study was not designed to give recommendations regarding the management of COVID-19. There is a need for future research to understand the scope of possible vaccines and prevention/treatment options in the setting of COVID-19.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) has left a significant impact on the world's health, economic and political systems; as of November 20, 2020, more than 57 million people have been infected worldwide, with over 1.3 million deaths[1]. While the global spotlight is currently focused on combating this pandemic through means ranging from finding a treatment among existing therapeutic agents to inventing a vaccine that can aid in halting the further loss of life.

The scientific community has been extremely busy with COVID-19. Thousands of research articles have been published to date, with centers worldwide trying to take the lead and find a solution to this problem.

Systematic reviews and meta-analyses represent the highest level of evidence given to a structured search method, with critical appraisals limiting bias and reaching a summative conclusion. Systematic reviews and meta-analyses are now an integral part of clinicians' daily practice. They help busy clinicians stay up-to-date by supplying aggregate data from multiple studies and facilitates evidence-based medicine. These studies are also often used in the synthesis of clinical guidelines[2].

Herein, we aimed to collect all systematic reviews and meta-analyses published related to COVID-19 to better identify available evidence, highlight gaps in knowledge, and elucidate further meta-analyses and umbrella reviews need that to be performed.

MATERIALS AND METHODS

Searching strategy

We extracted systematic reviews and meta-analyses covering a range of aspects related to COVID-19 (coronavirus disease) assessment, prevention, management, testing, analysis, and epidemiological findings. We accessed full-text articles in the English language on COVID-19 across databases including Embase, Medline, and Cochrane. We focused on extracting coronavirus disease data and findings published between January 01, 2020, and October 30, 2020. We formulated various combinations of keywords, including severe acute respiratory syndrome (SARS), SARS virus, coronavirus disease, COVID-19, and SARS coronavirus-2 (SARS-CoV-2), to fetch the articles of interest.

Inclusion/exclusion parameters

We did not consider the retrieval of rapid reviews, scope reviews, opinion papers, guidelines documents, panel reviews, and other review articles for our narrative review—the included articles are based on full-text and freely accessible studies available in the public domain. Our articles specifically included systematic reviews and meta-analyses on coronavirus disease. We excluded systematic reviews/meta-analysis with COVID-19 as the secondary focus or data/analyses of morbidities/comorbidities other than coronavirus disease.

Selected studies

The full-text publicly accessible studies were copied into our centralized database for their data assessment and thematic analyses.

RESULTS

Searching Embase, Medline, and Cochrane databases resulted in 1906, 669, and 19 results, respectively, that comprised 2594 studies. 515 duplicates were subsequently removed, leaving 2079 studies.

The inclusion criteria were systematic reviews or meta-analyses. Of 860 results were excluded for being a review article, scope review, rapid review, panel review, or guideline that produced a total of 1219 studies (Figure 1).

After screening, articles were then categorized into clinical presentations, epidemiology, screening and diagnosis, severity assessment, special populations, and treatment. Subsequently, the articles were then divided into another subclassification of the following groups: gastrointestinal, cardiovascular, neurological, stroke, thrombosis, anosmia, and dysgeusia, ocular manifestations, nephrology, cutaneous manifestations, D-dimer, lymphocyte, anticoagulation, antivirals, convalescent plasma, immunosuppressants, corticosteroids, hydroxychloroquine (HCQ), renin-angiotensin-aldosterone system (RAAS), technology, diabetes mellitus (DM), obesity, pregnancy, children, mental health, smoking, cancer, and transplant, as seen in Table 1.

DISCUSSION

Epidemiology

We have reviewed 171 systematic reviews regarding the epidemiology of COVID-19 infection. The incubation period of COVID-19 showed a median of 5.1 d with the 95th percentile of 11.7 d. The incubation period of COVID-19 that induced severe acute respiratory distress syndrome had an average of 6.0 d[3].

Several systematic reviews showed mortality with hospitalization was invasive mechanical ventilation was 13%[4]. Higher mortality was seen in patients with the following factors: Living in the European region, male sex, older age, active smoking, alcohol use, intensive care unit admission, comorbid conditions such as DM, obesity, hypertension, chronic lung disease, cerebrovascular disease, coronary heart disease, chronic renal disease, chronic liver disease and presence of malignancies[5,6]. The fatality rate was approximately 0.68%, with very high heterogeneity[5]. Some reviews described specific gene susceptibilities and recommended further genetic research[6]. O blood group is thought to be protective against COVID-19 with regard to mortality and susceptibility[7].

Several systematic reviews showed the most common laboratory or radiological finding of COVID-19 was lymphopenia, bilateral ground opacities in lungs, elevated C-reactive protein (CRP), lactate dehydrogenase (LDH), procalcitonin, D-dimer, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and/or creatinine. Fever, cough, and fatigue were the most common presenting clinical symptoms[8]. Dyspnea, anosmia, diarrhea, and myalgia were also frequently found in patients[9]. Healthcare workers (HCWs) who were positive with real-time reverse transcription polymerase chain reaction (RT-PCR) showed no symptoms in 40% of cases, 5% showed severe clinical complications, and 0.5% unfortunately died[9].

Regarding personal protective equipment (PPE), face mask users had decreased transmission, especially with N95 or similar equipment level[10]. Maintaining a physical distance of 1 meter or more and eye protection also revealed lower transmission rates[11]. Reusing masks did not yield a statistically significant result. Duration of PPE usage was recommended for no more than six hours of continuous use, with a break needed every two to three hours. PPE use is advised with appropriate hydration and skincare. The use of a powered air-purifying respirator (PAPR) is associated with greater heat tolerance but lower scores for mobility and communication ability. However, the reviews do not indicate a difference in HCW infection utilizing PAPR devices *vs* other compliant respiratory equipment. PPE can also be reused if they receive the appropriate dose of ultraviolet germicidal irradiation (UVGI) treatment[12].

Systematic reviews regarding disinfectants recommended using UVGI with vaporized hydrogen peroxide, non-thermal plasma, and air filters with photocatalytic disinfection[13]. Irritability of skin with propanol and isopropanol use was noticed but was less than frequent hand washing with detergent[14].

Systematic reviews regarding transmission showed inconclusive evidence about the viability and infectivity of SARS-CoV-2 in aerosol-generating procedures, but some studies showed an increased risk of infection with endotracheal intubation[15]. Clusters of infection played an important role. Frequently touching the T-zone (eyes, nose, mouth, chin) increases the chance of COVID-19 infection[16]. Transmission from an asymptomatic/pre-symptomatic patient is possible and more significant with pre-symptomatic patients. Quarantine is an essential factor in reducing the incidence of transmission[17]. No sexual or vertical transmission was observed and was not related to the route of delivery or breastfeeding[18]. A warm and humid climate reduced the

Table 1 Classification of the systematic reviews

No.		Clinical presentation	Epidemiology	General	Screening and diagnosis	Severity assessment	Special populations	Treatment	Grand total
1	Mental	15	6		2	2	34	4	63
2	Gastrointestinal	33	3		6	6	9		57
3	Cardiovascular	15	3		3	7	9	2	39
4	Children	4		1	1		33		39
5	Neurology	33				1	2		36
6	Diabetes	5	2		1	8	11	2	29
7	Hydroxychloroquine							28	28
8	Technology		1		5		14	4	24
9	RAAS					5	1	15	21
10	Antiviral							20	20
11	Cancer						20		20
12	Pregnancy	1	1	1	1		16		20
13	Transmission		18						18
14	General	16		1					17
15	Smoking	1	3			3	8	1	16
16	Thrombosis	13			2	1			16
17	Obesity		1			7	6		14
18	Smell	14							14
19	Corticosteroids							13	13
20	Stroke	7	1			2	3		13
21	D-dimer				9	3			12
22	Renal	9				1	1		11
23	Lymph				6	3			9
24	Cutaneous	8							8
25	Ocular	8							8
26	Anticoagulation							7	7
27	Convalescent plasma							6	6
28	Immunosuppressive							6	6
29	Transplant						5		5
30	Hospital	1				1	2		4
31	Incubation	1	3						4
32	PPE		4						4
33	Traditional Chinese							4	4
34	Chinese							3	3
35	Older adult	2					1		3
36	Rehab						2	1	3
37	Sex		2			1			3
38	Asthma	2							2
39	Asymptomatic	2							2
40	Cytokine					2			2

41	Disinfection		2						2
42	Fatality		1				1		2
43	Fibrin				2				2
44	Guillain-Barre	2							2
45	Oral	1					1		2
46	Thrombocytopenia	2							2
47	Kawasaki	1							1
48	Multisystem inflammatory	1							1
49	Obstructive sleep apnea						1		1
50	Unclassified	27	120	14	139	62	82	155	579
	Grand Total	223	171	17	177	115	263	271	1219

RAAS: Renin-angiotensin-aldosterone system; PPE: Personal protective equipment.

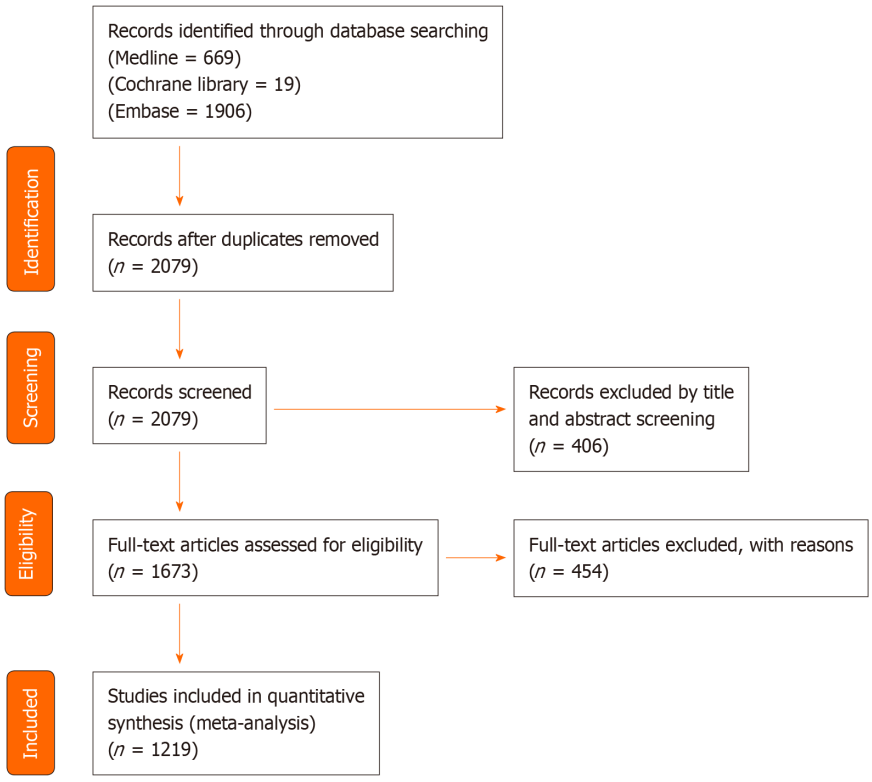

Figure 1 Shows a PRISMA flow diagram of our literature search.

spread of COVID-19. Viral carriage on the outer surface of surgical masks worn by HCW who treat patients with clinical respiratory illness is low[19].

Transmission

In a review of the 18 systematic reviews regarding the transmission of SARS-CoV-2, the consensus remains that respiratory infection *via* droplet and aerosolization in the human-to-human setting remains the most likely form of infection and that other forms such as contact with fomites and vertical transmission played a small role in contamination with the disease[20,21].

In one review article, the aerosolized transmission of SARS-CoV-2 showed that the virus was still viable. However, no correlation was shown regarding aerosolized transmission and disease[20]. If infection were to occur, contact would have to be in an

enclosed environment. For aerosol transmission, the highest risk was related to health care workers involved in aerosol-generating procedures with the high-risk involving direct airway manipulation such as manual ventilation with intubation[15].

With the onset of the SARS-CoV-2 pandemic, data regarding all transmission forms has been researched, leading to one systematic review that found no direct virologic evidence of vertical transmission. Another review concluded that neonatal infection with SARS-CoV-2 was uncommon and that the rate of infection was no more significant than when the baby was born vaginally, *via* cesarean section, or if the baby breastfed and remained with the mother[22].

As human-to-human contact is responsible for SARS-CoV-2 infection, one systematic review analyzed 108 cluster infections from 13 countries[23]. This concludes that because the most common places associated with infection were family contact, community transmission, transportation, and healthcare-related facilities[23], minimizing unnecessary contact and social distancing should be strictly implemented to contain infection clusters.

Screening and diagnosis

One hundred seventy-seven articles were reviewed regarding the screening and diagnosis of COVID-19. The most common presenting symptoms to screen for were fever and cough. Studies also showed associated anosmia and loss of taste[24]. The most susceptible patients seem to be those with hypertension, diabetes, coronary artery disease, cerebrovascular disease, and chronic obstructive pulmonary disease. Males older than 50 with comorbidities were more likely than females to have severe pneumonia and dyspnea[25]. The pediatric population presented more commonly with diarrhea, and the infection was notably less severe. Innovations in technology such as contact tracing applications and telemedicine have been able to help promote surveillance of the disease and have been integrated into traditional medicine. Contact tracing tools have been shown to reduce transmission but can be challenging to implement in large populations[26]. There are privacy concerns, and further studies would be needed to show their effectiveness. Furthermore, infrared thermal screening was studied against the traditional thermometer, and it showed a low positive predictive value during the initial outbreak but continued to have a high negative predictive value throughout the pandemic's early and later stages[23].

Many labs were significantly appropriate for screening purposes. Elevated inflammatory markers, biochemical biomarkers, and hematological markers such as CRP, erythrocyte sedimentation rate (ESR), troponin, decreased lymphocyte count, fibrinogen, fibrinogen degradation products level, LDH, ferritin, and D-dimer on admission were associated with a poor outcome and were predictive of the severity and mortality of COVID-19 pneumonia[27]. An elevated D-dimer was linked to an overdiagnosis of disseminated intravascular coagulation (DIC) and led to inappropriate treatment with anticoagulation therapy[28]. Platelet count did not correlate with the severity of the disease. High levels of ferritin and interleukin-6 (IL-6) were elevated due to the cytokine storm from COVID-19, which correlated with increased intensive care unit (ICU) stays and mechanical ventilation requirements. The most common abnormality for the liver function test was hypoalbuminemia. However, some studies have shown elevated ALT, AST, and total bilirubin. An elevated Red Cell Distribution Width also correlated with the disease's severity[29].

There were numerous tools used for diagnosis. Stool studies exhibited more viral shedding than respiratory tract samples, which were easier to collect from the pediatric population. Studies showed that the RNA strains could be found in the saliva within seven to ten days of onset. Nasopharyngeal swabs showed more sensitivity than oropharyngeal swabs. Salivary samples are questionable, with inadequate sample power to comfortably substitute for the PCR based nasopharyngeal swab. The Reverse Transcriptase PCR has a sensitivity of 64% and specificity of nearly 100% but should not be used alone as the gold standard due to bias in measurements[30]. No evidence has shown a practical point of care serologic test at the time of this analysis. The virus uses Angiotensin-converting enzyme-2 (ACE2) receptors to enter the body. ACE2 receptors are found in the respiratory epithelium, gastrointestinal mucosa, and liver cholangiocytes. A portion of the adult patients presented with gastrointestinal symptoms, where computed tomography (CT) scans of the abdomen showed findings including small and large bowel wall thickening and pneumatosis intestinalis. The indications that the Infectious Disease Society of America recommends for serologic testing for antibodies are patients with clinical symptoms who have a negative diagnostic test and children with multisystem inflammatory syndrome to estimate antibody levels against the disease. The virus is positive in blood, urine, and stool, with higher viral shedding in blood and stool. Urine has been shown to have increased

viral shedding in more severe patients with systemic disease and those requiring ICU admissions[31].

COVID-19 has predominantly been diagnosed using a real-time PCR test and/or chest CT with no consensus on which method is superior[32,33]. CT scans are sensitive but not specific, as imaging cannot differentiate coronavirus from other respiratory diseases. A chest CT with no pathologic findings cannot exclude COVID-19 alone, even if the patient has no symptoms. Also, positive CT findings with no symptoms may still warrant further testing because 90% of those patients later developed symptoms. The most common findings are ground-glass opacities with a distribution in the peripheral regions[34,35]. Other common findings are interlobular septal thickening and air bronchograms. COVID-19 lung pathology has consistently shown histology consistent with acute respiratory distress syndrome (ARDS) with frequent microthrombi[36]. Patients do not usually present with lymphadenopathy or pleural effusions. Lung ultrasounds of COVID-19 patients most commonly show the B-line interstitial pathology pattern[37]. CT scans of the chest still seem to be the most helpful imaging modality in pediatric patients and can detect pneumonia even before presenting symptoms.

Clinical presentation

We included 204 systematic review articles looking at clinical presentations of COVID-19. This disease can present in many ways. Nearly all organ systems can be affected, including the respiratory, cardiovascular, renal, gastrointestinal, endocrine, reproductive, central nervous system, bone marrow, and skin. Although most COVID-19 patients typically present with respiratory symptoms, ranging from upper airway disease to severe ARDS with multiorgan failure, many systematic reviews examined other organ systems implicated in the body. Several review studies concluded that cardiac and renal complications following respiratory complications are the most common clinical complications of COVID-19[38,39]. The most prevalent comorbid condition in patients with COVID-19 is diabetes, with it being both a significant risk factor and an indicator of poor prognosis[40]. Also, studies have shown that the elderly population has been affected by COVID-19 with high severity[41].

High rates of cardiovascular disease have been reported, and several studies showed a significant association with increased mortality and ICU admissions. Arrhythmias are prevalent and are reportedly the second most common cardiovascular complication[42,43].

Many reports are available that look at COVID-19 infection and the prothrombotic state, explained by coagulation activation, endothelial dysfunction, and formation of in situ thrombi rather than embolization of peripheral thrombi. This cascade of events causes deep venous thrombosis, pulmonary microthrombi, pulmonary embolism, cerebral venous thrombosis, and acute ischemic strokes that in some cases lead to a critical condition with poor long-term outcomes, residual disability, and prolonged rehabilitation[44]. Some studies showed ischemic strokes to be the most important prognostic marker and indicator of severity and poor clinical outcome. Prompt evaluation and early treatment with anticoagulation were associated with reduced mortality and better clinical outcome[45].

It has been found that many patients experience digestive symptoms as the primary complaint. Some studies have reported that patients with digestive symptoms had a trend to develop severe critical illnesses. Several available meta-analyses suggest that acute liver injury and elevated liver enzymes were significantly associated with COVID-19 severity and predicted worse outcomes.

Acute kidney injury (AKI) is a common complication of COVID-19. AKI's incidence is between 8%-20% depending on the study. In some studies, the presence of AKI is associated with a 13-fold increased risk of mortality[46]. Age, DM, hypertension, and baseline serum creatinine (SCr) levels are associated with increased AKI incidence. Concerns have emerged about the potential impact of COVID-19 on male reproductive organs and male fertility.

There is strong evidence of neurological involvement ranging from Guillain–Barré syndrome, delirium, and encephalitis, to cerebral venous thrombosis and ischemic strokes, with acute ischemic strokes being the most frequently reported complication with the highest mortality rate. Neurologic manifestations are shown to develop approximately one to two weeks following the onset of respiratory symptoms[47]. Olfactory and gustatory dysfunction have been described and used to aid in clinical diagnosis as they present reasonably early after the disease's contraction. Cutaneous involvement has been described in several review articles and was shown to manifest occasionally in asymptomatic carriers. Several ongoing studies investigate the role of early identification of cutaneous involvement, which may be vital to early diagnosis

and lead to a possible better prognosis[39].

Finally, several systematic reviews have looked at the implication of COVID-19 on mental health and reported increased suicide risk, depression, and anxiety levels in the general population irrespective of COVID-19 status.

Gastrointestinal

We included 57 systematic review articles regarding the association of COVID-19 with the gastrointestinal system. These studies found that those with high rates of digestive symptoms, acute liver injury, and elevated liver enzymes are more likely to develop severe/critical illnesses. Common presenting gastrointestinal symptoms included abdominal pain, nausea, vomiting, and diarrhea. Frequent abnormalities in liver function tests included hypoalbuminemia, derangements in gamma-glutamyl-transferase, increased bilirubin values, prolonged prothrombin time, and deranged aminotransferases[48]. Patients with preexisting liver abnormalities such as hepato-cellular carcinoma, metabolic associated fatty liver disease, and chronic liver disease had an increased risk of COVID-19 disease progression, more severe COVID-19 infection, and increased mortality rates[49]. Two systematic reviews found that in patients with inflammatory bowel disease and COVID-19, diarrhea occurred more frequently; however, these patients did not appear to be at a higher risk of developing COVID-19 than those in the general population[50,51]. One systematic review investigated the relationship between COVID-19 and fecal nucleic acid testing in the pediatric population and found that the positive rate for fecal nucleic acid testing in COVID-19 children was relatively high, suggesting that fecal nucleic acids can be used as a method of detecting COVID-19 in this population[52]. Additionally, several studies observed that the gastrointestinal manifestation of COVID-19 raises the question of possible transmission through the fecal-oral route in both the adult and pediatric populations, indicating that healthcare workers should exercise caution when collecting stool samples.

Cardiovascular

We included 39 systematic reviews about patients' cardiovascular manifestations with COVID-19 infection and the clinical significance of biomarkers, preexisting cardiovascular disease, and thromboembolic disease risk.

The most common comorbidities associated with increased mortality among patients with COVID-19 infection were hypertension, coronary artery disease, and heart failure. These factors were positively correlated with an augmented risk of hospitalization, poor outcomes, and death. Although preexisting conditions consistently demonstrated increased complications and mortality, COVID-19 also increased cardiovascular disease by inducing cytokine storms[53]. There was an association between COVID-19 infection and direct cardiovascular complications, including myocardial injury, heart failure, myocardial infarction, myocarditis, arrhythmias, and blood clots, leading to increased mortality and adverse outcomes. There was poor documentation about the incidence and nature of arrhythmias in the setting of COVID-19. A systematic review reported Takotsubo syndrome could be associated with COVID-19 infection and demonstrated a higher prevalence in older women with higher rates of complications[54].

The biomarkers associated with increased mortality were LDH, creatinine kinase, brain natriuretic peptide, and troponin I. Lastly, the coagulopathies observed in patients with COVID-19 infection ranged from mild laboratory alterations to DIC. It is proposed that the endotheliopathy could be from direct endothelial infection with SARS-CoV-2 and indirect damage caused by the inflammation.

Cardiovascular complications added to the elevated morbidity and mortality in patients with preexisting cardiovascular risk factors. Further studies could help better to identify the role of SARS-CoV-2 in this population.

Neurology

The most common neurological symptoms in our analyses of COVID-19 patients included chemosensory dysfunction, vascular events, neurologic syndromes, encephalopathies, and inflammation[55,56]. Strokes were associated with the highest mortality rate. Neurological manifestations developed one to two weeks after the onset of respiratory disease, but they were also seen in patients who did not have any respiratory disease. One systematic review article regarding the severity assessment of COVID-19 patients with neurological symptoms described that the predominant central nervous system symptoms were headaches and dizziness, while the most common peripheral nervous system symptoms were dysfunction in taste and smell.

Neurologic similarities were seen in the symptomatology of COVID-19, SARS, and Middle East respiratory syndrome[53,57]. All three viral syndromes were associated with similar neurologic complications, such as fatigue, headache, and smell and taste disorders. The myriad of neurological manifestations in COVID-19 syndrome should be further explored to elucidate the pathogenesis of COVID-19 related neurologic disease.

Stroke

According to 13 articles, including multiple meta-analyses and systematic reviews, COVID-19 infection has been associated with significant neurological manifestations within the central nervous system, including stroke, intracranial hemorrhage, encephalomyelitis, and acute myelitis. We included one systematic review and meta-analysis that suggests an increased risk of stroke in hospitalized patients with preexisting cerebral vascular disease. This increased risk of stroke was associated with an increase in adverse outcomes, most notably mortality. One multicenter study and meta-analysis suggest that more severe COVID-19 infections are associated with a higher risk of stroke, with an overall pooled risk estimated at 2.9%[58].

One systematic review and analysis showed COVID-19 patients who suffered from stroke symptoms developed neurologic symptoms after an average of nine days from the onset of their respiratory illness[59]. Multiple studies suggest that pro-inflammatory markers associated with COVID-19 infection, including D-dimer, IL-6, ferritin, and fibrinogen, may contribute to an inflammatory process mediating cerebrovascular accidents. One study found correlations with large vessel thrombosis and anterior circulation strokes[60], but more data and institutional collaboration of information are needed to understand the significance of the anatomic locality of strokes associated with COVID-19 infection. This multi-centered approach may elucidate future guidelines for certain patients, risk factors, or clinical findings unique to COVID-19 patients with neurologic symptoms.

Thrombosis

We included 16 systematic review articles regarding arterial and venous thromboembolism in patients with COVID-19 infection. Thromboembolic events in the deep veins of the lower extremities, pulmonary arteries, and cerebral veins suggested that the high rate of pulmonary artery occlusion was secondary to both embolic events from deep veins of legs and in situ thrombosis in pulmonary arteries. The proposed mechanism for these thrombotic events includes a combination of endothelial injury, platelet activation, hyperviscosity, blood flow abnormalities, and immune reactions. Segmental and subsegmental arteries were more commonly involved than the main pulmonary arteries[61]. The severity of COVID-19 infection correlates directly with thrombotic events, with a higher incidence of pulmonary embolism reported in ICU patients than general medical floor patients.

Additionally, it was discovered that ICU level patients also experienced a higher incidence of failed or inappropriate dosing of anticoagulation, whether preventive or therapeutic. Overall, higher mortality was seen in COVID-19 patients with thromboembolic events. However, data is limited in addressing whether or not there are mortality benefits seen with anticoagulation.

Anosmia and dysgeusia

There are 14 systematic review articles about chemosensory dysfunction. Ten articles discussed anosmia and dysgeusia, three articles for anosmia only, and one article for dysgeusia. Anosmia and dysgeusia are common early symptoms of COVID-19. There is a discrepancy in the prevalence of anosmia and dysgeusia according to sensitivity [62]. The prevalence of anosmia in an average individual was shown to vary according to age, sex, and testing technique[63]. The prevalence of anosmia, dysgeusia, or both as a symptom for COVID-19 was less often seen in male and geriatric patients. Anosmia was shown to be highly specific for COVID-19 and should be included in the evaluation process of suspected patients. As these dysfunctions were often seen early in the disease course, their presence may indicate early disease onset and indicate the clinician recommended isolation to prevent transmission. The mechanism of viral pathogenesis and causality remains a topic of study.

Ocular manifestations

The most common ocular presentations of COVID-19 are conjunctivitis, hyperemia, photophobia, dry eyes, chemosis, epiphora, blurry vision, foreign body sensation, ocular pain, floaters, and eyelid dermatitis. Patients may present with mild eyelid

edema and/or tender, palpable preauricular and submaxillary lymph nodes. Rare presentations include herpes-like pseudo-dendritic infiltration of the cornea and bilateral pseudomembranous conjunctivitis.

COVID-19 virus entry is mediated by the binding of viral surface spike (S) glycoprotein to the ACE2 receptor[64]. Two primary mediators (the ACE-2 receptor and cell surface protease enzyme, TMPRSS2) are involved in this mechanism. Immunohistochemistry has shown the presence of ACE-2 receptors on the conjunctiva, limbus, and cornea. The ocular manifestations' treatment options include topical and systemic preparations, with antibiotic eye drops and artificial tears with or without corticosteroid eye drops.

COVID-19 patients treated with proning have had additional side effects, including exposure keratopathy, microbial keratitis, increased intraocular pressures, occlusion of the central retinal artery, and in some severe cases, orbital compartment syndrome. The prevention of acute orbital compartment syndrome was achieved with surgical interventions such as lateral canthotomy and cantholysis. Keratopathy can be prevented by lubricant ointments, moisture chambers, and polyethylene films. Topical viscous lubricants and mechanical closure of the eyes with hypoallergenic tapes or topical dressings may also be used.

This meta-analysis emphasizes the need for PPE for all healthcare workers involved in the care of COVID-19 patients to prevent exposure and infection. Along with a gown and gloves, wearing eye protection is also essential to prevent the spread of COVID-19 infection as eye mucous membranes can play a crucial part in transmitting COVID-19 viral particles.

Nephrology

A review of 10 literature publications showed that kidney injury incidence varies widely across studies, depending primarily on the severity of the disease. In one pooled analysis, AKI was detected in 8.3% of patients with COVID-19 *vs* 19.9% in critically ill patients[65]. Moreover, the association between renal impairment and poor outcomes is well established, with significantly increased mortality and need for ICU level of care. These findings were irrespective of age, sex, or other comorbidities such as hypertension, diabetes, and respiratory diseases. The association of mortality between preexisting chronic kidney disease (CKD) and the severity of COVID-19 infection has been studied[66]. The need for renal replacement therapy (RRT) correlated with poor outcomes. Mortality was also significantly increased in renal transplant recipients. Several studies found a statistically significant rise in SCr and blood urea nitrogen in patients with COVID-19, as well as the severity of proteinuria, hematuria, and decline in estimated glomerular filtration rate. Viral RNA positivity was detected in the urine of 5.7% of patients in some studies[67], but it is unclear if this correlated with disease severity. While both the occurrence of and mortality due to kidney injury seem to be declining (≥ 75% in critically ill patients with mortality of 67% in studies published before the end of May 2020), it still constitutes a significant morbidity and mortality factor in COVID-19 patients. For this reason, health experts call for the early detection of renal dysfunction in patients with COVID-19 to prevent further kidney damage and provide appropriate renal support.

Cutaneous manifestations

While respiratory and gastrointestinal manifestations are the predominant presenting features of COVID-19 patients, reports on cutaneous manifestations are increasingly noted. In some studies, the estimated prevalence of cutaneous manifestations in COVID-19 ranges between 0.2%-20%. The cutaneous manifestations recorded are urticaria, chilblain-like lesions, livedo reticularis, petechial rash, and finger/toe gangrene, with the majority of lesions localized on the trunk, followed by extremities [68]. Cutaneous involvement usually follows the respiratory symptoms; nonetheless, in the minority, it preceded systemic features.

Histopathological analyses suggested a predominance of spongiosis, perivascular infiltrate of lymphocytes, and thrombogenic vasculopathy, but the potential mechanisms remain to be investigated. One small systematic review of 507 European patients suggested that the presence of the ACE-2 receptor on skin keratinocytes proposes that skin might be a potential target for the virus[69].

A systematic review from Switzerland mentions vesicular rashes during an initial diagnosis of COVID-19, suggesting cutaneous involvement as a valuable prognostic factor for disease progression and correlation to disease severity[70].

Overall, developing a comprehensive understanding of all clinical manifestations of COVID-19 infection will require knowledge of all possible disease presentations. It is suggested that accurate and rapid identification of cutaneous manifestations may be

vital to early diagnosis and can portend a better prognosis in COVID-19 patients. Notably, the majority of these studies failed to report any correlation between COVID-19 severity and skin lesions.

Severity assessment

Although the reported mortality rates through the pandemic's progression have decreased, ICU mortality rates remain higher than those seen in ICU admissions for non-COVID viral pneumonia. Over 110 systematic review articles investigating the aspects of severe COVID-19 infection were analyzed with the goal of risk stratification and the mitigation of poor outcomes.

Predisposing factors which increase the patient's risk of severe disease should be assessed at the onset of admission. Multiple studies found that individuals with obesity, cardiovascular disease, hypertension, diabetes, chronic lung disease/smoking history, CKD, chronic liver disease, history of cerebrovascular events, male sex, or older age were found to have a more severe illness (with hypertension, cardiovascular disease, and diabetes conferring the most significant risk). Specific clinical assessments were found to have a greater likelihood of severe disease burden, including dyspnea at presentation, elevated pro-inflammatory markers, evidence of coagulopathy, signs of cardiac damage, acute kidney injury, lymphopenia, or neutrophilia[71].

Next, physical and lab assessments that have been found to indicate a greater likelihood of more severe disease are patients presenting with dyspnea, elevated inflammatory markers (CRP, ESR, somatostatin analogue, IL-6, IL-8, PCT, D-dimers, ferritin, interferon-α, tumor necrosis factor-α), evidence of coagulopathies (elevated D-dimers, thrombocytopenia, DIC, thromboembolic events), signs of cardiac damage (elevated LDH, IL-6, creatine kinase MB, elevated Pro-B-type natriuretic peptide, right ventricular dilation/evidence of right heart strain, emerging arrhythmias), acute kidney injury, and white blood cell findings consistent with either lymphopenia or neutrophilia (the high neutrophil-to-lymphocyte ratio is positively associated with disease severity)[72,73].

Critical care management of diffuse alveolar damage and pulmonary vasculature microthrombi can be achieved through mechanical ventilation or extracorporeal membrane oxygenation (ECMO). However, there have been increasing incidents of complications seen in patients requiring ECMO with concurrent COVID-19 infection, likely due in part to the hematological dynamic changes inherently involved in ECMO, coupled with the pro-inflammatory and pro-coagulopathic nature of the COVID-19 disease itself. Due to the reliable association with IL-6 and COVID-19 disease severity, tocilizumab and monoclonal antibody therapy early in the disease course have shown promising results. Additionally, RAAS inhibitors in hypertensive patients showed an improved prognosis in many cases[74].

The severity of COVID-19 infection in adults with the comorbidities mentioned above differs from pediatric and immunosuppressed patients. While the severity of the organic disease is severe, the psychological burden develops as the global pandemic continues. Studies show that isolation and social distancing measures necessary in epidemic states to minimize the spread of communicable diseases have significant psychological impacts on parents, children, and adolescents. Although long-term and persistent effects of the pandemic are currently unknown in instances of anxiety, depression, stress, and other mental strains yet to be discovered, what has been observed is that the duration of the isolation is having a more significant impact on the mental well-being of children and adolescents than the intensity of isolation. Solutions to mediate psycho-social burdens associated with the pandemic are evolving, but the child and adolescent developmental impact remain to be seen.

D-dimer

D-dimer elevations as a sign of pro-inflammation are significantly related to COVID-19 infections. The activation of the coagulation cascade is a common feature of DIC and adverse clinical outcomes in COVID-19. D-dimer is an important prognostic tool that is often found to be elevated in patients with severe infection. Regardless of the D-dimer reference value, the studies show that D-dimer concentrations were significantly higher in patients with more severe infection than non-severe forms and were associated with an increased risk of mortality[75]. Early integration of D-dimer testing can be practical for better risk stratification and guidance in clinical decision-making. Further investigation is warranted to evaluate the appropriateness of D-dimer monitoring as a management tool for this disease.

Lymphocytes

Meta-analyses evaluating the feasibility of specific markers in assessing the severity and prognosis of COVID-19 patients demonstrated a significant inverse association of peripheral lymphocyte levels with progression and mortality. Those patients with a low lymphocyte count at baseline were found to have a higher risk of disease severity. A meta-analysis reported that lymphopenia and neutrophilia on admission were significantly associated with increased risk of progression to severe disease and death, suggesting that these variables may help risk stratification models[76].

However, the results of studies regarding the prognostic value of lymphocyte subsets are inconsistent. A meta-analysis concluded that increased neutrophil/lymphocyte levels and a low lymphocyte-to-CRP ratio might indicate a poor prognosis in COVID-19 patients[77]. COVID-19 predictive equations were generated in another meta-analysis based on CRP and D-dimer levels and lymphocyte or the neutrophil count. These equations exhibited high specificity, sensitivity, positive and negative predictive values and suggested that the equations could predict the severity of outcomes of COVID-19 patients[78].

Treatment

Treatment articles reviewed included those studying antiviral drugs such as Lopinavir/Ritonavir, Favipiravir, and Remdesivir. Lopinavir/Ritonavir did not show any significant difference in mortality or progression to a more severe course or cure. However, some benefits were seen in the duration of hospital stay. Favipiravir may have some role in improving clinical and radiological imaging but has no benefit on oxygen requirements or viral clearance. Remdesivir is the only antiviral drug that has been shown to improve recovery and reduce serious adverse events[79-81]. Additionally, it may reduce mortality, though some studies did not show mortality benefits. It was also seen that five-day treatment with Remdesivir might provide similar benefits with fewer harmful effects than a 10-d course[82].

The prevalence of venous thromboembolism in COVID-19 patients has been reported to be about 10%-35%, with autopsy results rising to 60%. Biomarkers related to platelet activation like D-dimer have been shown to have prognostic value in COVID-19 patients. There was a slight tendency to reduce the mortality rate using therapeutic anticoagulation in patients with COVID-19 on mechanical ventilation.

Current evidence shows no benefit in using HCQ in patients with mild to moderate COVID-19 infection. Additionally, HCQ has been associated with higher adverse events, including skin pigmentation, ocular toxicity, QT prolongation, and worsening psoriasis lesions[83].

The effect of convalescent plasma products is based on randomized controlled trials (RCT). In these trials, convalescent plasma did not decrease all-cause mortality, but early initiation may decrease mortality rate compared to late initiation. Convalescent plasma and immunoglobulin were both effective in relieving symptoms of COVID-19 [84,85].

Corticosteroids may reduce mortality in patients with COVID-19 and ARDS, but the evidence is inconsistent for patients with COVID-19 without ARDS. Excessive inflammatory response and lymphopenia were both associated with severity, leading to the recommendation that, if not contraindicated, steroids should be considered in the absence of adverse effects.

Tocilizumab is an interleukin-6 receptor antagonist that has been used in the treatment of severe COVID-19 infections[86]. There are indications that tocilizumab can reduce mortality and prevent mechanical ventilation in severe COVID-19 infections, but results need to be confirmed with high-quality clinical trials before the drug is implemented as a standard of care.

Anticoagulation

Anticoagulation methods studied in the treatment of COVID-19 included unfractionated and low molecular heparin, apixaban, clopidogrel, dipyridamole, and tissue plasminogen activator. The association between mortality rate and incidence of thromboembolic events in patients with COVID-19 infection receiving venous thromboembolism prophylaxis was evaluated as combination therapy or single-drug therapy, with studies showing no superiority of any anticoagulant.

According to one article that looked at three separate studies[87], thromboembolism in COVID-19 infected patients does not warrant a change in guidance on thromboprophylaxis among hospitalized patients, but the studies have overall poor quality due to methodological limitations. The rest of the articles emphasize the importance of anticoagulation. Overall, the findings indicate that therapeutic doses might be associated

with better survival compared to prophylactic doses. Data suggests that prophylactic and therapeutic anticoagulation may reduce mortality in COVID-19 patients[88].

Antiviral

Favipiravir, Lopinavir/Ritonavir, and Remdesivir were evaluated in our analysis, being studies in combination or as single-agent therapy. These articles compared antiviral drugs with other medications used for COVID-19 or standard of care. Favipiravir has a more favorable safety profile than other antivirals with mild and manageable side effects. The available data about combination therapy of Favipiravir with Lopinavir/Ritonavir is not enough to favor this combination over other treatments[89]. Remdesivir showed a positive impact on the hospitalized patients compared to the standard of care. The safety profile of Remdesivir in COVID-19 patients requires further studies with adequate design and power.

Convalescent plasma

Articles focusing on the effectiveness of convalescent blood product (CBP) therapy in COVID-19 infection and severe acute respiratory infections of viral etiology showed that most of the included studies had a critical risk of bias, leading to their exclusion from the analysis. From the remaining studies, a decline of all-cause mortality was observed only in observational studies, where using CBPs earlier compared to using CBPs later was associated with a significant reduction in all-cause mortality. Additionally, CBPs did not increase the risk of adverse events between intervention and control groups. However, in RCTs, the all-cause mortality showed no difference between the interventional and control groups.

Overall, the certainty of the evidence was low to very low. The effectiveness of CBPs in COVID-19 infection has poor validated results, but their use appears to be safe[90]. This observation contrasts with SARS coronavirus infection, which was similarly low to very low-quality studies that have effectively proven CBPs reduce both all-cause mortality and symptom duration.

Immunosuppressants

The role of immunosuppressants has been proposed as a possible treatment for the hyper-immune response in later stages of the infection, developing acute respiratory distress syndrome, multiorgan failure, and increasing mortality. The most discussed drug in our evaluation was the interleukin-6 receptor inhibitor monoclonal antibody tocilizumab (all six reviews), followed by corticosteroids, calcineurin inhibitors, and other immunosuppressants (one systematic review). The efficacy, mortality reduction, complication prevention, and their use alone or with the standard of care were discussed. The majority of the reviews show that the use of tocilizumab (either alone or in addition to the standard of care) has the potential to treat effectively, reduce mortality, and prevent mechanical ventilation[91]. One of the reviews evaluated provided no conclusive findings based on the low quality of evidence.

Corticosteroids

The administration of systemic corticosteroids compared with usual care or placebo in critically ill patients with COVID-19 was associated with lower 28-d-all-cause mortality[92]. Most studies show that the late administration of steroids in the course of the disease provides benefit in most patients and more severe, critically ill patients; however, other studies instead demonstrated that there was no survival benefit found with the use of corticosteroids[93] and that mortality risk was increased with the use of corticosteroids, likely secondary to a longer length of stay, a higher rate of bacterial infection, hypokalemia, and several other adverse effects[94]. Therefore, although the use of corticosteroids in some cases improved the clinical features of viral pneumonia, there was no confirmed evidence of corticosteroid therapy reducing the mortality in all COVID-19 patients. Results from the RECOVERY trial highlighted that steroids could potentially be an excellent therapeutic weapon against the coronavirus[95]. There was also a significant reduction in death with dexamethasone, which was seen only in severe cases of patients on ventilators or moderate cases requiring supplemental oxygen therapy[96]. However, there was no benefit observed in mild to moderate patient cases without hypoxia who did not require oxygen. Nonetheless, further studies are required to account for the range of co-variables and confounders, which would detail the dosing regimen and the duration of corticosteroid therapy and the stage at which therapy should be initiated to benefit patients and avoid adverse effects of corticosteroids.

HCQ

HCQ is an antimalarial drug used to treat rheumatological conditions, such as rheumatoid arthritis and systemic lupus erythematosus. It has been shown to have antiviral properties against COVID-19 "*in vitro*," but the evidence regarding its efficacy "in vivo" lacks the scientific support for its use[97]. Although considered a commonly well-tolerated drug, adverse effects to the gastrointestinal, cardiac, ocular, nervous, and dermatological systems were noted. The use of HCQ to treat SARS-COV-2 infection lacks efficacy in decreasing the risk of hospitalization or short-term mortality. This finding challenges the proposed association with increased mortality (either alone or in combination with azithromycin) and elicits the need for high-quality data from multicentric randomized control trials.

RAAS

A review of 21 systematic reviews of dozens of studies across the globe has, for the most part, failed to find a significant association between RAAS blockade by either agent–or even by both combined in some studies–with the risk, severity, or mortality of COVID-19 infection.

A massive debate on the role of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) in patients with COVID-19 and whether these agents should be continued in infected individuals was a significant question in the medical community at the onset of the pandemic[64,98]. Many global studies have mostly failed to find a significant association between RAAS blockade with risk, severity, or mortality in COVID-19 patients. While the viral spike coat protein of SARS-CoV-2 binds to the human ACE-2 cell surface receptor to cause infection, those hypertensive patients currently managed on ACEi or ARBs had a small, albeit significant, decrease in mortality. Some studies found this mortality benefit to be associated only with ARBs, not ACEi, while others have reduced death rates with both classes of medicines. This fact has encouraged some health experts to suggest that RAAS inhibition may be protective against COVID-19; however, such a recommendation requires more robust evidence. Current guidelines advise that hypertensive patients should continue taking ACEi or ARBs if they become infected with COVID-19; they do not recommend the initiation of ACEi or ARBs to treat COVID-19 infection.

Technology

We included 24 systematic review articles about the technology used to deliver quality healthcare during the COVID-19 pandemic. As face-to-face encounters have become more restricted over the past several months, the rise of telemedicine has allowed many health care professionals to provide continuity of care for patients. The introduction of various telecommunication modalities, including videoconferencing, telerehabilitation, tele-neuropsychology, teledermatology, telemonitoring, teletherapy, and telementoring, have been a great way to curb the transmission of COVID-19, increase access to healthcare, and triaging patients suffering from various ailments [99]. Analysis of these articles has revealed that the general population has adopted an overall favorable telemedicine response because of its convenience and comfort.

Unfortunately, telemedicine also has several drawbacks. Many patients do not have adequate internet access and/or are not technology savvy. Furthermore, patients may have limited care because telemedicine services do not always guarantee the same medical care team. Additionally, telemedicine is more beneficial for patients with chronic conditions that do not always require a physical examination. Although telemedicine benefits are irrefutable, we will need more RCT to determine the long-term effects and costs of telemedicine on society.

Special populations

Regarding special populations with COVID-19, we included 263 systematic reviews. Of these, children were the most common, with 33 articles reported. These articles' overall consensus pointed out that most children tend to have mild disease and a better prognosis. Physicians needed to be mindful of this population: Multisystem inflammatory syndrome cases in children (MIS-C), which is fatal if early recognition, supportive management, and early immunomodulators are not implemented. Morbidity and mortality were low overall In the neonatal population[100-102]. The second special population we looked at was pregnant women, with 16 articles evaluated. Most pregnant women were reported to be asymptomatic, although it was observed that preterm birth rates in some women were higher compared to COVID-19 negative pregnant women. Some unique findings included decreased white cell count

and possible impairment in follicular and luteal phases. Vertical transmission of COVID-19 was reported to be low.

Patients with malignancies and COVID-19 were reviewed, included in 19 articles. Cancer patients tend to have higher inflammatory markers at presentation, more severe symptoms clinically, greater risk of complications, and increased ICU admissions. Chemotherapy in these patients has not been associated with an increased risk of severe COVID-19. Delay in cancer treatment in COVID-19 positive cancer patients has been favored by many oncologists, although no evidence of interactions of COVID-19 treatment and chemotherapy has been identified[103].

Regarding diabetic patients with COVID-19 infection, they were reported to have a higher prevalence of progression to ARDS, which led to a higher rate of ICU admissions and subsequent mortality. It was also noted that diabetic ketoacidosis (DKA) in COVID-19 patients tended to lead to mortality that approached 50%. Eight articles regarding smoking and COVID-19 were analyzed. There seems to be a positive correlation between smoking and disease severity. This applies to both current and past smokers, although further studies need to be performed before establishing this connection. The obese population was reviewed in five articles. Findings showed obese individuals had a higher risk of contracting COVID-19, increased risk of ICU hospitalizations, severe disease, as well as diminished prophylactic and therapeutic responses to standard treatment. Lastly, we evaluated transplant patients with COVID-19 infection, covered in five articles. Higher mortality rates were observed in patients with solid organ transplants older than 60 years of age. There was a low incidence of reported COVID-19 infections in renal transplant patients; however, patients were reported to have more severe disease progression in those who were infected. This particular population was at greater risk of acute renal failure, ICU admission, and RRT[104]. As we look at the diverse populations studied, we find that COVID-19 not only impacts our ability to diagnose and treat early but may affect each population differently.

DM

We included two systematic review articles regarding the epidemiology of COVID-19 patients with DM. The articles found diabetes had a negative effect on the health impact of COVID-19, and the prevalence of DM in patients with severe COVID-19 was significantly higher than moderate patients with COVID-19. Five systematic review articles were evaluated regarding the clinical features of COVID-19 in patients with DM; these articles concluded that those with severe COVID-19 had higher blood glucose levels, especially with the use of corticosteroids. It was observed that DM was associated with increased mortality, severe COVID-19 infection, ARDS, and disease progression[105,106].

We included three articles regarding the treatment of COVID-19 patients with DM. Due to media coverage of dexamethasone benefits in COVID-19, this may increase patients' self-medicating themselves[107]. Studies have shown that corticosteroid use in diabetic patients puts them at a higher risk of hospitalization due to diabetic complications. Therefore, healthcare workers should be avidly aware of the potential risk of using these medications[107].

One systematic review demonstrated COVID-19 patients with combined DKA and hyperosmolar hyperglycemic state (HHS) had higher mortality than DKA alone, with DKA/HHS representing 20% of the total cases of DKA, concluding the importance of differentiating between these two groups[108]. One meta-analysis showed an increased risk of ICU admission for COVID-19 in diabetic patients and increased mortality in these populations[109]. The majority of studies were performed in COVID-19 patients with DM type 2; DM type 1 poses unique challenges, with research showing that in COVID-19 patients with DM type 1, modified management and telemedicine have been practical tools for patient care.

Obesity

We reviewed a total of 14 systematic review articles on the relationship between obesity and COVID-19. We included one systematic review article regarding the epidemiology of obesity pertaining to COVID-19[110]. Obese patients with hypertension, type 2 DM, active smokers, lung disease, and/or cardiovascular disease are at higher risk for ICU admissions, severe COVID-19, and disease progression. The six articles discussed patients suffering from obesity and COVID-19 show a positive correlation between higher body mass index and severe COVID-19 cases. COVID-19 patients with obesity were significantly affected and had a worse prognosis than those without. In one particular review article, the mechanistic pathways in obese individuals were investigated, evaluating factors linked with COVID-19 risk, severity,

and the potential for diminished therapeutic and prophylactic treatments among these individuals. These studies concluded that individuals with obesity are linked with a significant risk of morbidity and mortality from COVID-19[111].

Regarding the severity assessment of COVID-19 in obesity, there were seven articles discussed. A meta-analysis suggested a linear association between the severity and mortality of COVID-19 and body mass index (BMI)[112]. Also, a BMI ≥ 30 kg/m^2 was associated with a higher risk of increased severity in COVID-19 and in-hospital mortality. Furthermore, the study revealed obesity and being overweight were represented as unfavorable factors for COVID-19 infection, where the higher the BMI, the worse the outcome. This occurred by worsening infection, resulting in increased hospitalizations, worse outcomes, and markedly significant mortality, especially when coinciding with other chronic conditions and in the elderly. BMI is an essential routine measurement that should be regularly assessed in the management of COVID-19 patients, and special attention should be given to patients with obesity. Obesity may serve as a clinical predictor for adverse outcomes; therefore, BMI in prognostic scores may play an essential role in predicting the clinical outcomes[113].

Pregnancy

We included one systematic review assessing the clinical presentation of COVID-19 infection in pregnant women[114], which found the most reported clinical symptoms were fever, cough, and dyspnea. Commonly reported laboratory abnormalities included elevated CRP or procalcitonin, lymphopenia, and elevated transaminases. Some complications also included preterm birth and maternal ICU admission. Maternal mortality, however, reported a low prevalence. Another systematic review article evaluated screening and diagnosis of COVID-19 during pregnancy[115], demonstrating the most common symptoms included fever and cough, and for accurate diagnosis, RT-PCR and CT scan can be used together. Further studies showed the clinical characteristics of pregnant women with COVID-19 are similar to those of non-pregnant adults[116], but available data only include pregnant women infected in their third trimesters.

One systematic review article regarding the epidemiology of pregnancy showed insufficient data to suggest if there is transplacental transmission to neonates, with only one neonatal death reported, and 3.4% of neonates suffered from COVID-19[117]. Regarding the special population of pregnancy, we included 16 systematic review articles that showed there is not enough evidence to suggest vertical transmission, most articles stating that it is unlikely although suggesting that it cannot be ruled out. However, the data suggested an increased risk of preterm birth and SARS-CoV-2, leading to an increased need for cesarean delivery. The clinical presentation might not be drastically different as compared to non-pregnant adults[118]. The effect on fertility is not apparent[119].

Children

We looked at 39 systematic review articles regarding the relationship between COVID-19 and the pediatric population. Compared to adults, children presented with milder symptoms. The most common symptoms included fever, cough, vomiting, and diarrhea. Approximately one-third of the pediatric population were asymptomatic, raising concerns for children unknowingly transmitting the virus to at-risk individuals [116]. Overall, the disease course for children was milder, with fewer hospitalizations, ICU admissions, mechanical ventilation, and mortality. There are multiple theories as to why the pediatric population is not severely affected by COVID-19. The theories are as follows: an immature immune system resulting in no cytokine storm, poorly developed ACE2-receptors, fewer comorbidities, and the development of antibodies due to children being infected by other respiratory viruses that can protect against COVID-19[117].

The primary avenue for children contracting the virus was through family members. If a family member tested positive, it is recommended that children be tested as asymptomatic carriers[120].

Children were rarely the cause of significant outbreaks, bringing into question the efficacy of school closures. According to public health experts, school closures could do more harm than good. School closures could increase childhood depression and anxiety rates and cut children from vital resources such as meals, school nurses, and quality education. A small percentage of children did develop severe symptoms of COVID-19 resulting in ICU admissions, mechanical ventilation, and death. Children with comorbidities were at increased risk of a more severe form of infection. The comorbidities most associated with severe COVID-19 infections included cardiac diseases, diabetes, chronic non-asthmatic pulmonary disease, asthma, and obesity.

Several MIS-C were reported, which resulted in shock, multiorgan failure, and death [121,122]. However, a clear correlation between COVID-19 and MIS-C does not exist. It must be determined if COVID-19 increases the risk of MIS-C or if there is simply a temporal relationship between both conditions. The primary form of treatment for severely ill children included intravenous immunoglobulins, corticosteroids, and immunomodulators. However, the efficacy of these treatments is not known. Therefore, the systematic review articles highlight the potential for additional studies to truly understand the effects of COVID-19 in children. The areas that need to be further studied include the possibility of vertical transmission, the extent to which children are transmitters of the virus, the efficacy of school closures in fighting the outbreak, and the ideal treatment course for severely infected children[120].

Mental health

Sixty-three articles were reviewed regarding the mental health impact of COVID-19. COVID-19 has caused many mixed emotions and has impacted people from a mental health perspective due to the isolation and "social distancing" components. It is estimated that a quarter of the population experienced significant stress due to the pandemic. Given that many stores had limited supplies, it created a panic buying atmosphere[123]. This was mainly due to the survival of the fittest mentality, the fear of the unknown, and coping mechanisms. Bereavement and grief were important aspects many dealt with due to the numerous deaths from the pandemic. Having afterlife ceremonies such as funerals benefited many family members as it was a more sentimental way of saying goodbye[124].

Studies showed that isolation could cause neuroendocrine-immune changes, which further exacerbates COVID-19 and mental health associations. The early stages of the virus caused delirium in a large proportion of the patients. Pulmonary insults of the disease tended to show high amounts of cognitive dysfunction. The elderly were at an increased risk from both a physical and mental perspective from the increased social distancing. It was recommended that this group have more activity and exercise planned before enforcing the lockdowns. The pediatric population's most common disorders were acute stress disorder, post-traumatic stress disorder, adjustment disorder, and grief[125]. The younger population of children also will have higher effects from depression and anxiety. Laws should be more intentional about providing counseling[126].

Though beneficial from a public health standpoint, quarantine has the propensity to impact an individual's psyche negatively and has in some ways passed the brink of being considered clinically relevant. Many of the mental health problems included depression, anxiety, mood disorders, and lack of self-control. Age and sex did not correlate with anxiety disorders. These conditions not only impacted the public and patients but healthcare providers as well[127].

Healthcare workers may be presented with anxiety, depression, and insomnia. Studies showed that HCWs who worked in areas with fewer resources and protective gear tended to be more stressed. Comparing HCWs to non-healthcare workers found their psychological stress level was similar in many factors, except that HCWs had higher amounts of insomnia. Physicians and nurses were the most impacted and resulted in many sleep disturbances. Other studies showed evidence that those with comorbidities had even more stress than health care workers. The virus has shed light on the importance of and need for solutions to improve the well-being of HCWs by providing more education and counseling through policy change[125].

Smoking

We included 16 systematic review articles that investigated the relationship between smoking and COVID-19 infection. Severity, progression, and adverse outcomes of COVID-19 infection were discussed in relation to smoking status, comparing past and current smokers to non-smokers[128]. Severe cases were defined as critical with the need for ICU, refractory, and non-survivors. Several studies found patients who are both current and former smokers are at an increased risk of severe infection and progression[129]. Meanwhile, a few studies suggested that current smokers have a reduced risk of infection than both former and non-smokers, evidenced by an unexpectedly low prevalence of current smokers among hospitalized patients with COVID-19. However, those hospitalized were at higher odds of adverse outcomes than non-smokers and lower odds than former smokers. Furthermore, due to the low prevalence of hospitalized current smokers, further exploration of nicotine as a therapeutic option is suggested as a potential treatment in COVID-19.

In particular, patients with the chronic obstructive pulmonary disease have a significantly higher odds ratio of severe infection *vs* current smokers[130]. Findings showed that these patients were older and predominantly males compared to non-severe cases. With most studies finding an increased risk between smoking and COVID-19 severity, a continued focus on smoking cessation efforts is recommended [131].

Cancer

Twenty articles were reviewed regarding the special populations of cancer patients with COVID-19 infection. It was hypothesized that the unique nature of the pathophysiology and the treatment protocols utilized for cancer patients might lend themselves to specific considerations regarding precautions, screening, treatment, and outcomes. Overall, cancer patients were more likely to experience severe COVID-19 disease when compared to those without malignancy. Additionally, cancer patients were found to have lower platelet levels with higher inflammatory markers, leading to increased susceptibility for complications[132].

Certain risk factors were found to be significantly predictive of increased mortality in this population. It was found that the male gender, age greater than 65 years, history of hypertension, and history of chronic obstructive pulmonary disease were associated with an elevated risk of death. Interestingly, many studies found that while symptoms on admission such as dyspnea, cough, and increased sputum were more likely to predict severity, the use of antibiotics, glucocorticoids, interferon, and invasive ventilation were associated with a higher probability of death. This finding likely elucidates the fact that severe infections were treated with complex, invasive medical care. Patients undergoing chemotherapy targeted radiotherapy or immunotherapy had a more aggressive infection course, with significant interactions seen with tocilizumab, ruxolitinib, and colchicine[103]. However, increased mortality was not significantly associated with recent anti-cancer treatments, except when patients were given chemotherapy within 28 d of infection or immunotherapy within 90 d of infection[133]. These findings led to the recommendation that deviations from the standard of care for more extended periods (*i.e.*, beyond four to six weeks) may not be necessary and indeed may lead to a significant impact on overall outcomes.

While many studies have proposed identifying outcomes in all-cause mortality within the general cancer population, some studies have focused on specific cancer types. Multiple myeloma patients were found to have a higher risk of a severe infection due to their immunocompromised status, while treatment protocols for prostate cancer patients with very low, low, or favorable intermediate-risk diseases were avoided or delayed[134].

Overall, it was found that compared to other types of cancer, lung and colorectal cancer patients were more susceptible to COVID-19 infection[135]. Cancer patients are a special population due to their immunocompromised status, cytotoxicity of their treatment regimen, nutritional status, and already increased inflammatory markers.

Cancer patients had worse outcomes from COVID-19 when compared with the general population. Despite all efforts, finding the ideal approach for cancer patients with COVID-19 is not evident. The approach to high-risk patients in this group should include vigorous screening and intensive surveillance. The approach must be dynamic and anti-cancer treatment should be modified based on the cancer type and the patient's prognosis[135].

Transplant medicine

Five systematic review articles evaluated the presentation of COVID-19 in kidney transplant recipients, who had poorer outcomes on average compared to the general adult population. This subpopulation of patients developed acute kidney injury at higher rates, subsequently requiring RRT. Kidney transplant recipients had increased rates of hospitalizations, ICU admissions, and mechanical ventilation. Compared to the general adult population, the kidney transplant subgroup was elder and had higher mortality rates. Presenting symptoms in this subgroup differed from the general population, presenting with atypical symptoms such as lack of fever and predominance of gastrointestinal symptoms[136]. An area that will need further exploration is the treatment of COVID-19 in kidney transplant recipients. The systematic review articles discussed the risks *vs* benefits of down-titrating immunosuppressive medications while patients are infected with COVID-19.

Additionally, HCQ showed no additional benefit in kidney transplant recipients in the treatment of COVID-19[108,137]. The systematic review articles highlight the need for additional studies to determine the optimal therapeutic approach for the treatment of COVID-19 in this population. A better understanding of treating kidney transplant

recipients could potentially lead to the development of guidelines for any transplant recipient's treatment.

CONCLUSION

Among the included articles, it is clear that further research is needed regarding treatment options and vaccines. With more studies, data will be less heterogeneous, and statistical analysis will be applied and provide more robust clinical evidence. This study was not designed to give recommendations regarding the management of COVID-19.

ARTICLE HIGHLIGHTS

Research background

The coronavirus disease 2019 (COVID-19) pandemic continues to impact the sociopolitical, economic, and health care systems across the globe. Robust strategies to control this pandemic's unchecked progression are vital to restore and normalize the health and wellness of human populations. This article summarizes facts and evidence from the current body of literature concerning multiple aspects of COVID-19.

Research motivation

No research studies so far have succeeded in recommending a definite remedy to contain coronavirus infection transmission. The current study's motivation emanated from the requirement of improving the current knowledge base about COVID-19 prevalence, progression, incidence, management, and outcomes.

Research objectives

The research objective included the critical assessment of COVID-19-related meta-analyses and systematic reviews to explore the current knowledge gaps and track the best evidence to inform the scientific community.

Research methods

The research method relied on a robust search strategy based on the combination of various key terms while excluding panel reviews, guideline documents, opinion papers, scoping reviews, and rapid reviews related to COVID-19.

Research results

The research results generated 1219 studies concerning the COVID-19 knowledge base based on fifty predefined attributes.

Research conclusions

The outcomes of our study emphasized the need for future research to understand the scope of possible vaccines and prevention/treatment options in the setting of COVID-19.

Research perspectives

Prospective studies should deploy multicentred approaches to unravel the clinical findings, risk factors, and prevention/treatment guidelines in COVID-19 scenarios.

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EVIDENCE REVIEW

Human papillomavirus infection and gastric cancer risk: A meta-epidemiological review

Jong-Myon Bae

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Jong-Myon Bae, Department of Preventive Medicine, Jeju National University College of Medicine, Jeju-si 63243, Jeju Province, South Korea

Corresponding author: Jong-Myon Bae, MD, PhD, Professor, Department of Preventive Medicine, Jeju National University College of Medicine, 102 Jejudaehak-ro, Jeju-si 63243, Jeju Province, South Korea. jmbae@jejunu.ac.kr

Abstract

Gastric cancer (GC) is a multifactorial disease, and several modifiable risk factors have been reported. This review summarizes and interprets two previous quantitative systematic reviews evaluating the association between human papillomavirus (HPV) infection and GC risk. The results of two systematic reviews evaluating the same hypothesis showed a statistically significant difference in summary odds ratios and their 95% confidence intervals. Thus, it is necessary to conduct a subgroup analysis of Chinese and non-Chinese studies. Additional meta-analyses that control for heterogeneity are required. Reanalysis showed that all the Chinese studies had statistical significance, whereas the non-national studies did not. The funnel plot asymmetry and Egger's test confirmed publication bias in the Chinese studies. In addition, the proportion of HPV-positive cases in Chinese studies was 1.43 times higher than that in non-Chinese studies and 2.81 times lower in controls. Therefore, the deduced evidence is currently insufficient to conclude that HPV infection is associated with GC risk.

Key Words: Papillomavirus; Stomach neoplasm; Case-control studies; Meta-analysis; Systematic review; Risk factors

Core Tip: Chinese studies showed that human papillomavirus infections increased the risk of gastric cancer; however, non-Chinese studies showed no statistical significance. Therefore, the deduced evidence is currently inadequate to conclude that human papillomavirus infection is associated with gastric cancer risk.

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INTRODUCTION

Gastric cancer (GC) is the fifth most common incident cancer according to Global Cancer Statistics 2018[1] and ranks third in absolute years of life lost[2]. GC is a multifactorial disease, and several modifiable risk factors have been reported[3,4].

Infection with *Helicobacter pylori* or oncogenic viruses has important implications for preventing and managing GC[5]. *Helicobacter pylori* eradication is one of the reasons behind the steady decline in global GC incidence[6]. Therefore, human papillomavirus (HPV), which is among potential oncoviruses posing GC risk reviewed by Niedźwiedzka-Rystwej *et al*[7], should be considered to control GC occurrence because HPV vaccines have been used to prevent uterine cervix cancer[8,9].

However, the International Agency for Research on Cancer did not suggest an association between HPV infection and GC risk in a monograph published in 2007 [10]. This review summarizes and interprets previous quantitative systematic reviews evaluating the association between HPV infection and GC risk.

PREVIOUS SYSTEMATIC REVIEWS

A PubMed (https://pubmed.ncbi.nlm.nih.gov) search, using "papillomavirus infection" and "stomach neoplasms" as the keywords of the hypothesis, identified two systematic reviews as of December 31, 2020[5,11]. Both selected case-control studies and their results are summarized in Table 1.

Zeng *et al*[11] reported that in 2016, a total of 15 case-control studies, including 12 studies on Chinese patients, and a meta-analysis showed that HPV infection increased the risk of GC by 7.39 times [95% confidence interval (CI) of summary odds ratio (sOR): 3.88–14.1]. Further, a study by Wang *et al*[5] published in 2020 selected a total of 14 case-control studies, including five studies on Chinese patients, and the sOR was 1.53 (95%CI: 1.00–2.33).

The results of two systematic reviews evaluating the same hypothesis showed a statistically significant difference in sORs and their 95%CI. These findings can be inferred from the following three reasons. First, there was a difference in selection criteria. Wang *et al*[5] included three serological studies, in addition to tissue tests. Therefore, it is necessary to limit future research to tissue studies and conduct a meta-analysis again. Second, there was a difference in search databases between the two systematic reviews. Zeng *et al*[11] and Wang *et al*[5] selected 12 and five Chinese studies, respectively. Whereas Zeng *et al*[11] did not report a subgroup analysis, Wang *et al*[5] showed different subgroup analysis results between Chinese and non-Chinese studies. Therefore, it is necessary to conduct subgroup analyses of Chinese and non-Chinese studies in all selected articles. Finally, potential bias is possible due to heterogeneity. Wang *et al*[5] found no statistical significance in subgroups with less than 50% of the I-squared value, such as non-Chinese studies, serum studies, and HPV-18 studies (Table 1). Therefore, additional meta-analyses that control for heterogeneity are required.

RE-ANALYSIS OF META-ANALYSIS

Both systematic reviews selected a total of 25 articles. After excluding three serological studies[12-14], three studies had no information on the control group[15-17], and one showed zero HPV positivity in both the case and control groups[18]; hence, 18 articles were selected for reanalysis[19-35].

Table 2 illustrates the information extracted for the reanalysis of each study. Xu *et al*[25] extracted the results for cardia as well as those for the entire region for use in subgroup analysis by GC site.

Figure 1 displays a forest plot showing the results of the reanalysis. The sOR for 18 studies was 5.80 (95%CI: 3.27–10.31), showing statistical significance. While the I-squared value was reduced from 60% in all studies to 0% in 12 Chinese studies, their sOR remained statistically significant at 7.86 (95%CI: 5.19–11.89). However, the sOR

Table 1 The summary odds ratio with its 95%CI from two systematic reviews

Ref.	Search to	Subgroup	Case-control studies	sOR (95%CI)	I^2 (%)
Zeng *et al*[11], 2016	Jun 2016	All	15	7.39 (3.88-14.1)	56.7
Wang *et al*[5], 2020	Apr 2020	All	14	1.53 (1.00-2.33)	59.8
		Chinese	5	1.98 (1.04-3.75)	73.7
		Non-Chinese	9	1.17 (0.68-2.02)	33.4
		Tissue	11	2.24 (1.13-4.43)	66.5
		Serum	3	1.04 (0.75-1.44)	0.0
		HPV-16	8	2.42 (1.00-5.83)	67.5
		HPV-18	3	1.08 (0.59-1.99)	0.0

HPV: Human papillomavirus; sOR: Summary odds ratio.

Table 2 Extracted information of the 18 selected case-control studies

Ref.	Year	Nation	Site	Test	Sample	PCa	NCa	PCo	NCo
Sha *et al*[19]	1998	China	Gastric	PCR	FFPE	27	38	4	61
Dong *et al*[20]	1999	China	Gastric	PCR	Other	10	27	0	20
Yu *et al*[21]	1999	China	Gastric	PCR	FFPE	30	102	3	101
Zhou *et al*[22]	1999	China	Gastric	PCR	FFPE	19	31	0	20
Zhu *et al*[23]	2000	China	Gastric	PCR	FF	11	31	0	42
Liao *et al*[24]	2001	China	Gastric	ISH	Other	26	24	2	28
Xu *et al*[25]	2003	China	Cardia	ISH	FFPE	50	24	10	40
Xu *et al*[25]	2003	China	Gastric	ISH	FFPE	111	125	10	40
Ma *et al*[26]	2007	China	Gastric	PCR	FFPE	15	25	2	38
Ma *et al*[27]	2007	China	Cardia	PCR	FFPE	32	61	0	21
Rong *et al*[28]	2007	China	Cardia	PCR	FFPE	16	5	2	19
Wang *et al*[29]	2013	China	Gastric	PCR	FFPE	20	72	4	82
Su *et al*[15]	2015	China	Gastric	PCR	Other	1	14	0	15
Anwar *et al*[30]	1995	Japan	Gastric	PCR	FFPE	23	28	2	10
Erol *et al*[31]	2009	Turkey	Gastric	PCR	FFPE	17	21	33	73
Cândido *et al*[32]	2013	Brazil	Gastric	PCR	FFPE	4	36	10	30
Türkay *et al*[33]	2015	Turkey	Cardia	PCR	FFPE	2	17	0	8
Bozdayi *et al*[34]	2019	Turkey	Gastric	PCR	Other	20	33	5	21
Leon *et al*[35]	2019	Ethiopia	Cardia	PCR	FF	11	51	0	56

FF: Fresh frozen tissue; FFPE: Formalin-fixed paraffin-embedded tissue; ISH: *In situ* hybridization; NCa: Negative in cases; NCo: Negative in controls; PCa: Positive in cases; PCo: Positive in controls; PCR: Polymerase chain reaction.

for six non-Chinese studies was 1.97 (95%CI: 0.79–4.89), which was not statistically significant. In other words, all Chinese studies showed statistical significance; however, the non-national studies did not. This finding was the same in the subgroup analysis by cardiac tissue, formalin-fixed paraffin-embedded tissue, fresh frozen tissue, and polymerase chain reaction (Table 3).

Twelve Chinese studies were examined for publication bias. The asymmetry of the funnel plot (Figure 2) and Egger's test (P = 0.013) confirmed publication bias. The trimming sOR from trim-and-fill analysis[36] was 6.78 (95%CI: 4.40–10.45).

Table 3 Subgroup analysis by nationality

		All	Chinese studies	Non-Chinese studies
All		5.80 (3.27-10.31) [60.0] <18>	7.86 (5.19-11.89) [0.0] <12>	1.97 (0.79-4.89) [56.8] <6>
Area				
	Gastric	4.83 (2.64-8.83) [62.4] <14>	7.08 (4.60-10.89) [0.0] <10>	1.54 (0.60-3.92) [62.6] <4>
	Cardia	10.88 (5.42-21,8) [0.0] <5>	11.17 (5.34-23.35) [0.0] <3>	8.62 (0.88-84.8) [14.2] <2>
Sample				
	FFPE	5.13 (2.55-10.34) [68.4] <12>	8.02 (4.74-13.6) [19.6] <8>	1.38 (0.45-4.16) [58.5] <4>
	FF	27.9 (3.70-211.7) <2>	31.0 (1.76-546.6) <1>	25.2 (1.45-439.1) <1>
Methods				
	PCR	5.88 (3.00-11.52) [62.2] <16>	10.93 (6.44-18.5) [0.0] <10>	1.97 (0.79-4.98) [56.8] <6>
	ISH	6.23 (1.56-24.9) [64.0] <2>	6.23 (1.56-24.9) [64.0] <2>	-

Study: Summary odds ratio (95% confidence interval) [I^2 value (%)] <Number of selected studies>; FF: Fresh frozen tissue; FFPE: Formalin-fixed paraffin-embedded tissue; ISH: *In situ* hybridization; PCR: Polymerase chain reaction.

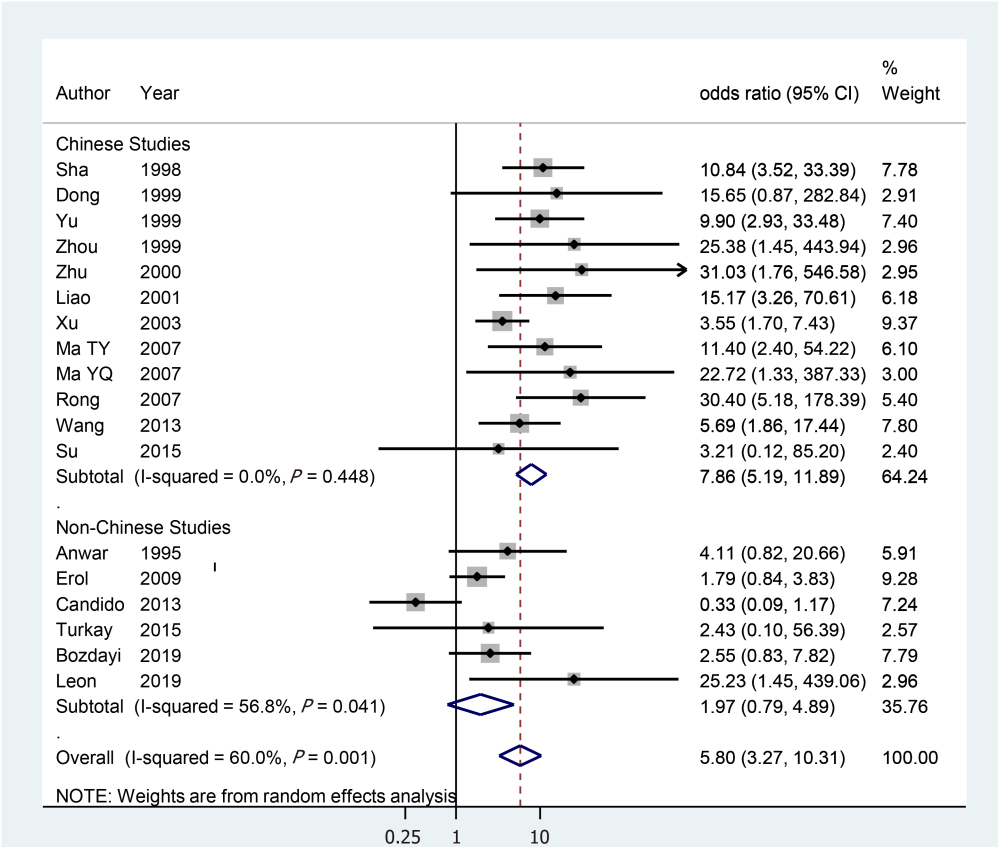

Figure 1 Forest plot for estimating summary odds ratio. CI: Confidence interval.

CONCLUSION

To summarize the above reanalysis results, Chinese studies demonstrated that HPV infections increased the risk of GC; nonetheless, non-Chinese studies showed no statistical significance. Therefore, the deduced evidence is currently insufficient to conclude that HPV infection is associated with GC risk.

The following interpretations and suggestions may be made based on the significant associations observed only in Chinese studies. First, there is a possibility that publication bias was involved in the selection of Chinese studies. After checking for

Table 4 Proportion of human papillomavirus positivity (%) by nationality

		Chinese studies	Non-Chinese studies
Total			
	Positive/Observe	335/1225	127/511
	PP (95%CI)	27.3 (24.9-29.9)	24.9 (21.2-28.8)
Case			
	Positive/Observe	298/711	77/263
	PP (95%CI)	41.9 (38.2-45.6)	29.3 (23.8-35.2)
Control			
	Positive/Observe	37/514	50/248
	PP (95%CI)	7.2 (5.1-9.8)	20.2 (15.4-25.7)

PP: Human papillomavirus positivity.

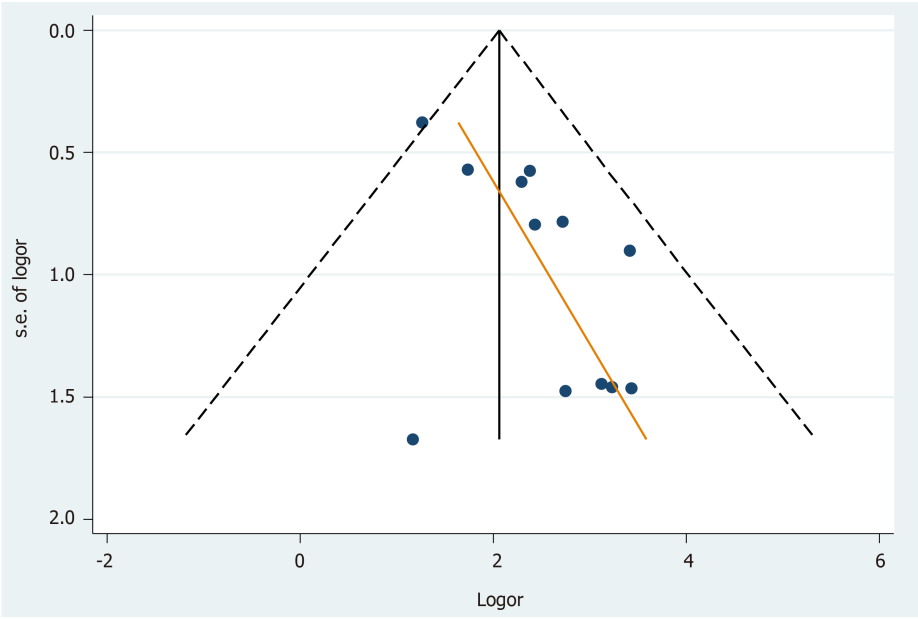

Figure 2 Funnel plot in 12 Chinese studies (*P* value of Egger test = 0.013).

publication bias using the funnel plot (Figure 2) and Egger's test, trim-and-fill analysis was performed. However, the trimming sOR in Chinese studies showed that HPV infections persistently increased the risk of GC. This mandated an alternative interpretation. The author attempted to infer that HPV positivity might have been different between Chinese and non-Chinese studies.

Using the information in Table 2, the proportion (%) of HPV positivity (PP) was obtained from both Chinese and non-Chinese studies (Table 4). On combining both the case and control groups, the PPs in Chinese and non-Chinese studies were 27.3% (95%CI: 24.9–29.9) and 24.9% (95%CI: 21.2–28.8), respectively. Their 95%CIs overlapped, showing no statistically significant differences. However, the case-group PP in Chinese studies was 41.9% (95%CI: 38.2–45.6), higher than that in non-Chinese studies (29.3%;95%CI: 23.8–35.2), and their 95%CIs did not overlap, showing a statistically significant difference. In contrast, the control-group PP in Chinese studies was 7.2 % (95%CI: 5.1–9.8), lower than the 20.2 % (95%CI: 15.4–25.7) in non-Chinese studies, and their 95%CIs did not overlap. In other words, the case PP in Chinese studies was 1.43 times (= 41.9/29.3) higher than that in non-Chinese studies and 2.81 times (= 20.2/7.2) lower in controls. This indicates a potentially significant relationship between HPV infection and GC risk in Chinese studies.

Given that the PP in the control group of the Chinese studies was significantly lower, descriptive epidemiological studies on HPV infection in the Chinese population are warranted. It is also necessary to conduct follow-up studies on whether the GC incidence rate due to HPV infection will change in the future due to the HPV vaccination project currently targeted at the Chinese population.

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EVIDENCE REVIEW

Scientific evidence in the COVID-19 treatment: A comprehensive review

Gorane Iturricastillo, Elena Ávalos Pérez-Urría, Felipe Couñago, Pedro Landete

ORCID number: Gorane Iturricastillo 0000-0001-8007-0874; Elena Ávalos Pérez-Urría 0000-0002-9988-4605; Felipe Couñago 0000-0001-7233-0234; Pedro Landete 0000-0002-9631-9408.

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Gorane Iturricastillo, Elena Ávalos Pérez-Urría, Pedro Landete, Department of Pulmonology, Hospital Universitario de La Princesa, Madrid 28006, Spain

Felipe Couñago, Department of Radiation Oncology, Hospital Universitario Quirónsalud Madrid, Pozuelo de Alarcón 28223, Spain

Felipe Couñago, Department of Radiation Oncology, Hospital La Luz, Madrid 28003, Spain

Felipe Couñago, Department of Radiation Oncology Universidad Europea de Madrid, Madrid 28670, Spain

Pedro Landete, Department of Pulmonology, Universidad Autónoma de Madrid, Madrid 28049, Spain

Pedro Landete, Department of Pulmonology, Instituto Investigación Princesa, Madrid 28006, Spain

Corresponding author: Gorane Iturricastillo, MD, Doctor, Department of Pulmonology, Hospital Universitario de La Princesa, Calle Diego de Leon 62, Madrid 28006, Spain. iturricastillo.gorane@gmail.com

Abstract

In December 2019, cases of unknown origin pneumonia appeared in Wuhan, China; the causal agent of this pneumonia was a new virus of the coronaviridae family called severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). According to the clinical severity, symptoms and response to the different treatments, the evolution of the disease is divided in three phases. We analysed the most used treatments for coronavirus disease 2019 and the phase in which they are supposed to be effective. In the viral phase, remdesivir has demonstrated reduction in recovery time but no mortality reduction. Other drugs proposed for viral phase such as convalescent plasma and lopinavir/ritonavir did not demonstrate to be effective. In the inflammatory phase, corticosteroids demonstrated reduction of 28-d mortality in patients who needed oxygen, establishing that a corticosteroid regimen should be part of the standard treatment of critically ill patients. There are other immunosuppressive and immunomodulatory treatments such as anakinra, sarilumab, tocilizumab, colchicine or baricitinib that are being studied. Other treatments that were proposed at the beginning, like hydroxichloroquine or azithromycin, demonstrated no efficacy and increased mortality when combined.

Specialty type: Respiratory system

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Core Tip: Severe acute respiratory syndrome coronavirus-2 is responsible for the unknown pneumonia that appeared in Wuhan, China, in December 2019. Lots of known drugs have been proved for coronavirus disease 2019. Corticosteroids demonstrated reduction of 28-d mortality in patients who needed oxygen and remdesivir proved to be effective reducing recovery time. Other drugs need more evaluation before establishing their effectiveness.

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INTRODUCTION

In December 2019, cases of unknown origin pneumonia appeared in Wuhan, a province of China. It was determined that the causal agent of pneumonia was a new virus of the coronaviridae family called severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)[1,2]. The spread of this virus was so fast that resulted in a pandemic in a few months, causing more than 2.5 million deaths worldwide as of the writing of this paper.

It has become a priority to establish a treatment that reduces mortality, the time of illness and the severity of the virus. For that reason, a wide variety of trials and studies have been developed to evaluate the effectiveness of different already known drugs. Boregowda *et al*[3] published a review of experimental treatments in coronavirus disease 2019 (COVID-19) in October 2020 concluding that the best method of dealing with the pandemic is to reduce the community spread. A lot of investigation has occurred since then, so we have reviewed the updated literature with focus on articles published in high impact journals.

Pathogeny

Siddiqi *et al*[4] proposed a three-phase classification of the evolution of COVID-19, according to the clinical severity, symptoms and response to the different treatments (Figure 1): (1) Viral phase or early infection: onset of infection and viral replication. The virus enters host cells through the angiotensin-converting angina 2 receptor, which is highly present in lung cells[5-7]. This phase includes the first seven days of symptoms; symptoms such as fever, myalgias and digestive inconveniences predominate. The polymerase chain reaction (PCR) of the virus is positive and there may be lymphopenia on laboratory tests and pulmonary infiltrates visible by computerized tomography; (2) Pulmonary phase: the virus continues to replicate and the host's humoral response develops. It appears approximately 7-14 d after the initial symptoms. It is technically divided into two sub-phases depending on whether the patient has respiratory failure (IIB) or not (IIA). The cytokine cascade is activated causing a severe inflammatory reaction in the lung tissue that can lead to respiratory distress. The most common manifestations are viral pneumonia, hypoxemia, cough and fever; and (3) Hyperinflammatory phase: it is the most severe phase and it is characterized by systemic inflammation with elevated blood levels of acute phase reactants and inflammatory cytokines[8]. It usually occurs 10-14 d after the initial symptoms. It can cause myocardial damage, shock, respiratory failure, *etc*. Only a few patients have this severe form of the disease. In this phase, treatment with immunomodulatory drugs or intravenous immunoglobulins may be useful.

Objective

The objective of this article is to do a brief review of the drugs that have been used the most to treat the disease since the beginning of the pandemic until today[9].

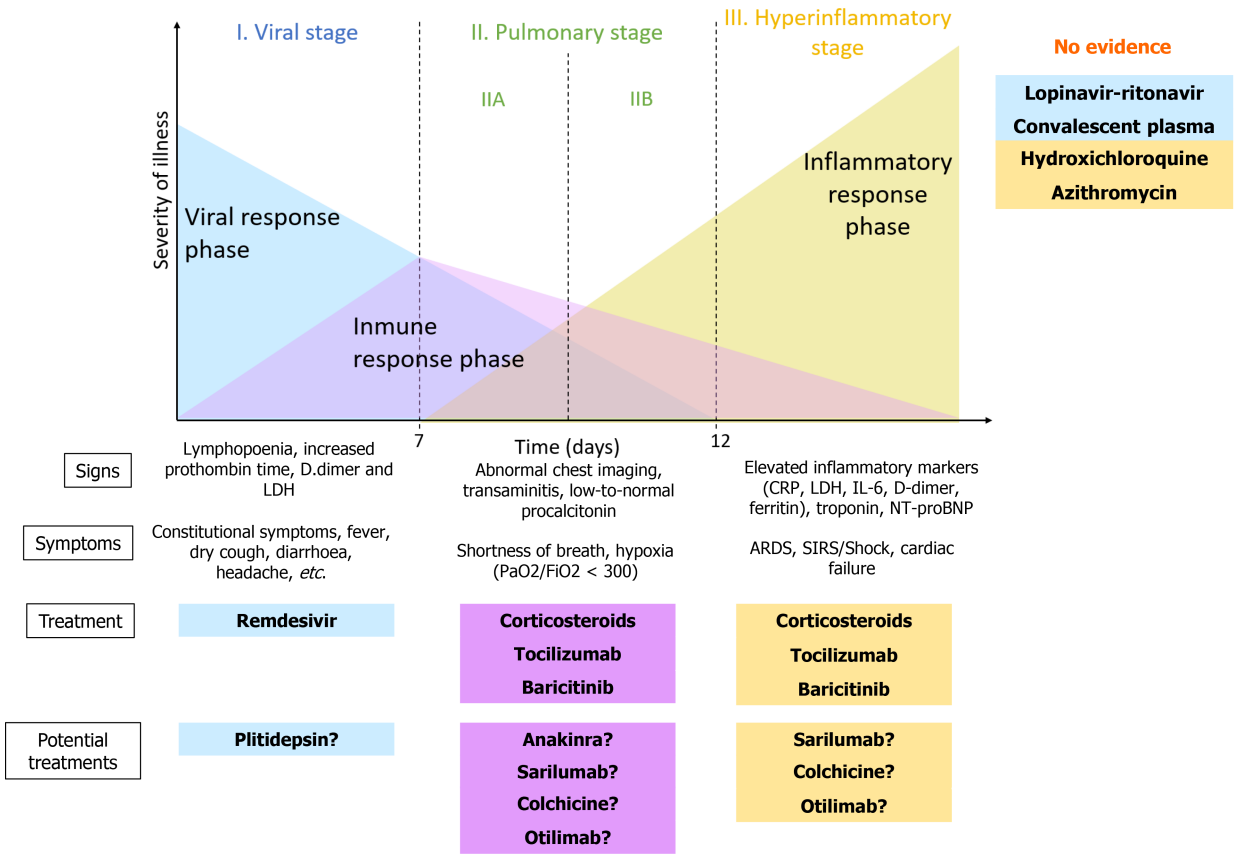

Figure 1 Classification of coronavirus disease 2019 states and potential therapeutic targets. Adaptation from Siddiqi *et al*[4]. LDH: Lactate dehydrogenase; CRP: C-reactive protein.

LITERATURE SEARCH

We performed a search in PubMed with the keywords "COVID-19" and the most frequent drugs (Corticosteroid, Hydroxychloroquine, Remdesivir, *etc.*) as well as "COVID-19 + TREATMENT". The most relevant articles have been selected in order of mention and by scientific relevance, prioritizing those published in journals with the highest impact factor.

VIRAL PHASE TREATMENTS

Remdesivir

This RNA inhibitor drug has been studied since an early stage of the pandemic for its inhibitory effect on the viral replication of SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV), demonstrating *in vitro* activity against SARS-CoV-2[10].

Since then, multiple studies and clinical trials have been conducted in order to prove its efficacy against COVID-19 infection. We highlight two of the largest: the Solidarity study and the Adaptive COVID-19 Treatment Trial (ACTT-1).

In November 2020, the final report of the clinical trial conducted by ACTT-1 group about the use of remdesivir for COVID-19 was published. In this clinical trial, 1062 patients with SARS-CoV-2 lower respiratory tract infection were enrolled. These patients were randomized to receive 10 d of treatment with remdesivir (200 mg as a loading dose, followed by 100 mg daily) *vs* placebo. The data obtained showed a significant reduction in recovery time compared to placebo (10 d *vs* 15 d). According to the results of this analysis, this effect was greater with the initiation of treatment in the early phase (first 10 d), and in patients in the 5th stage of severity. No clear results were obtained on its effect on mortality[11].

The Solidarity study carried out by the World Health Organization (WHO) confirmed the absence of effect of remdesivir on mortality in comparison with placebo

and in comparison with hydroxychloroquine, lopinavir/ritonavir and interferon[11].

Review articles on this drug have also been published, including information from the current literature and from smaller studies. A systematic review carried out by the American College of Physicians suggested that, according to the reviewed bibliography, there are studies that would demonstrate a similar benefit between the 5-d *vs* the established 10-d treatment regimen, with a consequent reduction in the reported adverse effects in patients with respiratory infection caused by SARS-CoV-2 who do not require mechanical ventilation or extracorporeal oxygenation[12].

Lopinavir/ritonavir

Lopinavir is a protease inhibitor antiviral drug used against human immunodeficiency virus; its combination with ritonavir increases its plasma half-life.

This drug has shown *in vitro* activity against SARS-CoV-1 and was used during the MERS epidemic, demonstrating efficacy in terms of clinical and radiological improvement and reduction of viral load[13].

Despite its initial compassionate use, clinical trials have shown lack of efficacy against SARS-CoV-2.

The RECOVERY clinical trial is one of the largest studies conducted to date. It included 26 hospitals in the United Kingdom, and has studied the efficacy and safety of various drugs against COVID-19 (hydroxychloroquine, azithromycin, dexamethasone and lopinavir/ritonavir). In this study, 1616 patients were randomized to receive lopinavir/ritonavir *vs* 3424 patients receiving the standard treatment at that time. This study confirmed lack of efficacy of this drug in terms of mortality reduction, clinical improvement or time to discharge, concluding with a recommendation against its use in COVID-19 patients[14].

Hyperimmune plasma

Convalescent plasma (hyperimmune plasma, with active antibodies against SARS-CoV-2) has been proposed as a treatment for COVID-19 due to its direct antiviral neutralizing effect, its ability to modulate viral activity in the acute moment and its ability to indirectly activate antiviral functions of the immune system such as the complement cascade, NK cells, *etc.* Hyperimmune plasma has been successfully used for the treatment of influenza pneumonia and, more recently, for SARS-CoV-1. The RECOVERY group has assessed mortality at 28 d with hyperimmune plasma in comparison with standard of care, concluding that there are no significant differences; neither when analysing by subgroups. They propose as a limitation for the study that only hospitalized patients are included, so most are not in the viral replication phase, where theoretically hyperimmune plasma would have more effect[15].

Piechotta *et al*[16] made a review of 20 studies comparing hyperimmune plasma and standard of care. In a preliminary analysis, they did not find any benefit in terms of mortality, death time or improvement of clinical symptoms, concluding that there is insufficient evidence on efficacy and safety[16].

Plitidepsin

The antiviral activity of plitidepsin is mediated by the inhibition of eukaryotic translation initiation factor 1, establishing it as a possible drug target. Thus, as observed both *in vitro* and *in vivo* in the article by White *et al*[17], plitidepsin can reduce viral replication by two orders of magnitude and lung inflammation *in vivo*, showing clinical potential against COVID-19. Clinical studies are needed to see if it is effective in human patients.

TREATMENTS IN THE INFLAMMATORY PHASE

Corticosteroids

Corticosteroids have been proposed as a possible treatment for COVID-19 due to their anti-inflammatory and immunosuppressive properties, being able to reduce the systemic damage produced in the inflammatory phase. In the systematic review by Budhathoki *et al*[18], 83 articles were included. It attempted to assess which patients would benefit the most from corticosteroid treatment according to the severity of the disease. It was observed that severely ill patients were more likely to receive corticosteroids in their treatment, with the groups receiving corticosteroids presenting a longer hospitalization and higher mortality; without being able to rule out bias because of the non-randomization of the patients[18].

The RECOVERY group assessed mortality from all causes at 28 d, comparing standard of care with the daily administration of dexamethasone 6 mg for 10 d. It demonstrated that mortality was lower in patients who received dexamethasone. In addition, they saw that this benefit was greater in those patients requiring oxygen therapy, with or without positive pressure therapy, and in those patients recruited after more than 7 d of symptoms. Likewise, it was observed in those patients with oxygen therapy that the administration of dexamethasone decreased their risk of needing invasive mechanical ventilation (IMV) and increased their possibility of IMV withdrawal if they were already receiving it[19].

Finally, it should be noted that a WHO work group has published a meta-analysis. Out of 1703 randomized patients, 678 received corticosteroids and 1025 received conventional treatment, showing an absolute risk of mortality at 28 d of 32% and 40% respectively. Also, mortality was lower in those patients who received low doses of corticosteroids (29%) than in those who received high doses (36%). No increase in adverse effects was perceived in the group receiving corticosteroids.

The Food and Drug Administration, WHO, European Medicines Agency and National Institutes of Health recommend the use of corticosteroids for the treatment of COVID-19 in patients requiring oxygen therapy. The WHO also established that a corticosteroid regimen should be part of the standard treatment of critically ill patients [20].

Tocilizumab

Hypoxia and severe respiratory failure that occurs in patients with COVID-19 infection have been related to a disproportionate increase in acute phase reactants and pro-inflammatory cytokines such as Interleukin-6 (IL-6) or IL-1[21].

Therefore, it is believed that specific immunomodulatory substances against these cytokines could stop the mentioned inflammatory cascade and slow down the clinical deterioration of these patients.

Tocilizumab is a monoclonal antibody used in rheumatological diseases such as Rheumatoid Arthritis. It blocks the IL-6 membrane and soluble receptors, with the consequent reduction of the associated inflammatory response[22].

Its efficacy in patients with COVID-19 infection is still uncertain. To date, multiple clinical trials have been conducted, with disparate results.

In October 2020, Stone *et al*[23] published the results of its randomized clinical trial, conducted in 7 hospitals in the city of Boston (United States). They included a total of 243 patients with moderate COVID-19 infection (who did not require mechanical ventilation), randomized with a 2:1 ratio to receive conventional treatment *vs* placebo, or a single dose of 8 mg/kg of tocilizumab (maximum 800 mg). This study did not demonstrate any beneficial effect on the use of tocilizumab in mortality, IMV requirements or decrease in clinical deterioration. It should be noted that, at the time of this study, the results of the RECOVERY study on the efficacy of dexamethasone had not been published, so corticosteroids were not included as standard treatment [23].

In February 2021, Malhotra's group published the results of its phase 3 clinical trial. This was carried out in 61 centers between the United States and Europe, in patients with severe COVID-19 infection, randomized with a 2:1 ratio to receive tocilizumab 8 mg/kg *vs* placebo. In this study, no results were obtained that demonstrated an additional benefit of tocilizumab on mortality, or improvement in clinical status according to the ordinal severity scale (Table 1) at 28 d. It suggests a possible reduction in hospitalization time and ICU stay time in the treatment group, but more extensive research is needed[24].

Salama *et al*[25] conducted a phase 3 trial in 6 countries, with 389 patients of different age groups and ethnicity. This trial has demonstrated a decrease in the progression of the clinical deterioration and the need for IMV, mainly in patients with moderate or severe disease without mechanical ventilation. No reduction in mortality was demonstrated compared to the placebo group.

The RECOVERY group has recently published the results of the randomized trial carried out in the United Kingdom, with the participation of 131 hospitals belonging to the National Health System. The trial included 4116 patients who were randomized to receive tocilizumab *vs* standard treatment. The results of this study have shown a significant decrease in mortality at 28 d in the group randomized to receive tocilizumab and in patients with hypoxia and elevated acute phase reactants. It also improved the odds of hospital discharge before 28 d and a lower rate of progression toward IMV. In this study, the use of corticosteroids was included as standard medical treatment against COVID-19, also suggesting a possible benefit of the synergy of these two drugs[26].

Table 1 Coronavirus disease 2019 treatments

Drug	Mechanism of action	Recommendation	Posology	Benefits
Remdesivir[1]	RNA replication inhibition	Hospitalized patients in the first 10 d of infection requiring supplementary oxygen, without mechanical ventilation or extracorporeal oxygenation	Loading dose of 200 mg, followed by 100 mg daily for 5 d	Reduction in recovery time compared to placebo (10 d *vs* 15 d)
Corticosteroids[1]	Anti-inflammatory and immunosuppressive effects	Hospitalized patients requiring oxygen therapy. Also beneficial in patients with higher requirements of respiratory support	Dexamethasone 6 mg daily for 10 d	Reduction of mortality at 28 d. Decrease the risk of IMV and days of IMV
Tocilizumab[1]	Antagonist of IL-6 receptor. Immunomodulatory effect	Hospitalized patients with hypoxia and elevated acute phase reactants	8 mg/kg in a single dose (maximum of 600 mg). A second dose might be administrated if lack of effect	Reduction of mortality at 28 d. Reduce progression to IMV
Anakinra[2]	Antagonist of IL-1 receptor. Immunomodulatory effect	Not clear recommendations. Hospitalized patients with hypoxia and elevated acute phase reactants	-	Some data show some effect on clinical improvement in patients with NIMV requirements.
Sarilumab[2]	Antagonist of IL-6 receptor. Immunomodulatory effect	Not clear recommendations. Hospitalized patients with hypoxia and elevated acute phase reactants	-	It might reduce mortality in critical patients (unclear data)
Bariticinib[2]	Janus kinase (JAK) 1/2 inhibitor. In-vitro activity against SARS-CoV-2, given its inhibitory effect on cytokine release and its inhibition of virus entry into pneumocytes	Not clear recommendations. Hospitalized patients with moderate-severe COVID-19 infection	-	In combination with corticosteroid, it improves SpO_2/FiO_2
Colchicine[2]	Lipid soluble alkaloid, with anti-inflammatory effect	Not clear recommendations. Non-hospitalized patients with COVID-19	-	Some data show reduction of mortality and hospitalization in patients with mild infection.
Otilimab[2]	Monoclonal antibody, anti-granulocyte macrophage colony-stimulating factor	Not clear recommendations. Hospitalized patients with severe disease	-	Might have beneficial effects in elderly patients with severe disease
Plitidepsin[2]	Inhibition of eef1a, reduce viral replication	More studies needed, not clear recommendations	-	-
Hydroxychloroquine[3]	RNA replication inhibitor	Not recommended		
Azithromycin[3]	Immunomodulatory effect	Not recommended		
Lopinavir-Ritonavir[3]	Protease inhibitor.	Not recommended	-	-
Hyperimmune plasma[3]	Convalescent plasma with active antibodies against SARS-CoV-2	Not recommended	-	-

[1]Recommended ones.
[2]Need more evidence.
[3]Not recommended treatments. COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; IMV: Invasive mechanical ventilation.

Anakinra

Anakinra is an antagonist of the IL-1 receptor, with the ability to inhibit the pro-inflammatory activity of IL-1 alpha and beta. This drug is approved for the treatment of rheumatologic diseases such as Still's disease or familial Mediterranean fever. It is believed that it could be a therapeutic target against the inflammatory cascade produced by COVID-19, and especially against macrophage activation syndrome[27].

So far, this drug has shown effectiveness in patients with sepsis criteria and signs of hyperinflammation[28].

In the retrospective study carried out by Cavalli *et al*[29], they analyzed 29 patients admitted to the San Rafaelle hospital in Milan with NIMV requirements. This showed a certain improvement of the clinical status of the patients, without finding a reduction in mortality.

The CORIMUNO-ANA-1 clinical trial included 153 patients across France with moderate-severe COVID-19 infection, without mechanical ventilation (category 5 on the WHO severity scale). It did not demonstrate any beneficial effect of anakinra, indicating the need for further studies in other groups of patients with greater severity [30].

Therefore, according to the literature, so far there is no clear evidence that supports the use of anakinra in any specific group of patients. Currently, there are ongoing clinical trials with this drug in different subgroups of patients.

Sarilumab

Several studies prove that elevated levels of interleukin-6 are related to greater severity of COVID-19 infection and higher mortality[31].

Sarilumab is a recombinant monoclonal antibody against the IL-6 receptor (soluble and membrane), approved for rheumatoid arthritis[32].

Many publications and trials have shown a benefit with the use of IL-6 antagonist drugs on severe COVID-19 infection. The study carried out by the REMAP-CAP group on 895 patients with COVID-19 demonstrated a reduction in mortality and a higher clinical improvement in critically ill patients randomized to receive an IL-6 antagonist. However, it should be noted that in this trial only 48 patients received sarilumab, while 366 patients received tocilizumab[33].

The results of the clinical trial carried out by Lescure *et al*[34] for the Sarilumab COVID-19 Global Study Group were recently published. In this Phase 3 trial, 431 patients with severe SARS-CoV-2 pneumonia (categories 5, 6 or 7 on the WHO severity scale) were randomized. This trial compared the use of sarilumab (200 or 400 mg) *vs* placebo. Sarilumab did not show to be effective in reducing mortality, improving the clinical severity scale, or reducing the length of hospital stay.

Baricitinib

Baricitinib is another drug used in rheumatology as a Janus kinase 1/2 inhibitor. Multiple *in vitro* studies have been carried out with this molecule. The results of these studies suggest *in vitro* activity against SARS-CoV-2, given its inhibitory effect on cytokine release and its inhibition of virus entry into pneumocytes[35].

Studies in animal models show a significant reduction in cytokine production by alveolar macrophages, which translates into a reduction in the local inflammatory cascade and neutrophil recruitment[36].

The Oxford study, carried out by Rodriguez-Garcia *et al*[37], suggests a beneficial effect of the combined use of baricitinib with corticosteroids in patients with moderate-severe COVID-19 infection, by observing an improvement in lung function measured by SpO_2/FiO_2. It might produce a certain lung protective effect, as lower D-dimer values are observed in this group of patients.

The study carried out by Kalil *et al*[38] suggested a benefit from the combination of baricitib together with remdesivir in patients with COVID-19 infection. In this clinical trial, 1033 patients were randomized to receive remdesivir in combination with baricitinib or placebo. The results demonstrated a greater benefit with the association of the two drugs in terms of improvement in clinical status and in the days to recovery, with a greater benefit in patients requiring high-flow therapy or NIMV at the beginning of treatment.

Right now, there are multiple ongoing studies about the efficacy of this drug, alone or combined with others.

Colchicine

Colchicine is a lipid soluble alkaloid that accumulates in granulocytes and monocytes. It reduces chemotaxis of inflammatory cells, blocks the expression of E-selectin, responsible for leukocyte binding to endothelial cells, and it is also in charge of the inflammasome activation and superoxide production. It has shown anti-inflammatory activity in pathologies such as pericarditis or gout.

McEwan *et al*[39] conducted a systematic review of the infectious complications of the use of colchicine and the use of colchicine for the treatment of infectious diseases, concluding in the case of COVID-19 that mortality at 21 and 28 d was lower in the colchicine group than in the standard treatment group. However, it is unknown whether this potential benefit is due to the antiviral or anti-inflammatory action of colchicine.

Likewise, the preliminary results of the COLCORONA study (Tardif *et al*[40]) were recently published confirming that in non-hospitalized patients with COVID-19, colchicine reduces mortality and hospitalization.

Otilimab

This monoclonal antibody that inhibits granulocyte macrophage colony-stimulating factor (anti-GM-CSF) is currently under investigation in patients with severe SARS-CoV-2 infection.

The OSCAR clinical trial, which is about to start Phase 3, has shown promising results in Phase 2, ensuring the safety goals and suggesting a benefit in groups with older patients[41].

OTHER TREATMENTS

Hydroxychloroquine

Hydroxychloroquine has shown *in vitro* antiretroviral activity against several viruses, including SARS-CoV-2, it has an acceptable adverse effect profile and is inexpensive. It has not shown clinical efficacy in animals, but there are several studies that have suggested clinical benefits from the association of azithromycin with hydroxychloroquine.

The Oxford RECOVERY group compared all-cause mortality at 28 d in two groups, one of which received hydroxychloroquine (n = 1561) and the other, standard treatment (n = 3155). The risk of progression to non-invasive mechanical ventilation was found to be higher in the group taking hydroxychloroquine. Likewise, mortality was higher in the group taking hydroxychloroquine, determining that hydroxychloroquine is not an effective treatment for COVID-19. In addition, there is a risk of cardiovascular toxicity, which is exacerbated by co-administration with azithromycin [42].

Tleyjeh *et al*[43] studied the cardiovascular risk of the use of chloroquine and hydroxychloroquine in patients with COVID-19, establishing a significant risk of drug-induced QT prolongation and increased incidence of Torsades de pointes, ventricular tachycardia and cardiac arrest. Therefore, they do not recommend this treatment by routine for COVID-19.

The meta-analysis by Kashour *et al*[44] establishes with moderate certainty that hydroxychloroquine, with or without azithromycin, does not reduce short-term mortality in hospitalized patients with COVID-19 or the risk of hospitalization in patients treated on an outpatient basis.

Fiolet *et al*[45] also analysed the mortality of hydroxychloroquine alone, hydroxychloroquine and azithromycin, and standard treatment, showing that hydroxychloroquine alone does not modify mortality over standard treatment. However, when it is combined with azithromycin, mortality increases.

Azithromycin

Once the benefit of the use of corticosteroids in COVID-19 had been evaluated, it was assessed whether other treatments that suppress or modulate the immune system could be effective against the disease. Azithromycin, besides being an antibiotic of the macrolide family, has shown an immunomodulatory effect by reducing the production of pro-inflammatory cytokines and inhibiting the activation of neutrophils.

The RECOVERY group studied mortality at 28 d, the time to discharge and the need for invasive mechanical ventilation in hospitalized COVID-19 patients. No significant differences between the azithromycin group and the standard treatment group were observed, nor were significant differences in subgroup analysis. Thus, they consider that azithromycin is not an effective treatment in hospitalized patients with COVID-19 and should be reserved for those who have an indication of azithromycin for antibiotic purposes[46].

Verdejo *et al*[47] conducted a systematic review on the use of macrolides in COVID-19, evaluating articles in which they are used alone or in combination with other drugs such as hydroxychloroquine. They evaluated all-cause mortality, the need for invasive mechanical ventilation and extracorporeal membrane oxygenation, hospitalization time, respiratory failure, serious adverse events, and SARS-CoV-2 PCR time to negativize. Although the quality of the evidence for most of the results was low, they concluded that macrolides do not show any beneficial effect compared to standard treatment.

Anticoagulation and thromboprophylaxis

So far, there is wide evidence that confirms a higher risk of thromboembolic events in patients with severe COVID-19. For this reason, despite not being a direct COVID-19 treatment, the use of anticoagulation in these patients has been a controvert topic.

These thrombotic events are cause by the infection itself, but also by the proinflammatory response, the hypoxia and the critical illness. Some of these mechanisms are still unknow.

Most of the recent guidelines recommend keeping a high level of suspicion of thromboembolic events in hospitalized patients, monitoring laboratory parameters such as D-dimer and blood count. It is important to point out also the risk of haemorrhage in some patients, with its consequent implications. Tools like Wells score and IMPROVE-bleeding score could be useful to predict the risk of thrombosis and bleeding.

According to the article published by Skeik *et al*[48], patients with low or no suspicion for VTE calculated by Wells score (0 for deep vein thrombosis or < 2 for pulmonary embolism), they recommend regular antithrombotic prophylaxis. In patients with higher risk, imaging should be considered. If the result is negative, or imaging is not available, we should consider the bleeding risk. If this one is high, also regular thromboprophylaxis is recommended; if it is low, we should consider anticoagulation. In patients with high suspicion of VTE (Wells > 2 for VDT or 6 for PE) and without imaging available, the anticoagulation is also recommended according to the bleeding risk. Direct oral anticoagulations are usually preferred[48].

Guidelines like the CHEST Guidelines or the American College of Cardiology also recommend thromboprophylaxis in hospitalized patients depending on the thrombotic and bleeding risk of each patient. More studies are still needed.

CONCLUSION

Currently, multiple pharmacological studies continue to be carried out. For the moment, the evidence recommends treating patients with remdesivir in the viral phase and with dexamethasone, tocilizumab or baricitinib in the inflammatory phase. Nevertheless, we are sure that in the following months we will be able to have more therapeutic weapons to tackle COVID-19.

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REVIEW

Oncolytic virus therapy in cancer: A current review

Jonathan Santos Apolonio, Vinícius Lima de Souza Gonçalves, Maria Luísa Cordeiro Santos, Marcel Silva Luz, João Victor Silva Souza, Samuel Luca Rocha Pinheiro, Wedja Rafaela de Souza, Matheus Sande Loureiro, Fabrício Freire de Melo

ORCID number: Jonathan Santos Apolonio 0000-0002-9463-8114; Vinícius Lima de Souza Gonçalves 0000-0002-6445-9318; Maria Luísa Cordeiro Santos 0000-0001-7078-9789; Marcel Silva Luz 0000-0003-1650-5807; João Victor Silva Souza 0000-0002-9474-1816; Samuel Luca Rocha Pinheiro 0000-0002-8877-892X; Wedja Rafaela de Souza 0000-0002-5135-7785; Matheus Sande Loureiro 0000-0002-5140-2996; Fabrício Freire de Melo 0000-0002-5680-2753.

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Jonathan Santos Apolonio, Maria Luísa Cordeiro Santos, Marcel Silva Luz, Samuel Luca Rocha Pinheiro, Wedja Rafaela de Souza, Matheus Sande Loureiro, Fabrício Freire de Melo, Universidade Federal da Bahia, Instituto Multidisciplinar em Saúde, Vitória da Conquista 45029-094, Bahia, Brazil

Vinícius Lima de Souza Gonçalves, João Victor Silva Souza, Universidade Estadual do Sudoeste da Bahia, Campus Vitória da Conquista, Vitória da Conquista 45083-900, Bahia, Brazil

Corresponding author: Fabrício Freire de Melo, PhD, Professor, Universidade Federal da Bahia, Instituto Multidisciplinar em Saúde, Rua Hormindo Barros, 58, Quadra 17, Lote 58, Vitória da Conquista 45029-094, Bahia, Brazil. freiremelo@yahoo.com.br

Abstract

In view of the advancement in the understanding about the most diverse types of cancer and consequently a relentless search for a cure and increased survival rates of cancer patients, finding a therapy that is able to combat the mechanism of aggression of this disease is extremely important. Thus, oncolytic viruses (OVs) have demonstrated great benefits in the treatment of cancer because it mediates antitumor effects in several ways. Viruses can be used to infect cancer cells, especially over normal cells, to present tumor-associated antigens, to activate "danger signals" that generate a less immune-tolerant tumor microenvironment, and to serve transduction vehicles for expression of inflammatory and immuno-modulatory cytokines. The success of therapies using OVs was initially demonstrated by the use of the genetically modified herpes virus, talimogene laherparepvec, for the treatment of melanoma. At this time, several OVs are being studied as a potential treatment for cancer in clinical trials. However, it is necessary to be aware of the safety and possible adverse effects of this therapy; after all, an effective treatment for cancer should promote regression, attack the tumor, and in the meantime induce minimal systemic repercussions. In this manuscript, we will present a current review of the mechanism of action of OVs, main clinical uses, updates, and future perspectives on this treatment.

Key Words: Oncolytic viruses; Antitumor response; Tumor lysis; Tumor cells; Mechanism; Therapy

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Core Tip: Oncolytic viruses are organisms able to infect and lyse the tumor cells beyond stimulating the immune system to combat the disease. The clinical use of oncolytic viruses has shown to have positive results in the treatment of some types of cancers, contributing to reducing the tumor. Furthermore, the combined use of these viruses and other antitumor therapies have contributed to better prognosis in the patient's clinical condition.

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INTRODUCTION

The first theories about the possible use of viruses to combat tumor cells date from the early 20th century with the description in 1904 of a woman with acute leukemia who presented remission of the clinical picture and a patient with cervical cancer in 1912 that demonstrated extensive tumor necrosis, both after a viral infection[1]. Thereafter, between 1950 and 1980, influenced by the possibility of developing a therapy for cancer, many studies were performed with different types of wild viruses aiming at an oncolytic action; however, the goal was not achieved due to the non-existence of necessary tools to control the viral pathogenesis and direct the virus to specific targets [2]. Viruses can be used to infect cancer cells, specifically over normal cells, to present tumor-associated antigens, to activate "danger signals" that generate a less immune-tolerant tumor microenvironment, and to serve transduction vehicles for expression of inflammatory and immunomodulatory cytokines[3]. Currently, in order to overcome these obstacles, the updates in the field of genetics seek to increase the specificity and efficacy of some viruses in infecting the abnormal cells through mechanisms such as gene deletion and the combined use of viruses and immune checkpoint inhibitors (ICIs)[4].

The oncolytic viruses (OVs) are organisms able to identify, infect, and lyse different cells in the tumor environment, aiming to stabilize and decrease the tumor progression. They can present a natural tropism to the cancer cells or be oriented genetically to identify specific targets[5]. Several OVs are being studied as a potential treatment for cancer in clinical trials[6]. Moreover, the OVs are capable of contributing to the stimulation of the immune system against the tumor cells, influencing the development of an antitumor response[7].

It is known that there are several evasion mechanisms in the tumor environment that contribute to the downregulation of the immune system, positively influencing the stability and progression of the disease even in immunocompetent patients[8]. Antigen presenting cells can be prevented from presenting tumor antigens to the T cells correctly, which contributes to the non-activation or discouragement of these cells [9]. Moreover, certain types of tumors can promote an abnormal stimulation of immune checkpoint receptors in T cells, like the cytotoxic T lymphocyte-associated antigen 4 and the programmed cell death protein 1/programmed death ligand 1(PD-L1), both related to the negative regulation of the inflammatory response and immune system homeostasis contributing to apoptosis and inhibition of proliferation of T cells [10]. In addition, the excess of tumor-associated macrophages, main lymphocytes regarding the inflammatory response against the tumor, are also an important mechanism of immune evasion since they have some similar functions and features to type M2 macrophages, which are responsible for tissue repair and immune response regulation. Thus, the abnormal rise of tumor-associated macrophages has been related to the downregulation of inflammation and increase of tumor growth rates[11].

Therefore, the clinical use of OVs emerges as an alternative to modifying the tumor environment from a state of immune desert caused by the evasion mechanisms that contribute to tumor progression, to an inflamed state, where the immune system is able to kill the abnormal cells[12]. In addition, the viruses present different mechanisms that would lead the infected cells to a cell lysis process, contributing to tumor cell death and increasing the efficacy of the immunotherapy[4]. This review will

encompass the viral mechanisms responsible for the oncolytic action of OVs, the clinical use of these viruses in certain tumors, and the future perspectives about their use.

MECHANISM

General mechanism

OVs are able to infect abnormal cells through specific targets, such as nuclear transcription factors and among them human telomerase reverse transcriptase, prostate specific antigen, cyclooxygenase-2, osteocalcin, and surface markers as prostate-specific membrane antigen, folate receptor, CD20, endothelial growth factor receptor, and Her2/neu, which are substances produced by the tumor cells[5]. Furthermore, the deletion of pathogenic viral genes in the laboratory in order to increase the selectivity to the tumor cells and decrease the aggressiveness of the OVs to normal tissues is also possible[13].

The administration route of OVs is intrinsically related to the type of tumor to be treated, given that the virus pathway directly influences the effectiveness of the therapy due to the virus availability on-site and the natural barrier of the organism of combat to antigens. The distribution can occur *via* intraperitoneal, intrathecal, subcutaneous, intratumoral, which provides greater control of viral quantity in the tumor environment and less adverse effects, and intravenous, which is related to the treatment of distant metastases[14].

Regarding the mechanisms of immune evasion by the tumor, the cancer cells can present certain alterations in the expression and activation of some mechanisms, such as protein kinase R and interferon 1 signaling pathway, which interferes in the response to viral infections, programmed apoptosis, and maturation of inflammatory cells. The modifications in the antiviral response, allied to viral factors capable of preventing apoptosis, allow OVs to survive longer in cancer cells and consequently to conclude the life cycle and maturation to the lytic phase[15].

The presence of viruses in the human organism stimulates the recognition of different immune signs related to the virus structure, such as viral proteins, RNA, DNA, and viral capsid, the pathogen-associated molecular patterns (PAMPs)[16]. Dendritic cells, upon recognition of the PAMPs through toll-like receptors (TLRs), which are pattern recognition receptors, stimulate production of inflammatory molecules with antiviral characteristics, like the type 1 interferons, tumor necrosis factor alpha (TNF-alpha) and cytokines such as interleukin 2 (IL-2), important mechanisms of recruitment of immune cells, and maintenance of the inflammatory environment[17].

TNF-alpha is related to response to the viral infection, positively regulating the expression of class 1 major histocompatibility complex in the cell membrane and positively influencing the action of caspase enzyme and cell apoptosis on some tumors [18]. This interferon is capable of stimulating cancer cell death through mechanisms that contribute to necrosis and apoptosis, generating thrombotic events through its antiangiogenic effects, which can lead to the destruction of some blood vessels responsible for the blood supply of the tumor[19]. TNF-alpha is also related to the stimulation of T helper cells type 1 (Th1) response, increase of the cytotoxicity of natural killer cells, and maturation of antigens presenting cells[18].

Studies have shown that IL-2 is related to the stimulation of cytotoxic lymphocytes and activation of T cell response, contributing to maturation and expansion of CD8+ T cells (TCD8) and natural killer cells, along with positive regulation of CD4+ T cells (TCD4). IL-2 is also capable of regulating T regulatory cell action and homeostasis, creating an inflammatory environment favorable for combating the tumor[20]. Furthermore, the Th1 inflammatory profile was also related to the decrease of T regulatory cells, increased rates of TCD4 and TCD8 effector cells, stimulation and differentiation of T lymphocytes as well as the maturation of dendritic cells, which contributes to the reversal of the immunosuppressive state of the tumor and promotes an inflammatory response[21].

In addition to the damage caused by the inflammatory response, the viral action inside the cell is also an important factor in the lysis and death of the aberrant cells. The presence of OVs could stimulate some dysfunction of organelles, such as the endoplasmic reticulum, mitochondria, or lysosome, compromising the normal cellular function. Moreover, the virus can stimulate oxidative stress through the production of reactive nitrogen species and endoplasmic reticulum stress, which is related to an increase of intracellular calcium levels[17], contributing to the stabilization and

decrease of the tumor.

The combined use of cell checkpoint blockers and OVs is an important mechanism to increase viral survival rates in the human organism, given that it contributes to the stimulation of an inflammatory response against the tumor. Through negative regulation of PD-L1, the tumor can circumvent the immune system, avoiding the maturation of T cells. In this way, PD-L1 inhibition was capable of stimulating a response with a Th1 profile, contributing to the appearance of TCD8 cells against the tumors and stimulating natural killer cell action[22]. Furthermore, studies have demonstrated that the administration of the OVs and monoclonal antibodies that inhibit the action of cytotoxic T lymphocyte-associated antigen 4 contributed to enhancing the effectiveness of immunotherapy[21].

The aforementioned mechanisms contribute to different types of elimination of the tumor cells, such as autophagic cell death, apoptosis, pyroptosis, and necrosis, leading to the production of immune signs related to the cell damage: damage-associated molecular patterns (DAMPs), like high mobility group box 1 protein and ATP. The DAMPs are important elements in the stimulation of the dendritic cell maturation process and contribute to the presentation of tumor-associated antigens to the immune cells through the cross-presentation between DAMPs and tumor-associated antigens, which leads to the perpetuation of the inflammatory response process[23]. Therefore, cellular lysis allows the liberation of the viruses in the extracellular environment and subsequent infection of other tumor cells, creating a chain reaction of combat to the tumor[16]. Besides that, the cell death contributes to the release of tumor antigens liable to be identified by immune cells in the inflammatory environment, stimulating a response against tumor cells, even in the uninfected ones, by the OVs[15].

The main mechanisms of action of OVs are represented in (Figure 1).

OVs

Adenovirus: The adenoviruses are non-enveloped organisms with double-stranded linear DNA and an icosahedral capsid with three main proteins, hexon, penton base, and fiber, which when identified by the immune system contribute to the emergence of an antiviral response. There are more than 80 human types of adenoviruses that belong to the *Adenoviridae* family[24]. These viruses have a high tropism for different tissues of the organism, including ocular, respiratory, enteric, renal, and lymphoid and are able to use several receptors, such as human coxsackie-adenovirus receptor, CD86, CD46, and CD80 to enter the host cells[25]. Moreover, due to its capacity of serving as a viral vector[24], allied to their chemical and thermal stability outside the cell, various mechanisms of cellular entry, and the great knowledge about their biology, the adenoviruses have been used for the development of different immune therapies[26].

The viral replication process starts inside the cellular nucleus, inducing the expression and liberation of some proteins in the cytoplasm such as E1a and E1b, which are related to the stimulation of the autophagy process. This mechanism induces the production of some autophagosomes that can later merge with lysosomes resulting in the death of organelles or even the full cell[27]. Furthermore, research has shown that in tumor cells the expression of E1a can be related to the stimulation of the production of autophagic complexes, and E1b possibly supports the potentiation of action of these complexes, both contributing to the stabilization and decrease of the tumor[28].

When identifying and responding to different proteins of the viral capsid of adenoviruses, the human organism starts producing several inflammatory cytokines, such as IL-12 and TNF-alpha[29], which are related to the stimulation of cytotoxic cells like natural killer cells and TCD8, besides contribution in the maturation of immune cells and against the tumor. The type 5 Ad is commonly used for oncolytic therapy, since it can be detected by TLRs in the cellular membrane (TLR-2) or inside the cell (TLR-9) teasing the stimulation of different mechanisms in order to create a Th1 profile inflammatory response[29]. Moreover, the *Adenoviruses* can activate other pathways of the immune system, such as the complement system stimulating the opsonization processes, increasing the migration rates of inflammatory cells and production of inflammatory cytokines[23], which contributes to destroying infected cells.

Finally, the cellular stress caused by the viral infection and the inflammatory process lead to tumor cell death through necrosis, autophagy, or apoptosis and further liberation of DAMPs or PAMPs in the inflammatory environment, stimulating the maturation and migration of inflammatory cells as well as the production of cytokines. Furthermore, in addition to the direct tumor cell killing, the adenoviruses are capable of initiating the formation of an antitumor immune memory that contributes to the combat in metastatic sites[25]. Table 1 shows some genetic modifications to improve the adenoviruses oncolytic action.

Table 1 Genetic modifications in the adenovirus

Ref.	Virus	Updates	Aim
Rojas *et al* [219]	COVIR -7/-15	Insertion of E2F-binding sites in the gene *E1A*	Specific targeting to the tumor cells, which express E2F and increase viral replication rate and antitumor action
Sarkar *et al* [220]	CTV-m 7	Insertion of the transgene MDA-7/IL-24	Expression of the protein MDA-7/IL-24 increases the cytotoxic action in the tumor sites and lyse the metastatic cells. The studies have shown greater effectiveness in the therapy of prostate cancer
Sarkar *et al* [220]	tCCN1 -CTV - m 7	Replacement of E1A by tCCN1	Specific targeting and cytotoxicity against the tumor cells, which express the promoter tCCN1 in prostate cancer
Choi *et al* [221]	Ads armed with inhibitors of tumoral angiogenesis	Incorporation of the gene *FP3*	Increase of the antiangiogenic capacity, which decreases the vascular endothelial growth factor production and suppresses the rate of tumor growth
Lucas *et al* [222]	Ad5 armed with the peptide CKS17	Replacement of HVR5 by the peptide CKS17	Specific target to the TGFBRII in the liver cancer cells, increasing the viral cytotoxic action and decreasing the liver sequestration
Garofalo *et al*[223]	AdV-D24-ICOSL-CD40L	Insertion of *D24*, *ICOSL* and *CD40* genes in the chimeric virus, AdV-D24, serotype 5/3	Selectivity to infect the cancer cells through DSG-2 receptor and stimulation of the immune system by ICOSL and ICOS, both contributing to the immunogenic cell death in melanoma
Vera *et al* [224]	VCN-01	Selectivity to the pRB pathway and ability to express hyaluronidase	Specific viral replication, decreasing the side effects and degradation of the extracellular matrix by the enzyme hyaluronidase in solid tumors
Yang *et al* [225]	Ad5/3-CXCR4-TIMP2	Replacing Ad5 knob with Ad3 knob and incorporating the gene *TIMP2*	Selective replication in the cancer cells, which reduces the action over the normal cells and the expression of inhibitors of metalloproteinases, contributing to the degradation and remodeling of the extracellular matrix, preventing tumor growth and metastasis

Ads: Adenoviruses; CD40L: CD40 ligand; DSG-2: Desmoglein 2; FP3: Farnesylated protein 3; HVR5: Hypervariable region 5; ICOSL: Inducible co-stimulator ligand; IL-24: Interleukin 24; MDA-7: Melanoma differentiation-associated gene-7; pRB: Retinoblastoma protein; tCCN1: Truncated cellular communication network factor 1; TGFBRII: Transforming growth factor-beta receptor II; TIMP2: Tissue inhibitor of metalloproteinases 2.

Protoparvovirus: The Protoparvoviruses are single-stranded DNA, non-enveloped viruses that belong to the *Parvoviridae* family. They are capable of infecting mammalian cells, including human beings, through fixation factors such as the transferrin receptor or glycosidic substances like the N-acetylneuraminic acid that is expressed on the cellular membrane and contributes to an environment favorable to viral fixation in the cell[30].

The major capsid protein VP1 is a protein that coordinates the penetration of protoparvoviruses in the host cell by an endocytosis process and enables the destruction of the endocytic vesicle inside the cell and further liberation of viral proteins in the cytoplasm. Moreover, VP1 has nuclear localization signals responsible for assisting the viral protein displacement to the cell nucleus[31]. From this point, the virus can remain inert until the beginning of the cellular division process when during the S/G2 phases through protein NS1 action, it can block the cell genome replication and allow the integration of viral material with the host genetic material to ensure the viral survival[31].

H-1PV can produce an oxidative stress state through the increase in levels of reactive oxygen and nitrogen species through NS1 protein action inside the cell. NS1 is also related to the regulation of RNA viral replication, leading to the destruction of genetic material and activation of apoptosis pathways with later cell death. Furthermore, the virus can stimulate the liberation of proteases from the lysosome to the cytoplasm causing cellular necrosis of tumor cells[17].

In addition, the protoparvoviruses are capable of triggering an inflammatory response with antitumor characteristics generating the production of cytokines with a Th1 profile like IL-2 and TNF-alpha, which[32] sets an inflammatory environment able to deal with the tumor cells. H-1PV also contributes to the stimulation of T lymphocytes like TCD8, cytotoxic cells, and the auxiliary cells TCD4 and formation of an immune memory against the tumor[33].

During the lytic phase, the viral action enables the increase of membrane permeability of lysosomes that allows the passage of the cathepsins enzymes to the cytoplasm and decreases the action of inhibitory agents of these proteases. Both factors play an important role in the gathering of cathepsins in the cellular cytoplasm, stimulation of their action, and contribution to the apoptosis pathways and to tumor cell death[34]. Moreover, the expression of NS1 contributes to cellular apoptosis

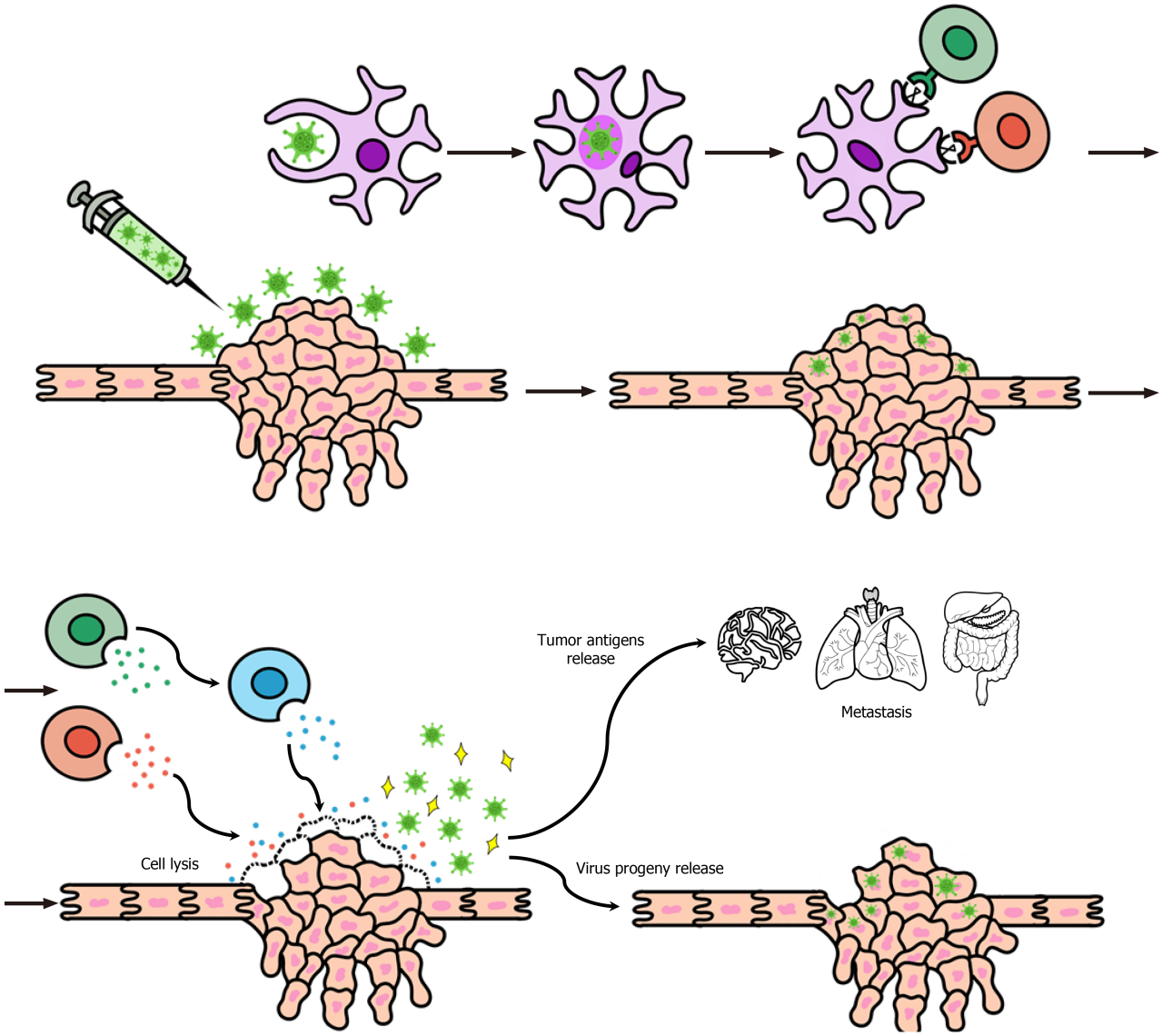

Figure 1 Mechanism of action of oncolytic viruses. Initially, oncolytic viruses can be administered by different pathways, such as intratumoral, subcutaneous, intraperitoneal, and intrathecal. Natural tropism and genetic targeting are responsible for favoring the arrival of oncolytic viruses to the tumor cells. Thereafter, the oncolytic viruses start to recognize the abnormal cells through substances expressed in the tumor environment and can use different receptors to connect and infect the host cell. From this point, the virus starts to use the cellular machinery for its replication process, producing viral proteins, reducing the cell function, stimulating oxidative stress states and contributing to the activation of some pathways related to the autophagic processes. At the same time, the antigen-presenting cells encompass some viral organisms, generating the formation of an endosomal vesicle that will merge with a lysosomal vesicle and will cause the digestion of the virus, providing smaller viral particles to be processed inside the cell. Later, the expression of the major histocompatibility complex class 2 together with the viral proteins on the cell surface occurs, creating a favorable environment for the antigenic presentation and subsequent activation and stimulation of the CD4+ T cells and CD8+ T cells, the first related to the production of cytokines responsible for contributing to the migration and maturation processes of inflammatory cells, and the second related to the direct action against the infected cells. Finally, the viral action and the immune response contribute to the destruction of the tumor cells releasing the viral progeny in the host organism allowing it to infect other abnormal cells and restart the process of combatting the tumor. Furthermore, cell death also releases tumor antigens that the immune system can identify, contributing to the formation of new inflammatory responses capable of acting both in the tumor environment and even in metastatic sites.

through damage to the genetic material, activation and stimulation of caspase action, and the generation of oxidative stress processes, bypassing the apoptotic evasion mechanism of the tumor cells[35].

Vaccinia virus: The vaccinia viruses (VACVs) are enveloped viruses with double-stranded linear DNA and belong to the *Poxviridae* family. They were used for smallpox vaccination in 1796, and currently after the eradication of this disease, their scientific use is aimed at the creation of vaccines and therapies for other pathologies[36]. One of the members of this family is the Pexa-Vec (pexastimogene devacirepvec, JX-594), which is genetically modified to possess the granulocyte-macrophage colony-stimulating factor (GM-CSF) along with thymidine kinase (*TK*) gene deletion in order

to increase the tropism to the tumor cells and limit the replication to the cells that express aberrant levels of TK[37].

The administration of VACVs in the tumor environment was related to the stimulation and expression of GM-CSF and IL-24, factors that together could contribute to stabilize and provide tumor cell death. GM-CSF is related to the maturation and differentiation of immune system cells like dendritic cells and neutrophils, which create an inflammatory environment that enables the combat of the tumor, and IL-24 inhibits tumor angiogenesis, positively influencing the apoptosis pathways and the formation of an antitumor response while inhibiting the formation of tumor metastases[38].

The viral action of some VACVs strains stimulate different cell death pathways such as necrosis and apoptosis, leading to the liberation of substances related to damage and danger, like ATP and high mobility group box 1 protein, that provides an immunogenic environment. Thereafter, the DAMPs support the cross-presentation between them and the tumor antigens, stimulating the TCD8 cell action and contributing to the stimulation of the antitumor response[39]. Furthermore, the Pexa-Vec has a tropism for endothelial cells that are responsible for tumor growth through the expression of vascular endothelial growth factor or fibroblast growth factor. It leads to the destruction of vasculature that irrigates the tumor and consequently a tissue necrosis process and decreasing of the tumor extension[40]. Some genetic modifications in the VACVs and updates in oncolytic therapy are listed in Table 2.

Reovirus: Respiratory enteric orphan virus (Reovirus) is a non-enveloped and double-stranded RNA virus that belongs to the *Reoviridae* family, which has a wide range of hosts (fungi, plants, fish, mammals, among others)[41,42]. This name is due to the isolation of the pathogen in the respiratory and gastrointestinal tract and the inability to cause any known human diseases[43,44]. Interestingly, this last characteristic is strongly correlated to the successful use of reoviruses in oncolytic therapy as well. The primary connection of reoviruses to an oncolytic role was found in 1977 when a study demonstrated that they have a tropism for "transformed cells" and that normal cells are resistant to the virus[45]. This information led, consequently, to further studies in order to evaluate the possibility of reoviruses as an alternative for cancer treatment.

There are three different reovirus serotypes: type one Lang, type two Jones, and type three Abney and Dearing[44]. Among them, the T3D is the most widely studied as a possible therapeutic for cancer treatment and is also known as Reolysin[46]. Furthermore, reoviruses are dependent on a mutation in the *ras* gene in order to replicate properly in the tumor cells[47], a fact that limits its use, given that only approximately 30% of the human tumors have these mutations. However, the Ras pathway can be activated by some elements, which means that more types of cancer can be subjected to viral oncolytic therapy by reoviruses (up to 80%)[48].

Regarding the mechanism in which reoviruses replicate in tumor cells, the Ras pathway plays an important part, given that it inhibits protein kinase R and therefore enables viral protein synthesis[49]. Moreover, studies also show that the epidermal growth factor receptor, more specifically the tyrosine protein kinase signaling pathways, increases reovirus infection and viral peptide synthesis[50]. In addition, reovirus-resistant NIH 3T3 cells capable of being infected and enhance protein production when transfected with the gene encoding epidermal growth factor receptor or with the v-erbB oncogene are also documented[51]. Thereby, these works on reoviruses clarified their possible use in oncolytic therapy, given that they are also non-pathogenic in humans, which makes it an attractive option.

The main mechanism of tumor lysis by reoviruses is virus-induced apoptosis, along with the immunomodulatory characteristics of the virus. The viral capsid proteins are able to activate an apoptotic pathway in the tumor cells through release into the cytosol of cytochrome *c* and smac/DIABLO from the mitochondria[52]. In regard to the immune response, once the reoviruses start protein synthesis, there is a secretion of proinflammatory cytokines and chemokines through PAMPs and DAMPs, which eases the generation of an adaptive antitumor immune response[15,53]. Then, cytotoxic TCD8 cells recognize the reovirus antigens and lyse the cells, along with a maturation of dendritic cells[54], consequent activation of natural killer cells, and further cytotoxicity[55].

Herpes simplex virus type I: The herpes simplex virus-1 (HSV-1) is a double-stranded DNA virus with a large genome of 150kb encoding for 70 or more genes that belongs to the alpha-herpesviruses subfamily[56,57]. Its large genome is very important, given that it can be easily modified in order to improve oncolytic properties and safety for the patient[56]. Unlike the reoviruses, HSV-1 is pathogenic to humans and can cause

Table 2 Genetic modifications in the vaccinia virus

Ref.	Virus	Updates	Aim
Parato *et al* [226]	JX-594	Express *GM-CSF* and *lacZ* transgenes	Increase lytic activity and antitumor immunity
John *et al* [227]	vvDD-GFP	Insertion of an Ab specific for the costimulatory molecule 4-1BB	Increase antitumor responses with myeloid cells, greater infiltration of CD8+ effector T and NK cells
Zhang *et al* [228]	GLV-1 h68	Insertion of three expression cassettes into the A56R, F14.5L, and J2R	Increased tumor targeting specificity and reduced toxicity
Yoo *et al*[229]	CVV	Deletion of viral thymidine kinase genes	Regression of liver tumorigenicity and metastasis to the colon
Ricordel *et al* [230]	deVV5	TK-deleted chimeric VV armed with the suicide gene *FCU1*	Union of different VV strains, with increased oncolytic properties, with more efficient replication in human tumor cells
Ge *et al*[231]	vvDD-IL-12	Oncolytic VV delivering tethered IL-12	Increase tumor infiltration of activated CD4+ and CD8+ T cells, decrease the transforming growth factor β and increase interferon γ
Deng *et al* [232]	VG9	The oncolytic potency of VG9 was evaluated in various cell lines	Evaluate replication and cytotoxicity in vitro, antitumor effects and process of biodistribution of VG9 in a B16 tumor model

Ab: Agonist antibody; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL-12: Interleukin 12; NK: Natural killer; TK: Thymidine kinase; VV: Vaccinia virus.

infections of the mucosa or skin and central nervous infections, which reveals the need of deletions and insertions of additional transgenes in order to produce a viable oncolytic virus therapy[58].

In that context, a large number of oncolytic HSVs-1 have been developed and tested, with good outcomes, and among them the Talimogene Laherparepvec (T-VEC) is approved by the Food and Drug Administration[59,60]. T-VEC is one of the most studied HSV-1 oncolytic virus; it is created through deletion of γ34.5 and ICP47 and insertion of GM-CSF to inactivate neurovirulence factors and enhance the virus replication and immunogenicity[61,62]. It was also found possible to link HSV-1 to the *ras* signaling pathway in order to provide viral replication[63].

The mechanism of action of these viruses, especially T-VEC, is dual. The first aim is to perform direct tumor cell killing in which the viruses are able to enter the tumor environment, normally by local injection, and then start replication and consequent lysis of the infected tumor cell, release of tumor antigens, and local immune response [64]. In addition, the GM-CSF expression enables an accurate migration and maturation of dendritic cells to the environment and further antigen presentation to CD4+ and CD8+, which are capable of reaching distant metastases[65,66]. Studies also demonstrate that interferon response increases PD-L1 expression, and consequent T cell infiltration in the tumor environment is also possible[66,67]. Table 3 lists some genetic modifications in HSV-1 and impacts in the oncolytic action.

CLINICAL USES

Pancreatic cancer

Worldwide, the occurrence of pancreatic cancer is low, and the disease is not recommended for screening by the World Health Organization[68]. The survival rate of pancreatic ductal adenocarcinoma, responsible for 95% of pancreatic cancers[69], is 6% in 5 years[70], and the only potential cure for pancreatic ductal adenocarcinoma (duodenopancreatectomy) does not offer a big change in mortality[69].

Reolysin® (Oncolytics Biotech Inc., Calgary, AB, Canada) is the name of a reovirus that is in a Phase II clinical trial in pancreatic cancer[71]. The studies are not yet conclusive. However, intraperitoneal administration of reovirus has been shown to be effective and safe in the control of peritoneal metastases in hamsters with pancreatic ductal adenocarcinoma carcinomatosis[72].

Measles viruses depend on overexpression of CD46, a viral entry receptor also found in many cancer cells[73]. In a previous study, a modified measles virus showed oncolytic activity in pancreatic tumor xenografts in mice with tumor regression and increased survival[74]. In another study, the virus was modified to target prostate stem cell antigen, which is a protein expressed in pancreatic cancer and was armed

Table 3 Genetic modifications in the herpes simplex virus-1

Ref.	Virus	Updates	Aim
Liu *et al*[61]	T-VEC	Insertion of GM-CSF and deletion of γ34.5, US12	Increase lytic activity and antitumor immunity
Ushijima *et al* [233]	HF10	Insertion of UL53, UL54 and deletionof UL43, UL49.5, UL55, UL56, LAT	Reduce neurovirulence and increase immunogenicity
Ebright *et al* [234]	NV1020	Incorporation of the HSV-1 TK gene and deletion of α0, α4, γ34.5, UL56, UL24	Reduce neurovirulence and provide susceptibility to antiviral chemotherapy
MacKie *et al* [235]	HSV 1716	Incorporation of γ34.5	Reduce neurovirulence
Mineta *et al* [236]	G207	Insertion of lacZ and deletion of γ34.5	Avoid ribonucleotide reductase encoding and reduce neurovirulence

GM-CSF: Granulocyte-macrophage colony-stimulating factor; HSV-1: Herpes simplex virus 1; LAT: Latency-associated transcript; T-VEC: Talimogene laherparepvec; TK: Thymidine kinase.

with the drug purine nucleoside phosphorylase. The authors concluded that viral therapy demonstrated antitumor activity in immunocompromised mice[75].

A study using H-1PV, a parvovirus, associated with gemcitabine in mice showed a reduction in tumor growth, in addition to increased survival and absence of metastases in imaging studies[76]. In another previous study using parvovirus, the infection increased natural killer-mediated cell death in pancreatic ductal adenocarcinoma[77]. However, many studies still need to be done to obtain a conclusive answer since current studies only suggest the viral oncolytic action of parvoviruses[76]. However, the myxoma virus demonstrated *in vitro* lysis of pancreatic ductal adenocarcinoma cells[78] and prolonged the survival of mice, especially when the therapy was combined with gemcitabine[79].

Adenoviruses are the main viral vectors used to treat cancer, as they are able to bind to a target cell receptor with great affinity[80]. This great affinity is due to the possibility of building the ideal selectivity using two techniques: excluding viral genes necessary for replication in normal cells and introducing fundamental proteins accompanied by specific tumor promoters[81]. In preclinical tests, ONYX-15, an adenovirus, had a deletion mutation of the E1B gene and showed increased survival and antitumor efficacy in murine animals[82], in addition to showing viability and tolerability when combined with gemcitabine. However, its development was interrupted due to its limited clinical activity[83]. The LOAd703 virus, a parvovirus with the deleted E1A gene, has shown that it can change the tumor microenvironment from immunosuppressive to immunocompetent[84]. Tests have also shown its ability to elicit immune responses by releasing tumor-associated antigens while positively regulating favorable chemokines as well as dendritic cells[85].

HSVs are recognized for infecting and killing tumor cells quickly[86]. In addition, HSV has exhibited strong tumor reactivity mediated by T cells, indirectly causing an immune response to cancer[87]. In 1999, preclinical data showed that G207, an HSV-1 virus with gene deletions and inactivations, lysed pancreatic ductal adenocarcinoma cells *in vitro*[88] and induced complete tumor eradication by 25% when injected into mice xenograft tumors[89]. L1BR1, an HSV-2 with deletion of the US3 gene, replicated in pancreatic ductal adenocarcinoma cells and induced apoptosis cytolysis, especially when combined with 5-fluorouracil and cisplatin[90]. In a phase I study, HF10, a natural HSV-1 mutant, was injected into pancreatic tumors in 6 patients. Biopsies revealed a greater number of infiltrating CD4+ and CD8+ lymphocytes. In addition, an objective response was observed in 1 patient, while disease stabilized in 3 patients, and in the remaining 2 cases there was disease progression[91]. Finally, two phase I trials were performed to test the safety of the intratumoral injection of T-VEC (OV HSV-1 with multiple deletions) and Orien X010 (OV hGM-CSF HSV-1 recombinants) in advanced pancreatic cancer patients[92-94]. However, unfortunately, the results have not yet been reported to the scientific community.

Melanoma

Melanoma is a potentially fatal malignant skin disease that continues to have greater incidences in the world, while the scenario of other tumors is the opposite[95]. The average risk of melanoma is 1 in 50 in several western countries[96] and is more frequent in light-skinned populations[97].

Regarding OV therapy, the vaccinia virus is a prototypical poxvirus with high clinical relevance, which can be easily attenuated by deleting virulence genes and inserting therapeutic genes[98]. Two phase I studies using JX-594, an OV vaccinia modified to activate local macrophages and dendritic cells[99], involved a total of 17 patients with unresectable cutaneous melanoma. The studies concluded that JX-594 replicated successfully in the tumor microenvironment, led to local oncolysis, and that increasing doses of JX-594 were safe and effective[100,101]. In two other similar phase I clinical trials, they used the vaccinia virus, which encodes B7.1 T cell co-stimulating molecules[102], in 25 patients with unresectable melanoma. As a result of these tests, the rate of complete objective response was 20% with limited toxicity and low-grade reactions[102,103].

The herpes simplex virus is an attractive option for OV in melanoma since the large genome has several non-essential genes that can be deleted in order to reduce pathogenicity and insert genes of interest[104]. Currently, T-VEC is the first oncolytic virus approved by the United States Food and Drug Administration for melanoma cancer therapy[105]. Phase I, II, and III clinical trials were concluded with positive results from the use of T-VEC in the treatment of melanoma[106-108]. Biopsies of injected lesions were performed in phase I and showed significant tumor necrosis caused by T-VEC[107]. In phase II, the overall objective response rate was 26% with a 1 year survival rate for all patients of 58% and mild side effects in 85% of patients[107]. Finally, in phase III, the objective response rate for the T-VEC arm remained at 26% with 11% complete responses, but unfortunately the final survival data are not available[108]. Even so, this was the first randomized clinical trial to reveal beneficial therapeutic use of OV for patients with advanced or unresectable melanoma[104].

HF10, a spontaneously mutated strain of HSV-1 with a deletion mutation in some viral genes[109], was used in an *in vitro* study that revealed that murine and human melanoma tumor cells had relevant cytolytic effects after HF10 infection[110]. In that same study, immunocompetent mice with advanced melanomas received HF10 intratumorally. Tumor growth was reduced in injected and non-injected tumors, which suggests direct oncolysis and induction of a systemic antitumor immune reaction [110]. HF10 was associated with dacarbazine to assess the oncolytic efficacy of the virus in mice prepared with subcutaneous melanoma models. The combined treatment of dacarbazine with HF10 showed a very fast and strong cytotoxic effect compared to monotherapy since a robust systemic antitumor immune response was induced and prolonged survival[111].

Other viruses with fewer highlights have been tested and have shown good results. Coxsackievirus A21 demonstrated in preclinical studies oncolytic activity in melanoma cells, maintaining tolerability and low viral pathogenicity[112]. CVA21, a commercial version of coxsackievirus A21, was studied clinically in phase I and II in patients with advanced and unresectable melanoma who received the virus intratumorally for 15 wk. As a result of these trials, the treatment was generally well tolerated with low-grade reactions, being able to observe complete therapeutic responses and an acceptable safety profile[113,114]. Finally, a phase II trial evaluated the oncolytic action of Reolysin® in 21 patients with metastatic melanoma who received intravenous injections[71]. All patients tolerated the injections well, and in 2 patients viral replication was evident when evaluating post-treatment biopsy samples from 13 patients. However, the study did not obtain observed objective responses nor did it achieve its primary efficacy objective, although the trial data support the use of reovirus in combination with other therapies to treat malignant melanoma[71].

Breast cancer

Breast cancer (BC) is a multifactorial and heterogeneous disease in which the interaction between family history, lifestyle, and hormonal components has a fundamental role in its development[115,116]. Worldwide, the numbers of the disease are increasing, partly due to the increase in life expectancy of the population but also associated with the increase in early diagnosis techniques. Currently, 1 in 8 women have a chance of being diagnosed with BC in the world, making it the most common cancer among women[117].

There are prospects for treatment of more advanced forms of the disease since to date oncolytic virotherapy has demonstrated a wide variety of options for action at the cellular and molecular level[118]. Among the options currently most sought for this purpose, there are double-stranded DNA viruses that replicate and transcribe in the cell nucleus, without the integration of its genetic material with that of the host cell [118]. In addition, it is essential that OVs are extremely selective to replicate in cancer cells[15], a fact corroborated by tests that show the good tolerability and selectivity of genetically modified viruses for this purpose, such as the vaccinia virus[119]. Another

important OV, adenovirus, one of the most studied for BC, is still controversial. Preclinical studies show efficacy in tumor reduction by inhibiting the growth of its cells in addition to controlling metastases in mice[118]; however, other phase I trials demonstrate low efficacy for BC either in monotherapy or in combination with other drugs[119]. In addition to these, T-VEC approved in the United States and Europe for use in some types of melanomas[120] has been clinically tested in BC and shows good tolerability by the patient as well as relative success in inducing tumor necrosis and immune response[119,121].

RNA viruses such as Pelareorep (Reolysin) have also been studied for BC[119]. Although inconclusive, the trials show that there is safety in its use, in addition to an efficiency in viral replication and in its induction of cell death[122]; however, they suggest that the administration of Pelareorep in combination with the drug paclitaxel is more effective when compared to its isolated use[123]. An important point of this virus is its optimized form of intravenous administration, which favors its development even more and extends its use when compared to most of the OVs that are still administered in clinical trials by intratumoral route[119]. Also very promising against BC is the marabá virus, a strain of rabdovirus. Its MG1 variant was developed to have a greater oncolytic action and also little replicative action in normal cells, achieving success in these objectives[118]. As for tumor control, trials have shown an important association of positive results in the use of MG1 for the prevention of metastasis in the preoperative period[124] as well as in the safety of its use and the possibility of having a good systemic efficiency[125].

Liver cancer

A highly malignant tumor type, liver cancer is still a major challenge to current medicine[126]. Its most common form is hepatocellular carcinoma (HCC)[126,127], which represents one of the six most prevalent and four most lethal types of cancer in the world[128-131]. Linked to this, HCC is attributed to an increase over the years [128], related to a high worldwide prevalence, concentrated mainly in underdeveloped countries[130]. The unfavorable numbers corroborate to a high rate of disease recurrence after conventional therapies currently used, with just over 10% of patients surviving after 5 years[129].

The literature shows OVs as promising in the possibility of overcoming HCC, especially in more advanced stages, in a safe manner and with the least possible chance of recurrence[129,131]. One of the most widely used is adenovirus, which shares a relevant tropism for liver cells[128]. Among this type of virus, there are several lines of studies with particular modifications aiming at a better viral adaptation to the obstacles found in tumor cells. One of them is the Ad5 viral vector integrated with the GP73 and SphK1-shRNA promoters[130], in which through preclinical tests it was able to induce cell apoptosis and inhibit tumor expansion considerably, improving the survival of mice[131]. The adenovirus ZD55 vector was modified to overcome the high resistance of HCC cells to tumor necrosis factor-related apoptosis ligand and successfully managed to reduce the tumor size by associating ZD55-tumor necrosis factor-related apoptosis ligand with ZD55-Smac, a variant that has a second mitochondrial caspase activator in its constitution[128].

The vaccinia virus has also been studied for HCC. The JX-594 variant has been proven safe and effective through preclinical studies in rabbits by eradicating lung metastases and liver tumors in these animals[126,128]. In addition to this, the vaccinia virus may also be associated with cytokines, such as recombinant VV-IL-37, which with interleukin 37 associated with its genome also inhibited liver tumor growth[130]. Among the therapeutic options, it is also worth highlighting the findings in trials using HSV. A study using mice developed Ld0-GFP, a more selective and more oncolytic vector for liver cells, which has safely demonstrated an important potential in the induction of cell apoptosis and in the release of DAMPs related to immunogenic cell death[129].

Glioblastoma

Glioblastoma is the most common malignant primary brain tumor in adults, with a median age of approximately 55 to 60 years and has a 10% survival rate after 5 years [6], even with important advances in recent years in cancer therapy. Thus, oncolytic therapy has been highlighted in the treatment of glioblastoma, once it kills tumor cells *via* direct oncolysis and *via* stimulation of antitumor immune response[132].

Regarding the use of OVs, studies have shown its use with combined therapy and monotherapy. A research conducted at clinicaltrials.gov, Martikainen *et al*[133] found more than fifteen clinical studies at different stages. A phase II study, using the modified DNX2440 adenovirus, combining oncolytic virus with tumor-targeting

immune checkpoint modulators, demonstrated that the virus was able to specifically increase T cell activation, facilitating tumor recognition. In other studies, HSV (phase I), vaccinia virus (phase I/II), poliovirus (phase I/Ib), parvovirus H-1PV (phase I/II), and unmodified human reovirus were also used[134-137]. The study using attenuated (Sabin) poliovirus with internal ribosomal entry site from human rhinovirus 2 was applied to 61 patients over a period of 5 years with the result of increasing their patients' survival rate by 24 and 36 mo compared with the rate among historical controls. On the other hand, the study with unmodified rat parvovirus indicated that H-1PV treatment was safe and well tolerated. It showed favorable pharmacokinetics, induced antibody formation in a dose-dependent manner, and triggered specific T cell responses. There was an increase in survival compared to recent studies. Furthermore, researchers who used unmodified human reovirus reported that 10 of the 12 patients had tumor progression and 1 had stabilized, while the median survival was 21 wk. Finally, the preclinical study involving HSV-1 and rats used the modern approach of viral redirection with IL-12, resulting in increased overall survival and complete tumor elimination in 30% of the animals.

Prostate

Prostate cancer is the most common cancer among men and the second type of cancer that kills men the most in Western countries[138]. In view of the therapies currently available, the OVs are an attractive way of treating prostate cancer, either as monotherapy or in combination with other immunotherapies (for example, anti-programmed cell death protein 1 and anti-PD-L1 inhibitors)[139]. This is due to the immunological events induced by the administration of OVs in cancer-bearing animals that bring down multiple tumor immune evasion mechanisms and induce strong, multiclonal, and protective anti-prostate cancer immunity. The effect of OVs on prostate cancer occurs because of abnormalities in antiviral defense pathways, including those attributed to impaired tyrosine-protein kinase Janus kinase, a signal transducer and activator of transcription signaling.

To date, there are several clinical trials in phase I and II using adenovirus, reovirus, HSV-1, vaccinia virus, fowl pox virus, and Sendai virus[140]. Among the studies with adenovirus, one was able to insert mk5 (the mutational kringle5 of human plasminogen) into a DD3-promoted (differential display code 3) oncolytic adenovirus, showing that mK5 has been proven to be able to inhibit the tumor angiogenesis and inhibit cell proliferation[141]. Currently, a number of Ad5-CD/TK OVs have been developed and tested as a therapeutic for prostate cancer. These viruses provide two suicide genes, cytosine deaminase and HSV-1 TK, to tumor cells. Studies using a reovirus in patients with metastatic castration-resistant prostate cancer, on the other hand, showed an increase in the secretion of inflammatory cytokines[138].

Colorectal cancer

Colorectal cancer is the third most common cancer in the United States and the second leading cause of cancer-associated mortality[142]. There is currently no effective treatment for this type of cancer, so OVs can be an interesting option in this way. Heavily pretreated colorectal cancer patients were treated with the oncolytic vaccinia virus alone or combined, by increasing the expression of GM-CSF (a hematopoietic growth factor) and reached stable disease in 67% of patients[143,144]. Another study using oncolytic HSV2 performed an *in vitro* and *in vivo* analysis. In the first, oncolytic HSV2 effectively inhibited the growth of CT-26 cells. In the second, hepatic metastasis was reduced in mice models with xenograft tumor[145].

FUTURE CHALLENGES AND PERSPECTIVES

A wide variety of OVs are going through studies in phase I/II clinical trials or in preclinical cancer models[2,146]. According to clinicaltrials.gov, there are currently 114 clinical trials listed at the time of this writing showing considerable progress in this field. Despite all the advances, some limitations still have to be surpassed to enhance OV-based immunotherapy[37,119,147]. Thus, to overcome these challenges, research scientists are creating new strategies, which will be presented below.

Choosing the optimal OV species

As aforementioned, a range of virus species has been developed as OVs recently. It is essential to comprehend the exclusive biological aspects to establish the most relevant antitumor oncolytic virotherapy, considering that distinct kinds of viruses have

different sizes, genetic materials, shapes, and pathogenicity[148]. First, the size of the virus must be considered; larger viruses are more suitable for the therapeutic gene insertion, but they are less inclined to infiltrate the physical barriers, whereas smaller viruses can penetrate and spread throughout the tumor more easily, though they are not as susceptible for genetic administration[148]. In addition, the viral genome is important; RNA viruses replicate faster than DNA viruses and are able to kill tumor cells because they do it in the cytoplasm and do not have to reach the nuclei of the target cells[149]. Nevertheless, they have shown fewer tumor-selective properties due to the same reason[150]. Likewise, the existence of a viral capsid is also a crucial factor in OV selection because enveloped viruses are less oncolytic and are more likely to be eliminated by the host immune system[149].

Therefore, during the past decade, some improvements have emerged in the area, such as capsid development, genome engineering, and chemical modifications[151]. The capsid can be altered to improve the binding between the virus and the entry receptors from the target cell. For example, researchers have noticed that genetically inserting protein domains or peptides into the viral capsid can benefit transduction efficacy in some cells and improve the attachment of the OVs to target tumor cells membranes, boosting viral tropism, and internalization[151-153]. Furthermore, viral cytotoxicity needs to be considered since the high capacity to generate cell injuries can decrease viral replication rates and consequently interfere in the effectiveness of therapy[154]. Meanwhile, all of those strategies still have limitations and need to be improved.

Effective delivery methods

Finding an ideal route for OV administration still constitutes one of the major challenging issues in virotherapy[60]. The two leading delivery platforms include local intratumoral, which the OVs are injected directly into the tumor site, and systemic method (intravenous or intraperitoneal)[4,55]. Local intratumoral is the most common delivery route in preclinical or clinical trials due to its safety and to decrease the chance that preceding circulating antibodies might overcome the virus before it reaches its target[2,155,156]. However, this platform cannot be utilized for inaccessible or multifocal tumors, such as pancreatic or brain tumors, so it is not always a viable option[157]. On the other hand, the systemic injection is, theoretically, an ideal delivery method, because of the broad distribution of viruses, allowing the OVs to reach not only primary but also metastatic tumors, and it is relatively non-invasive and highly repeatable[155,157]. Nonetheless, its bioavailability and efficiency at the moment is unsatisfactory, and the viral particles in this route do not specifically target cancer because they can be rapidly sequestered and degraded by the host immune system before they reach the tumor[158].

In this way, several strategies have been studied to overcome these hurdles. For example, capsid modifications have been explored as a way to deliver OVs to tumor sites, like the changing of the viral envelope by polyethylene glycol polymers that prevent its recognition by macrophages[151,157,159]. Thus, considerable new approaches such as the use of nanoparticles, complex viral particle ligands, liposomes, polymeric particles, and immunomodulatory agents have been used and designed [160-163]. Another hopeful strategy is the utilization of ultrasound image guiding and magnetic drug-targeting systems[164-166]. These are all different kinds of approaches for improving the delivery methods.

Immune response

The immune response is an obstacle capable of preventing the effectiveness of OVs, given that it can limit infection and viral replication, whether by the specific immunity from viral infections or by pre-existing immune memory[167,168]. There are many cases in which antiviral immunity already exists from previous infections or vaccinations since many of the OVs used in anticancer therapy are originally pathogenic to humans[159,169]. Besides that, the excessive administration of OVs can induce antiviral immunity that eliminates it more quickly than supposed[159]. The presence of coagulation factors FIX, FX, and complement protein C4BP and the large number of immune cells infiltrated into the cancerous stem cells impair selective viral replication as well[149,170].

To overcome such problems, new treatment strategies were developed and showed promising results as genetic manipulation of OVs, cytokines, nanoparticles, complex viral particles binders, immunomodulatory agents, use of decoy viruses for sequestering pre-existing antibodies, and multiple administration of different serotypes[120, 168]. However, it is relevant to emphasize that viral immunity can be beneficial in some cases by recruiting immune cells for tumor microenvironment (TME) and

reversing the immunosuppressive TME. Therefore, there must be an adjustment in the balance between OV-induced antitumor immunity and antiviral immunity[147,169,171].

Physical barriers

Another major challenge that OVs need to overcome is physical barriers, as viruses must pass through the endothelial layer to reach target cells. Studies have identified several physical barriers that limit effectiveness, such as chemotherapeutic agents, monoclonal antibodies, antitumor immune cells, and genetic therapies[149,172,173]. Furthermore, abnormal lymphatic networks and epithelial cell tumors are protected by extracellular matrix, which results in interstitial pressure and may impair the ability of OVs to spread themselves throughout the tumor mass, negating its effectiveness[174,175].

Therefore, strategies to achieve efficient penetration and dissemination of OVs are highly necessary for significant improvements in this therapeutic modality[176]. To increase the viral spread, oncolytic adenovirus genetically modified to express molecules such as relaxin and hyaluronidase were generated in order to stop angiogenesis of the extracellular matrix and have shown promising preclinical results [174]. An intravenous administration of the OVs can bring numerous benefits for the vascularization of the tumor, being able to be superior to intratumoral injections[176]. Studies show efficiency in the spread of OVs in solid tumors through changes in the viral envelope or by increasing the diffuse transport of the virus through changes in the interstitial space[177]. These data provide strong evidence of the significant antitumor effects of the therapy.

Clinical use of OVs allies to other therapies

Since OVs showed limited efficacy in monotherapy, the combination of immunotherapy drugs and virotherapy has become a potential direction and appealing choice [158,178]. In this way, some preclinical studies in animal models and early clinical trials have confirmed the therapeutic responses increased with combination approaches, showing considerable response rates and tolerable safety profiles[120,179]. The following sections discuss these diverse combination strategies.

Combination with chemotherapy

The combination of virotherapy with chemotherapy agents is a promising approach. For example, adenovirus combined with chemotherapeutic agents such as cisplatin, 5-fluorouracil, doxorubicin, temozolomide, irinotecan, and paclitaxel has successful results and enhanced antitumor effects compared to the response rate of the virus alone[179-181]. Concomitantly, a combination strategy also showed less risks and higher safety, extending the patient's survival[182]. Likewise, vaccinia virus combined with paclitaxel also revealed a harmonious effect[183]. In some models, the combination of sorafenib and vaccinia virus demonstrated good antitumor results, while patient trials showed remarkable safety and clinical response, and it has been approved for use in kidney, liver, and thyroid cancers[184].

Combination with radiotherapy

Radiotherapy combined with OVs has shown potential effects in cancer treatment[185-187]. Initially, the propitious result was observed in studies with oncolytic HSV[188-190]. In addition, the forceful combination effects can also be observed in radiotherapy and vaccinia virus. For example, a study reported that VACV-scAb-vascular endothelial growth factor was able to boost the radiation therapy's sensitivity of tumor locations, increasing the antitumor response[191].

Combination therapy with adoptive cell therapy

Another promising strategy is the combination of OVs and adoptive cell therapy since OVs can kill cancer cells specifically and have the potential of turning the TME into an immunostimulatory environment that is susceptible to T cell entry and activation [192]. A recombinant oncolytic adenovirus, OAd-TNF-a-IL-2 combined with meso-chimeric antigen receptor T cells in an animal model of pancreatic ductal adenocarcinoma caused considerably better tumor regression and expanded the antitumor effectiveness of chimeric antigen receptor T cells[193]. Furthermore, a preclinical trial of this combination approach utilizing GD2-chimeric antigen receptor T cells and a recombinant oncolytic adenovirus in a mouse model revealed substantial elevated overall survival of mice as with both monotherapy ways[194]

Combination therapy with OVs and immune checkpoint inhibitors

One of the most common strategies to increase the effectiveness of OVs is to combine them with ICIs as the combination of the two therapies relieves the tumor immunosuppressive environment. The infection caused by OVs triggers an anticancer immune response, increasing the effectiveness of ICIs, which in the process interrupt the ligand-receptor interaction of cancer cells exposing T cells to attack[169,194,195]. In short, the objective of this combination is to make the local microenvironment more conducive to the proper functioning of ICIs through infections caused by OVs[195-197]. This synergistic relationship has led to the development of several studies with promising results.

For example, a phase II study (clinicalTrials.gov: NCT02978625) studied how biological therapy T-VEC and the immunotherapy with monoclonal antibodies nivolumab worked in 68 patients with lymphoma who have not responded to treatment or non-melanoma skin cancers that have spread to other parts of the body or have not responded to treatment. In addition, the combination of ICIs with various OVs, such as vaccinia virus, coxsackievirus, adenovirus, marabá virus, reovirus, and vesicular stomatitis virus, is being evaluated in different phase I or phase II clinical trials[167,198]. Thus, new treatment options through this combination continue to be awaited with expectations of promising paths.

Combination therapy with OVs and bispecific T cell engagers

In recent decades, there has been great clinical progress in immunotherapy with bispecific antibodies and effective therapeutic applications[199]. By definition, bispecific T cell engagers (BiTEs) are proteins that, through DNA recombination, form bispecific antibodies with two variable fragments of single chain antibodies, one directed to a cell surface molecule in T cells (for example, CD3) and the other targeting antigens on the surface of malignant cells[172,200]. BiTE-mediated interaction triggers the formation of immune synapses, which ultimately result in tumor specific cell death and release of effector Th1 cytokines[201]. However, BiTEs have low penetration in solid tumors, in addition to the risk of toxicity in hematological cancers[172,200]. In this sense, the combination of BiTEs and OVs is considered in order to increase therapeutic efficacy since OVs are able to selectively replicate and infect malignant cells, thus alleviating the immunosuppressive state of the TME[172,201].

Currently, several BiTEs delivered by OVs have been tested on several types of hematological and solid tumors reported by preclinical research, and promising tests were obtained with a BiTE that recognizes fibroblasts associated with cancer (*via* fibroblast activation protein)[202]. In addition, preclinical studies also provided evidence of the effectiveness of OVs in combating the side effects of therapy with BiTEs through the redirection of T cells, in addition to improving antitumor activity [203]. Such efforts should lead to the development of new anticancer agents as it is believed that this combination is powerful to address unmet clinical needs[199].

Biosafety on oncolytic virotherapy

Although OV therapy has shown potential to be a safe treatment for cancer patients, some biosafety issues *in vivo* still remain a concern as a treatment strategy. Primarily, some adverse events were associated with this therapy[14]. A few symptoms, such as mild flu-like syndromes[204,205], local reactions commonly manifested as pain, rash, peripheral edema, and erythema, are the most common events linked to the treatment [124,206]. Some of them disappeared without intervention after a few days or with the administration of nonsteroidal anti-inflammatory drugs during the treatment course[4, 207]. In addition, other common adverse events, like leukopenia, liver dysfunction, anemia, lymphopenia and more, were noticed in the trials of HSV, reovirus, and adenovirus[208,209]. Besides this, few OV therapies have caused severe adverse reactions that brought harm to patients' health[162,210-212], and they have been manageable and rarely caused a severe impact on the patients or threatened their lives [162,213]

Moreover, the transmission and shedding of OVs during the treatment is also a potential safety issue. During the therapy, viruses such as T-VEC, Ad5- Δ24-RGD, HSV, adenovirus, pox, and reovirus, can be transmitted to people in close contact with the patient, such as the family and health care staff who are more likely to be exposed to the patient's fluids, such as saliva or urine, or be shed to other parts of the patient's body[214-216]. Another challenge in the biosafety of the use of OVs is the application of the treatment in specific populations, given that the studies in this area are currently limited[14]. Therefore, in order to reduce risks, the viruses observed are highly attenuated, in addition to being of the utmost importance that the health professionals

who administer OVs carefully follow the safety standards for the procedures[215,216]. On the other hand, the trials and preclinical studies of several viruses, like the T-VEC, HSV-1, and H-1PV indicate that pregnant women and people with low immunity should avoid using them[214,217].

Lastly, aiming to improve the biosafety of oncolytic viral therapy and decrease its side effects, the use of viruses that do not present pathogenicity to humans are being evaluated. H-1PV, for example, demonstrated no inducement of the production of specific antibodies when inoculated in humans, which means little chance of generating an active infection. Nevertheless, the virus has shown specificity to the tumor cells[218]. Furthermore, the recombinant therapies between different OVs, such as adenovirus and parvovirus, have shown satisfactory results in terms of biosafety since the synergistic action generated from the viral specificities, such as the infectivity of adenoviruses to the tumor cells and the lack of harmfulness of parvovirus to the normal cells, contributes to greater therapeutic efficacy and reduction of collateral damage[14].

CONCLUSION

OVs emerge as a way of bypassing the immune evasion mechanisms of the tumor, aiming to improve the clinical condition of patients through the stimulation of the host immune system or direct lysis of abnormal cells. The modern techniques of genetic engineering have made it possible to improve the construction of OVs, increasing the safety and the efficiency, targeting the virus to the tumor, and decreasing the adverse effects of their use. Furthermore, it is possible to observe significant effects of the clinical use of OVs, whether in single or combination therapy, to the treatment of tumors. Therefore, upgrading antitumor therapies and consequently improving patient prognosis with contributions from the areas of molecular biology, structural biology, immunology, genomics, and bioinformatics lays a solid foundation for future clinical success of OVs.

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MINIREVIEWS

Ambisense polarity of genome RNA of orthomyxoviruses and coronaviruses

Oleg Zhirnov

ORCID number: Oleg Zhirnov 0000-0002-3192-8405.

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Oleg Zhirnov, Gamaleya Microbiology and Epidemiology Research Center, Ivanovsky Institute of Virology, Moscow 123098, Russia

Corresponding author: Oleg Zhirnov, DSc, MD, PhD, Professor, Gamaleya Microbiology and Epidemiology Research Center, Ivanovsky Institute of Virology, 16 Gamaleya Street, Moscow 123098, Russia. zhirnov@inbox.ru

Abstract

Influenza viruses and coronaviruses have linear single-stranded RNA genomes with negative and positive sense polarities and genes encoded in viral genomes are expressed in these viruses as positive and negative genes, respectively. Here we consider a novel gene identified in viral genomes in opposite direction, as positive in influenza and negative in coronaviruses, suggesting an ambisense genome strategy for both virus families. Noteworthy, the identified novel genes colocolized in the same RNA regions of viral genomes, where the previously known opposite genes are encoded, a so-called ambisense stacking architecture of genes in virus genome. It seems likely, that ambisense gene stacking in influenza and coronavirus families significantly increases genetic potential and virus diversity to extend virus-host adaptation pathways in nature. These data imply that ambisense viruses may have a multivirion mechanism, like "a dark side of the Moon", allowing production of the heterogeneous population of virions expressed through positive and negative sense genome strategies.

Key Words: Virus genome; Ambisense RNA; Influenza; Coronavirus; Virus diversity; Virus genes

Core Tip: A novel genes identified in viral genomes in opposite direction, as positive in influenza and negative in coronaviruses, are considered. The identified novel genes colocolized in the same RNA regions of viral genomes, where the previously known opposite genes are encoded, a so-called ambisense stacking architecture of genes in virus genome. It seems likely, that ambisense gene stacking in influenza and coronavirus families significantly increases genetic potential and virus diversity to extend virus-host adaptation pathways in nature. These data imply that ambisense

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INTRODUCTION

Orthomyxo- and coronaviruses are two families of enveloped viruses containing single stranded linear RNA genomes. Orthomyxovirus family includes seven genera: Alphainfluenzavirus, Betainfluenzavirus, Deltainfluenzavirus, Gammainfluenzavirus, Isavirus, Thogotovirus, and Quaranjavirus. These viruses infect wide range of hosts including mammals, birds, rodents, fish, ticks and mosquitoes. Orthomyxoviridae viruses contain six to eight segments of negative-sense single stranded RNA with a total genome length of 10-15 Kb[1]. Coronaviridae is divided into the four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. Alpha- and betacoronaviruses infect mammals, while gamma- and deltacoronaviruses primarily infect birds. The size of genomic positive sense RNA of coronaviruses ranges from 26 to 32 kilobases, one of the largest genome among RNA viruses[2]. Here we mainly consider alphainfluenza viruses and betacoronaviruses as a typical members in both families.

INFLUENZA A VIRUS AMBISENSE GENES

Genome of influenza A viruses is composed of 8 segments of single-stranded RNAs with mol. wt. 0.7-2.8 × 10^3 kilobases/segment. Each segment encodes one or several unique polypeptides through the canonical negative sense genome strategy (Table 1). It means that genome RNA of negative sense polarity is transcribed by the virus polymerase to produce positive sense mRNAs, which recognized by ribosomes to translate individual viral proteins (Figure 1). In addition to the negative sense genes, influenza A virus genome segments were found to contain long open reading frames (ORFs, genes) in opposite positive sense orientation. These ORFs have all ribosome translation elements: canonical start codon AUG or noncanonical CUG, termination codons (UAG, UAA, or UGA), internal ribosome entry sites (IRES), and Kozak-like sequences at the initial start codon[3-9].

There are three groups of data showing *in vivo* expression potential of these negative stranded genes. (1) The template function of the full length "negative sense" genome RNA of segment 8 (NS) was demonstrated in a cell-free translation system of rabbit reticulocyte lysate. It was shown that influenza A virion RNA of segment 8 can initiate synthesis of major polypeptide negative stranded protein (NSP8) (mol.wt. 23 kD) specifically reacted with antibody to the central domain of the NSP8[10]; (2) The NSP8 encoded in the 8'th influenza A virus segment NS could be expressed in vivo, in insect cells (ovary cell line of *Trichplusia ni*) infected with recombinant baculovirus (insect nuclear polyhedrosis virus) carrying influenza virus sequence NSG8 in the virus DNA genome. This gene appeared to express ~20 kD influenza-specific polypeptide NSP8, which was intracellularly stable and accumulated in the perinuclear zone of infected cells[11]. Later, it was also supported that influenza A virus NSP8 could be efficiently expressed from either a plasmid or a recombinant vaccinia virus in mammalian cells and the synthetized NSP8 was localized in the perinuclear endoplasmic reticulum (ER) and post-ER cellular compartments[12]; and (3) There are data that mice infected with influenza virus produce CTL response specific to epitopes presented in the influenza NSP8 protein[12-14]. These findings also demonstrate that translation of sequences locating on the negative RNA strand of a single-stranded RNA genome of influenza A virus can develop *in vivo* and can initiate antiviral CTL response and immunosurveillance.

Table 1 RNA segments of influenza A virus genome and encoded polypeptides

Viral RNA segments and their length (nt)[1]	Positive sense polypeptides (mol. wt., kDa)[2]	Negative stranded polypeptides, NSPs (mol. wt.; a.a.)[3]
PB1 (2341)	PB1 (86.6); PB1-N40 (89.4); PB1-F2 (10.5)	NSP1 (174, 239)
PB2 (2341)	PB2 (85.7); PB2-S1 (55)	NSP2 (116, 121, 130, 137)
PA (2223)	PA (84.2); PA-X (29); PA-N155 (62); PA-N182 (60)	NSP3 (95, 109)
HA (1778)	HA (61.5)	NSP4 (n.d.)
NP (1565)	NP (56.1); eNP (56.8)	NSP5 (117, 154)
NA (1413)	NA (50.1); NA43 (48.6)	NSP6 (91, 154)
M (1097)	M1 (27.8); M2 (11); M42 (13)	NSP7 (99, 102, 109)
NS (890)	NS1 (26.8); NEP (14.2); NS3 (21); tNS1 (17)	NSP8 (93, 167, 216)

[1]RNA segments and nucleotide (nt) calculations were made for the A/PR8/34 (H1N1) virus.
[2]Canonical influenza A virus polypeptides synthesized through the negative genome strategy (Figure 1; for review see[1]).
[3]Negative stranded genomic open reading frames (ORFs) and predicted negative stranded proteins (NSPs) have been calculated for A/PR8/34 (H1N1) and A/Aichi/2/68 (H3N2) viruses[3-8]. Negative stranded ORFs were identified by in silico approach using the Open Reading Frame Finder program (https://www.ncbi.nlm.nih.gov/orffinder/). These ORFs can be realized through the positive genome strategy. The amino acid length (a.a.) of NSPs were based on the data presented mainly in ref.[8]. A.a. values reflect variations among human, avian and other mammalian virus strains. N.d. means the absence of ORFs longer than 90 a.a. NSP: Negative stranded protein.

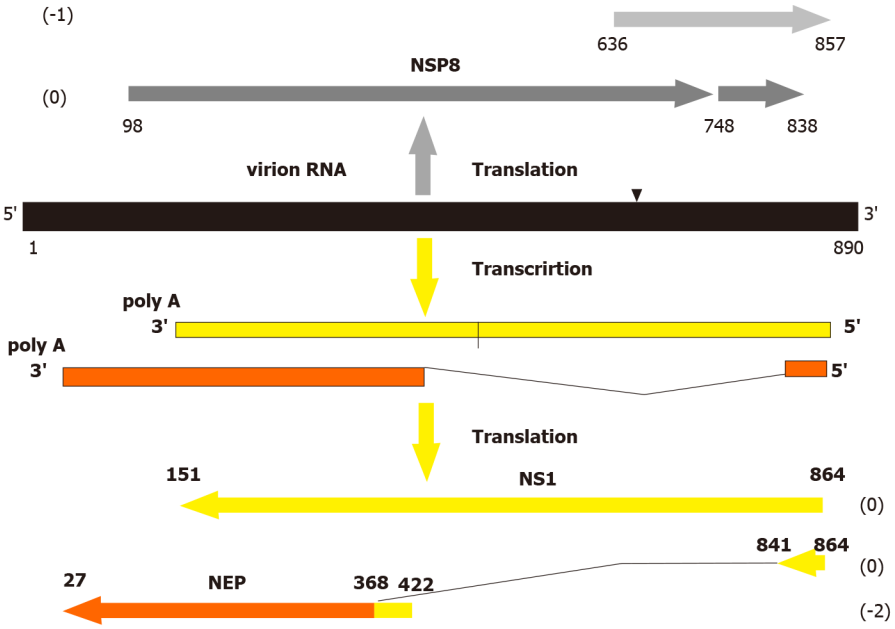

Figure 1 The scheme of expression of the genome negative sense segment of influenza A virus. The negative sense (NS) segment of influenza A/Aichi/2/68 (H3N2) virus is displayed. The horizontal arrows show the open reading frames (ORFs) of the negative strand protein 8, non-structural anti-interferon protein (NS1), and nuclear export protein (NEP). Numbers in brackets indicate the ORF translation phase. Numbers under the lines indicate nucleotide positions from the 5' end of the virion genome RNA. The broken line shows the splicing segment of the *NEP* gene mRNA. Triangle in the virion RNA molecule shows a site position of possible translation frameshifting[10]. NS: Negative sense; NSP8: Negative strand protein 8; NS1: non-structural anti-interferon protein.

The mature product of the NSP8 gene has not been yet identified in biological systems such virus-infected cells and animals. The failure to detect NEG8 protein could be due to a number of factors other than the complete absence of translation from genomic RNA. The properties of the NSP8 as an "escaping protein" may be explained either by its low synthesis and a short period of life or/and strong tissue-specific expression in certain cell types containing factors which are necessary for the regulation of expression of these "negative sense" genes. It would not be surprising if negative polarity genes are only expressed physiologically under special circumstances *in vivo* determining host cell tropism of influenza viruses.

NOVEL NEGATIVE SENSE GENES IN THE RNA GENOME OF CORONAVIRUSES

Recently, similar ambisense polarity has been revealed in coronaviruses genomes[15]. It is well known that these viruses possesses a linear positive sense genome RNA of 25-29 × 10^3 kb length[2]. The coronavirus genome RNA contains two groups of genes expressing proteins through the positive sense strategy. The first ones (nonstructural genes for nsp1-nsp19 proteins) are localized at the 5'-region of the virion genome RNA and directly translated by host ribosomes. The second ones (mostly the structural proteins genes N, S, HE, M, E and several accessorial proteins, such as 3a/b, 6, 7a/b, 8a/b, 9b, *etc.*) occupy a 3'-region of the virion RNA and express proteins through the translation of subgenomic mRNAs, which was transcribed on the anti-genomic RNA template[16] (Figure 2A). In addition to the positive sense genes, we have identified numerous long open reading frames in negative sense orientation (Table 2; Figure 2B). Like in the case of the ambisense genes of flu viruses, coronavirus negative sense genes have all elements characteristic of the mRNA molecules which are recognized by host ribosomes: classical AUG or alternative CUG[17] start codons, termination codons, IRES, and Kozak-like sequences at the start area[18,19]. However, unlike to influenza A viruses, coronavirus ambisense polarity has opposite configuration: a positive sense genome strategy and a negative sense orientation of the novel negative sense genes, so called a negative sense genes or negative gene proteins (NGPs).

The identification of coronavirus negative-polarity genes implies two possible mechanisms of their expression and synthesis of the corresponding mRNAs and proteins. These mechanisms include either direct translation of a replicative (-)copy of genomic (+)RNA (replication pathway II) or the transcription of genomic (+)RNA by viral polymerase with the formation of subgenomic mRNAs of "negative polarity" for their subsequent translation to synthesize specific viral polypeptides (transcription pathway I). To realize pathway I coronavirus genome contains poly A sequence (positions 11935-1194 nt) functioning as a viral polymerase binding site and transcription initiation signal (Figure 2B).

BIOLOGICAL SIGNIFICANCE OF THE AMBISENSE GENES

The function and role of the newly discovered ambipolar viral genes have not yet been determined. In the case of influenza viruses, there are indirect data that the identified new ambisense genes can be involved in the regulation of the host immune response against viral proteins and/or in the regulation of the stability of viral proteins in infected cells through the protein deubiquitinating system[5,12]. The possible functional significance of the novel ambisense genes is not yet generally clear. However, the stability and retaining of these type of genes in field viruses genomes for more than 100 years at the high variability of virus population suggest the functional necessity of these genes and their biological evolutionary determination[20]. Notably, the influenza NSP8 has high synonymous/nonsynonymous (dN/dS) mutations rate (> 1.5), which was similar to that one for the most variable surface virus glycoproteins HA and NA representing major target for antiviral host adaptive immune response. The elevated variability of the NSP8 implies that it undergoes positive selection and host adaptation, which influence its evolution[5].

The discovery of new ambisense genes has raised a number of important questions regarding its origin, functions, and evolutionary variability. One of the essential questions is how the novel genes have emerged in the genomic region to encode two opposite sense genes. The appearance of the ambipolar gene suggests the existence of yet unknown correspondence principle (or reverse determination rule) for the expression of oppositely directing genes locating in the same region of RNA molecule. This principle implies that a certain pre-existing gene can predetermine the emergence mechanism and the properties of a new ambipolar gene[5]. Without this rule, chaotic accumulation of mutations will result in the appearance of a new functional gene and its further evolutionary selection, that seems to be unlikely. Moreover, the probability for such chaotic event is low, considering the ambipolar overlapping of several preexisting genes, when changes in one of them would cause changes in the coupled ambipolar genes. In this case, gene variability and selection of mutations should be interconnected in all opposite viral genes (in the case of influenza virus for NS1, NEP, and NSP8). These considerations incline to the assumption of the existence of a rule of reverse determination, when both ambipolar genes can have linked structural motives and functions. Further studies are necessary to clarify this idea.

Table 2 Negative sense genes in genomes of coronaviruses

Virus genera	Viral genomes	Number of NSGs in virus genome[1,3]	M.W. range of the NGPs[2]
Alpha-coronaviruses	HCov-229E: https://www.ncbi.nlm.nih.gov/nuccore/NC_002645.1	29/1/29/5	12.4-14.4
Beta-coronaviruses	SARS-CoV-1: https://www.ncbi.nlm.nih.gov/nuccore/NC_004718.3	34/0/35/2	11.5- 15.0
	SARS-CoV-2: https://www.ncbi.nlm.nih.gov/nuccore/MT635445.1	21/1/26/4	10.9- 17.2
	MERS: https://www.ncbi.nlm.nih.gov/nuccore/NC_019843.3	32/8/23/3	11.1- 18.6
	Pangolin-CoV: https://www.ncbi.nlm.nih.gov/nuccore/MT040335.1	29/3/17/4	10.8-19.9
	HCov-HKU1: https://www.ncbi.nlm.nih.gov/nuccore/NC_006577.2	15/1/13/2	11.5- 15.0
	Bat coronavirus RATG13: https://www.ncbi.nlm.nih.gov/nuccore/MN996532.1	17/2/29/1	10.9- 19.7
	Bovine coronavirus BCoV-ENT: https://www.ncbi.nlm.nih.gov/nuccore/NC_003045.1	25/1/26/0	20.8
	Murine hepatitis virus A59: https://www.ncbi.nlm.nih.gov/nuccore/FJ884687.1	29/5/42/7	11.2-36.8
Gamma-coronaviruses	Avian infectious bronchitis virus: https://www.ncbi.nlm.nih.gov/nuccore/NC_001451.1	20/6/8/3	12.7- 26.5
Delta-coronaviruses	Porcine coronavirus HKU15: https://www.ncbi.nlm.nih.gov/nuccore/NC_039208.1	26/5/29/3	11.2- 17.4

[1]Negative sense genes (NSGs) were identified by in silico approach using the Open Reading Frame Finder program (https://www.ncbi.nlm.nih.gov/orffinder/). First and second digits show overall and numbers of the large gene open reading frames (ORFs) starting with classical AUG, respectively. Third and fourth numbers show overall and large gene numbers ORFs having noncanonical CUG, respectively. Large genes were assumed to have more than 300 nt long. GenBank ac.n. of the viral genomes are indicated.

[2]A range of mol. wt. (kDa) of negative gene proteins encoded by the large negative sense genes (≥ 300 nt) starting either with AUG or CUG codons are outlined.

[3]The data were partially presented in[15]. These partial elements were used here with the Publisher's permission. NSGs: Negative sense genes; SARS-CoV: Severe acute respiratory syndrome coronavirus.

Ambisense stacking of genes revealed in coronavirus and influenza virus genomes significantly increases virus diversity, genetic potential and extend virus-host adaptation pathway possibilities. Existence of numerous ambisense genes opens up a new avenue for virus reproduction where one virus genome can produce a multiple progeny population of virions possessing identical genome RNA and different protein compositions. In this case, a part of virions decorated with one of the NGPs proteins (in the case of coronaviruses) could be hidden from us, as "the dark side of the Moon". The expression of coronavirus "negative" and flu "positive" genes may have a host (tissue)-dependent regulation facilitating immune escape of overcovered virions and specific pathogenetic pathways in the host(s) where the up-expression of the virus NGP or NSP genes occurs. Further studies will shed light on this ambisense concept of human and animal orthomyxo- and coronaviruses.

For the current time, there are four ambisense virus genera (phlebo-, tospo-, arena-, and bunyaviruses), which are well known to realize both positive- and negative-sense genome RNA strategies to encode viral proteins[12,21]. Ambisense genes of these virus genera locate in separate areas of the genome RNA without their overlapping and stacking. The ambisense genes locating in the genome in the stacking manner were found in influenza viruses, in which, similarly to coronaviruses, direct expression of these genes has not yet been identified, but there are indirect signs of such expression during natural viral infection *in vivo*[12-14]. Location of genes with opposite polarity in the same region of the RNA molecule makes it possible to significantly increase the genetic capacity of the viral genome and opens new ways for virus diversity, increasing virus adaptability to the host and biological evolution in nature[15]. The presence of potential ambisense genes in genomes of influenza and coronaviruses raises the question of the classification of these families. The detection in infected cells or infected organisms of protein products expressed by the ambisense manner will give grounds for classifying the coronavirus and orthomyxovirus families as the ambisense viruses with a bipolar genome strategy.

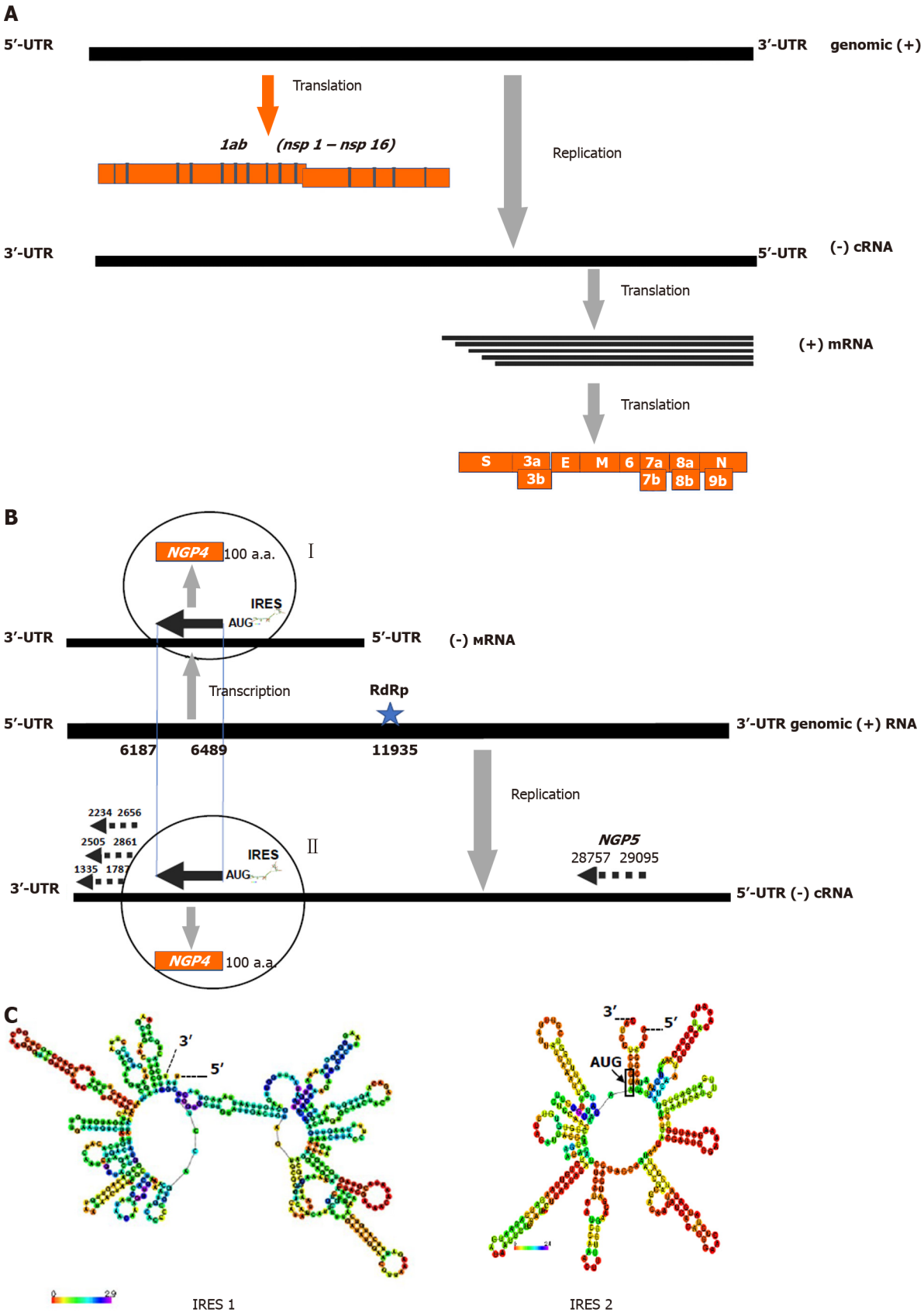

Figure 2 Positive sense genome strategy and translation cassette unit at the 3' end of the negative sense complimentary RNA of coronavirus severe acute respiratory syndrome coronavirus 2 genome. A: Replication scheme of the RNA genome of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) coronavirus (ac.n. MT890462.1). UTR means untranslated RNA region; B: A 3' end area of the subgenomic (-)cRNA complimentary to the virus genome 5' end (+)vRNA of SARS-CoV-2 (ac.n. MT635445.1) is displayed. Five ORF containing cassette for NGP1-NGP5 beginning either with classical AUG (NGP4) or noncanonical CUG (NGP1-3, NGP5) codons are shown by arrows. Nucleotides counting from the 5' end of (+)vRNA are shown for each ORFs. Phases of the translation frame (fr) are estimated regarding the frame of NGP4 (fr.0) as follows: NGP1and 2 (fr. +1), NGP3 (fr.0). Poly A tract (11935-

11940 nt) functioning as a viral RNA dependent RNA polymerase binding site is shown by star; C: IRES-like structures enriched with 16 and 10 canonical "hair-pins" RNA elements in the regions 8100-8599 nt (IRES 1) and 6488-6792 nt (IRES 2), respectively, were predicted by the IRESpred program[22]. The IRES-like structures 1 and 2 have significant free energy value as low as -99,4 and -73,8 kkal/mol, respectively. The data were partially presented in[15]. These partial elements were used here with the Publisher's permission.

CONCLUSION

The manuscript data suggest that ambisense gene stacking in influenza and coronavirus families significantly increases genetic potential and virus diversity to extend virus-host adaptation pathways in nature. These data imply that ambisense viruses may have a multivirion mechanism, like "a dark side of the Moon", allowing production of the heterogeneous population of virions expressed through positive and negative sense genome strategies.

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MINIREVIEWS

COVID-19 in dialysis units: A comprehensive review

Gabriel Martins Nogueira, Moisés Santana Oliveira, Ana Flávia Moura, Constança Margarida Sampaio Cruz, José A Moura-Neto

ORCID number: Gabriel Martins Nogueira 0000-0002-8819-6874; Moisés Santana Oliveira 0000-000-5002-3874; Ana Flávia Moura 0000-0001-7368-4704; Constança Margarida Sampaio Cruz 0000-0002-3885-4314; José A Moura-Neto 0000-0003-1339-3731.

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Gabriel Martins Nogueira, Moisés Santana Oliveira, Department of Medicine, Bahiana School of Medicine and Public Health, Salvador 40290-000, Brazil

Ana Flávia Moura, Constança Margarida Sampaio Cruz, José A Moura-Neto, Department of Internal Medicine, Bahiana School of Medicine and Public Health, Salvador 40290-000, Brazil

Constança Margarida Sampaio Cruz, Department of Internal Medicine, Hospital Santo Antônio, Salvador 40415-006, Brazil

Corresponding author: José A Moura-Neto, MD, FASN, Professor, Department of Internal Medicine, Bahiana School of Medicine and Public Health, Av. Dom João VI, 275 - Brotas, Salvador 40290-000, Brazil. mouraneto@bahiana.edu.br

Abstract

The coronavirus disease 2019 (COVID-19) pandemic has been challenging for healthcare professionals worldwide. One of the populations affected by the pandemic are patients on renal replacement therapy, as kidney disease is an independent risk factor for severe COVID-19 and maintenance dialysis (a life-sustaining therapy) cannot be interrupted in the vast majority of cases. Over the past months, several authors and medical societies have published recommendations and guidelines on the management of this population. This article is a comprehensive review regarding the measures to prevent, contain and deal with a COVID-19 pandemic in the dialysis setting. We recapitulate the epidemiology and pathophysiology of COVID-19 in kidney dysfunction and present the main recommendations concerning the screening of healthcare personnel, dialysis patients and visitors as well as measures to improve the safety of the dialysis facilities' environments. In addition to preventive measures, this article briefly describes actions directed towards management of an outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within a dialysis facility, the management of complications in dialysis patients with COVID-19 and overall data regarding the management of children with kidney disease.

Key Words: COVID-19; SARS-CoV-2; Renal dialysis; Renal replacement therapy; Hemodialysis units, Hospital

Specialty type: Urology and nephrology

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Grade B (Very good): B
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Core Tip: Dialysis patients are more vulnerable to develop severe coronavirus disease 2019 (COVID-19) infection. To minimize risks, some measures should be followed by dialysis units, healthcare personnel, patients and visitors. Until vaccination against COVID-19 is widely available to dialysis patients worldwide, an evidence-based approach is required to avoid the spread of the virus and consequently more death of patients.

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INTRODUCTION

The outbreak of the coronavirus disease 2019 (COVID-19) pandemic in early 2020 proved to be a massive challenge for healthcare professionals all around the world. Clinically, its symptoms range from pulmonary (*e.g.*, cough and dyspnea) to extrapulmonary manifestations (*e.g.*, fever, myalgia, anosmia and ageusia), revealing the systemic nature of the aforementioned malady[1,2].

Due to the aforementioned variety of clinical manifestations attributed to the infection by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), different areas of the medical field became highly interested in the better comprehension of COVID-19, one of them being nephrology. The glomerular epithelium, the proximal tubular cells of the nephrons and endothelial cells have considerable levels of angiotensin-converting enzyme 2, which explains why COVID-19 patients may develop renal injury[3-6].

Such interest emerged as doctors recognized the necessity for guaranteeing the safety of patients treated with renal replacement therapy (RRT) during the pandemic, focusing on preventing an outbreak in dialysis units. The attentiveness to COVID-19 by nephrologists was reinforced when multiple studies from different countries suggested that patients who acquire the disease have a significant risk for developing acute kidney injury (AKI)[7-9].

In this article, we review the epidemiology of COVID-19 in dialysis centers as well as the main recommendations concerning the screening of healthcare personnel (HCP), dialysis patients and visitors, the safety of the dialysis facilities' environments, the conduct regarding an outbreak of SARS-CoV-2 infection within a dialysis facility, the management of complications in dialysis patients with COVID-19 and the conduct directed towards children with kidney disease.

EPIDEMIOLOGY OF COVID-19 IN DIALYSIS UNITS

As of March 2021 there have been over 125 million confirmed cases of COVID-19 and over 2.7 million deaths, giving the disease a case fatality rate of 2.22%[10]. The currently available literature suggests that the frequency of COVID-19 among dialysis patients is approximately between 2% and 20%, a difference possibly explained by the region in which each study was conducted[11-14]. Meanwhile, the proportion of infected individuals appears to be lower in other health services, for both HCP and patients, and also in the general community[13-17].

Infection by SARS-CoV-2 in dialysis units does not seem to depend on sex, ethnicity, time of dialysis or presence of diabetes but is likely associated with in-center dialysis and older patient age; the higher risk of infection in healthcare facilities has been attributed to a higher rate of self-reported illness among the staff[11,18]. Chronic kidney disease (CKD) patients, especially those in dialysis, are more vulnerable to SARS-CoV-2 infection, given that a decrease in the estimated glomerular filtration rate has been associated with death by COVID-19 in one large cohort study that obtained data using OpenSAFELY[19]. The mortality of dialysis patients who contracted COVID-19 is approximately between 21% and 33%, being above both the general population's death rate due to SARS-CoV-2 infection[12,14,20,21]. Some studies have also shown that hemodialysis (HD) patients are more likely to contract the disease

than peritoneal dialysis patients, something that is at least partially explained by the fact that HD patients cannot perform dialysis at home, while peritoneal dialysis patients can[13,14].

COVID-19 can also cause AKI. It has been documented that about one fifth of patients with the disease end up developing AKI treated with RRT (AKI-RRT). CKD is associated with higher risk of developing AKI-RRT among COVID-19 patients as well as diabetes mellitus, hypertension, higher body mass index and high levels of D-dimer. The mortality is extremely high among AKI-RRT patients with COVID-19, even more than in the previously mentioned group, reaching levels above 60%[22].

HEALTHCARE PERSONNEL, PATIENTS AND VISITORS

It is known that AKI and RRT increase the risk of complications and death in COVID-19, so it is necessary to follow specific rules to avoid infection[23,24]. In addition, HD units are classified as high risk of contagion, hence the need to further tighten these measures in these environments[25]. It is possible to divide protective actions into measures for HCP, for patients and visitors.

The first group includes doctors at the HD unit, nurses, technicians and cleaning staff[26], and they must receive the following instructions: (1) The use of personal protective equipment (PPE: surgical or N95 masks, gloves, hair caps and clothing with waterproof insulation) must be mandatory and constant[26-28]; (2) Educational actions on how to properly use PPE, how to properly sanitize hands and how to dispose of contaminated items should be promoted[26]; (3) Updates and training on new knowledge related to the epidemic need to be encouraged[26,27]; (4) Nurses must be trained to collect the nasopharynx swab to perform the COVID-19 polymerase chain reaction (PCR)[26]; (5) Groups of face-to-face activities, including discussion groups, ought to be avoided and should be done digitally[27,29]; (6) Teams from different parts of the health unit must have meals at different times in order to avoid contact [27]; (7) The team should, if possible, avoid using public transport as well as participating in large agglomerations[27,29]; (8) The presence of COVID-19 symptoms in the team as well as in their family members should be monitored closely. Members with suspected infection should notify the unit, perform the PCR for COVID-19 and quarantine themselves in order to avoid contaminating patients[26,27]; and (9) HCP vaccination against SARS-CoV-2 should be implemented on a large scale as soon as possible[30,31].

Patients also need to take several protective measures in order to further mitigate the possibility of contagion, such as: (1) The use of surgical masks, N95 or similar should be mandatory and the use of homemade cloth masks should be discouraged. However, due to economic reasons and the low availability of surgical masks, N95 or similar, some emerging countries recommended universal use of cloth masks for dialysis patients[32]. Although these are a better option than not using masks, surgical masks are about three times more effective in blocking the transmission of the virus [26,27,29,33-35]; (2) Educational measures, such as avoiding the use of public transport, practicing social isolation, wearing appropriate face masks, not traveling, staying away from agglomerations, preventing contact with people outside your residence, must be promoted[26,27,29]; (3) It is necessary to instruct, even in the dialysis units, on proper hand hygiene, on the cough etiquette and on the main symptoms of COVID-19[26,27,29,33]; (4) The medicines previously prescribed must be continued, with due medical follow-up. This includes angiotensin-converting enzyme inhibitors, other medications for the treatment of hypertension, glucocorticoids, immunosuppressants, medications for diabetes and anemia and any other necessary for the patient[27,36]; (5) Vaccination against influenza should be encouraged in dialysis units[29]; (6) Measures of attention to psychosocial care must be taken, as dialysis patients are predisposed to problems such as anxiety, depression and insomnia during the pandemic[27]; (7) Vaccination against SARS-CoV-2 in patients with kidney disease should be implemented on a large scale as soon as possible. So far, this is the most effective measure in the prevention and containment of COVID-19[30, 31]; and (8) If possible, the patient should be transferred to a home dialysis program [37].

Dialysis units should be encouraged to decrease the flow of people during the pandemic; therefore, it is not indicated that other individuals accompany patients on dialysis[26,29]. It can be allowed in situations of extreme need, judged on a case-by-case analysis. In this matter, it is recommended that the companions wear surgical masks, N95 or similar and obey the same basic rules as dialysis patients, *e.g.*, social

distancing[26,28,29,33].

SAFETY OF DIALYSIS FACILITIES

The pandemic reinforced the importance of a safe environment for dialysis. Although current recommendations advise prioritizing the use of telehealth whenever is deemed possible[38], dialysis patients' demands are not always solved by those services alone. Thus, the ongoing scenario required that dialysis units adapted themselves to minimize SARS-CoV-2 infection rates within their installations.

General measures include the patient assessment for COVID-19 symptoms or exposure to diseased individuals in every dialysis session and planning for SARS-CoV-2 viral detection testing. In general, testing for COVID-19 (and other respiratory diseases) in outpatient HD facilities and home dialysis should be considered if the individual presents any signs or symptoms of the illness, even mild and atypical ones, or if there is suspicion of exposure to someone potentially infected with the virus.

It is also the role of the facility to ensure that the screening of staff, patients and visitors is being adequately done, including body temperature checking at entrance (and at both start and end of the dialysis session for patients) and that all rooms are well ventilated[26,29,39-41].

Also, safe patient placement is an important component of the strategy that dialysis facilities have been following. It is highly advisable that the minimum separation of six feet (approximately 180 cm) between patients, either in a waiting area or in the treatment area, is ensured in the whole facility. The same guidance applies to cohorting patients unless the individuals in question are confirmedly infected with the disease, in which case they can be cohorted together. Whenever possible, patients with suspected or confirmed SARS-CoV-2 infection should go through dialysis in a separate room. Also, single use of dialyzers is highly recommended in patients with confirmed or suspected cases of COVID-19; the once widespread (and still a reality in emerging countries) practice of reusing dialyzers should be avoided in patients with SARS-CoV-2 infection[29,42].

Given that the coronaviruses can persist on surfaces like glass, metal and plastic, cleaning and disinfection (C&D) has been frequently recommended to counteract SARS-CoV-2 transmission. The standard C&D course of action is considered satisfactory for COVID-19 cases, but the chemical product used for surface disinfection has to be capable of inactivating SARS-CoV-2, *e.g.*, ethanol, sodium hypochlorite and hydrogen peroxide[42,43]. It has been recommended that bed linens get changed between shifts and that the used ones are correctly contained or laundered, that constantly touched surfaces within the dialysis units are cleaned and disinfected regularly and that the adequate PPE is equipped when C&D is being performed[42,44].

However, it is arguable that too much focus is being directed towards C&D. Studies have suggested that the risk of infection by fomites is low and often exaggerated due to the inapplicability of the circumstances obtained in an artificial lab environment in daily life situations[45-47]. The reasons why C&D remains a constant aspect of many guidelines despite its apparent low impact on the dissemination of SARS-CoV-2 vary from public expectation and reliance on C&D protocols, as seen in cases in which people fumigate and/or wash the streets and sidewalks, measures which have been deemed by health authorities as ineffective[48].

As previously mentioned, telehealth plays a pivotal role in the current pandemic and should be used wherever and whenever possible. Even though it does not satisfy every need a dialysis patient may have, given that it is a complementary practice and not a substitutive one. Its benefits must not be downplayed, especially on the subject of home dialysis. There have been reports regarding the benefits of telehealth in dialysis in patients, released both before and during the pandemic and especially concerning peritoneal dialysis[49-51]. However, the quality of the obtained evidence is disputed[52,53]. Special attention must be given to specificities of home dialysis care, such as the likelihood of shortages of PPE and peritoneal dialysis fluid and the higher possibility of developing a more severe form of COVID-19. Dialysis facilities should also provide useful guidance to patients who are dialyzed at home and update their HCP on the clinical knowledge of COVID-19[27,54].

DEALING WITH AN OUTBREAK

Despite all the protective measures being taken, it is still possible for a case of COVID-19 to appear in the dialysis units precisely because of the current pandemic. During an outbreak period, several patients and doctors visit dialysis units in general, which makes them a high-risk environment for nosocomial coronavirus infection[55]. In this scenario, it is possible to deal with two types of cases: (1) Suspected infection in patients or visitors; and (2) In HCP.

Still at the entrance to the dialysis unit, patients and visitors must undergo both symptomatic (*e.g.*, presence of fever, dyspnea, myalgia, coughing and sneezing) and epidemiological screening (*e.g.*, contact with people positive for COVID-19 in the last 14 d)[42,56-58]. If the patient is at low risk of infection, he must be referred to dialysis and must obey the protective measures already addressed in this article, *e.g.*, wearing PPE and keeping a minimum distance of 6 feet from other people[42,58]. If the patient has symptoms of COVID-19 or has had contact with someone who is positive for the virus, he must do the PCR for the disease and has to be treated as moderate/high risk for infection. Also, the monitoring of the evolution of the symptoms is mandatory, even if it is absent. In such cases, as previously mentioned, patients have to wear PPE and must be dialyzed in separate environments from other patients, with the door closed. If this is not possible, treatment should be carried out at the end of the day, in places away from the main passage of personnel, such as at the end of the corridor or in a corner[42,56,58,59]. If the patient already has a positive PCR for COVID-19, care must be increased and dialysis in a separate location is highly recommended[42]. These recommendations can be seen in Figure 1.

If the visitors are symptomatic or were in close contact with people with COVID-19 in the last 14 d, their entry should be prohibited. If they are classified as a low risk of infection, they should continue to follow protective measures against COVID-19 within healthcare centers. In addition, it is recommended that only patients confirmed for COVID-19 are dialyzed together whenever the facility's infrastructure enables, thus patients with suspicion of SARS-CoV-2 infection (not yet confirmed) ought to be treated separately from them[42,56,59]. The healthcare team responsible for the treatment of patients suspected or confirmed disease should use N95 or equivalent or higher-level respirator, eye protection, glove and isolation gown[42]. Recommendations towards visitors are shown in Figure 2.

In a situation in which the outbreak originates from the healthcare team, two fronts of action should be adopted. First, the healthcare worker must be immediately and temporarily relieved from work and has to self-quarantine for 14 d or 10 d as long as remains asymptomatic for 3 consecutive days from the last exposure to a contaminated individual[42,60,61]. Furthermore, special attention should be given to patients who were under the care of this HCP. If the patient had contact with the infected individual at a distance of less than 6 feet for more than 15 min, the situation should be treated as a potential exposure. If the patient wears a surgical mask at the moment of contact, he will be considered as a low risk for infection and other symptoms should be monitored without further concern. However, if the mask used is homemade or even without a mask, the patient will be considered at high risk for infection and all the measures described previously must be taken into action (Figure 3)[42].

Finally, it is important to note that the perception of a nosocomial transmission of COVID-19 is challenging due to the large circulation of people and the possibility that they have acquired the infection outside the units. Still, if this type of transmission is identified, the situation must be considered an outbreak and containment measures must be taken immediately[42,55].

MANAGEMENT OF COMPLICATIONS

It should be reiterated that dialysis patients cannot interrupt RRT. Therefore, aside from the previously mentioned conducts related to preventing the dissemination of the virus within the facility, HCP must be able to know how to deal with possible renal complications in COVID-19 patients. The nephrologist plays a crucial role in the correct management of aggravations such as AKI, electrolyte imbalance and acid-base disorders.

The physiopathology of AKI in COVID-19 is not thoroughly known, but it is believed that it originates from a multitude of factors. Some of the proposed pathophysiological mechanisms relate to both prerenal and renal AKI, such as direct viral-related injury, corporal fluid disbalance, cytokine release syndrome, overstimu-

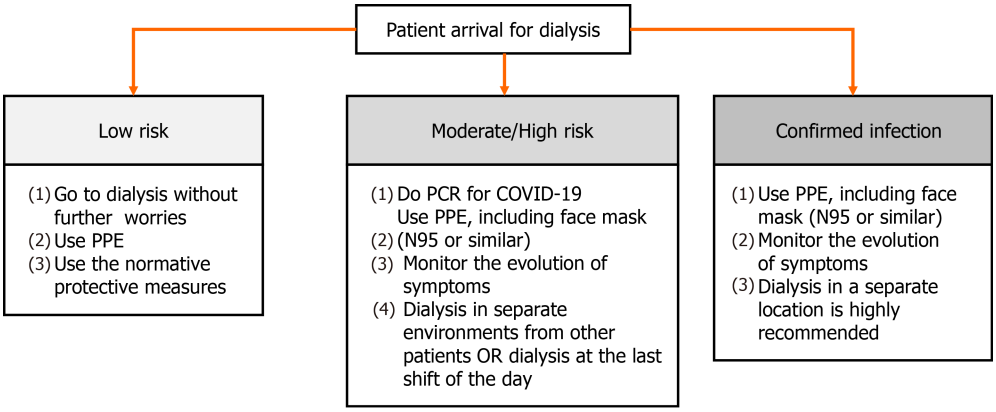

Figure 1 Conduct related to dialysis patients, stratified according to their risk or current status of being infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19: Coronavirus disease 2019; PCR: Polymerase chain reaction; PPE: Personal protective equipment.

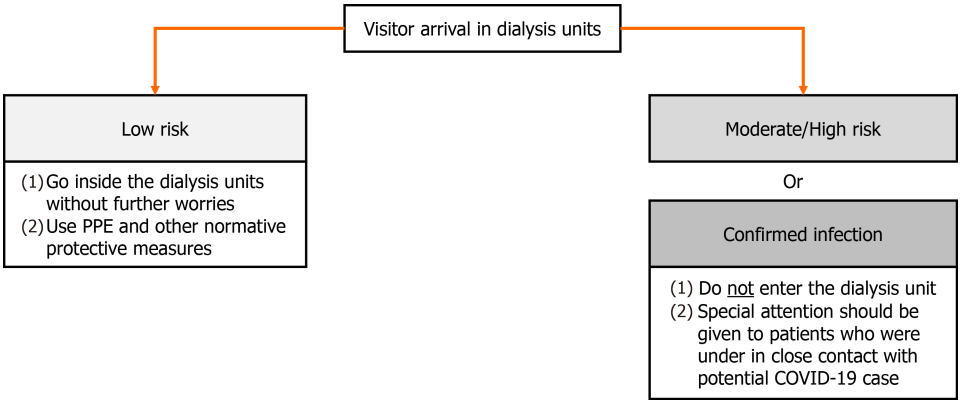

Figure 2 Conduct in regard to visitors in a dialysis facility. COVID-19: Coronavirus disease 2019; PPE: Personal protective equipment.

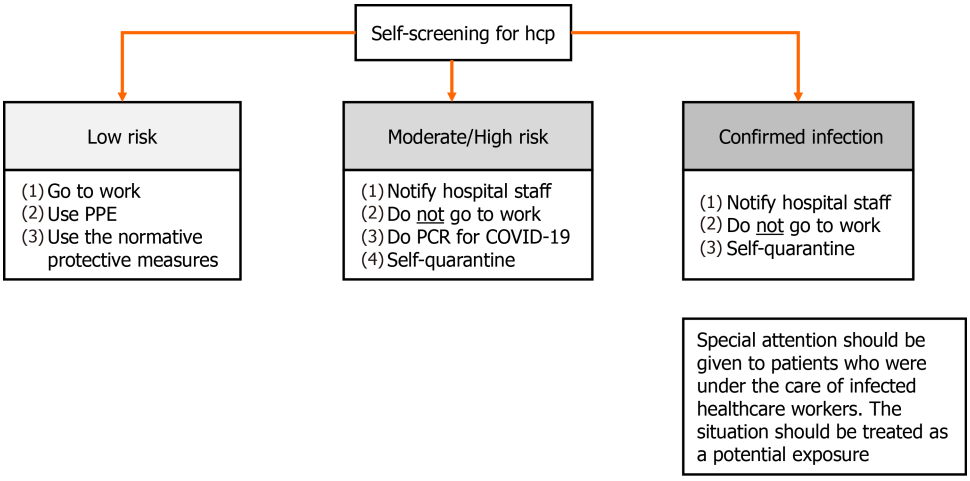

Figure 3 Self-screening for healthcare personnel, stratified according to their risk or current status of being infected by severe acute respiratory syndrome coronavirus 2. COVID-19: Coronavirus disease 2019; HCP: Healthcare personnel; PCR: Polymerase chain reaction; PPE: Personal protective equipment.

lation of the renin-angiotensin-aldosterone system, hypercoagulation, complement system dysregulation and multiple organ dysfunction syndrome[62,63].

Regarding RRT, the basic principle that guides all the others is that the entry of HCP in isolated areas must be limited and preference should be given to those who have already developed an effective immune response to SARS-CoV-2. When it comes to

choosing the dialysis modality for AKI patients, continuous RRT offers some considerable benefits regarding less physical contact between HCP and patients. However, due to the variability of resources in each healthcare setting, continuous RRT might not be available for a wide population. Therefore, other modalities might be more logistically adequate to use in certain areas[64]. Vascular access for RRT is usually done in the right jugular vein. While the left jugular vein comes as a natural second option, the femoral access has been suggested for consideration in order to reduce the likelihood of HCP contamination[65]. Also, the intensity of RRT in AKI related to COVID-19 should not be any different compared to the usual one, unless proven different[66]. It has been suggested that early RRT intervention in COVID-19 patients may provide benefits[67], but that assumption is not yet scientifically supported since one previous study detected no significant differences between early and delayed RRT start in general dialysis patients[68].

COVID-19 IN THE NEPHROPEDIATRIC POPULATION

Although CKD is considerably more frequent in the adult population, children are also susceptible to the development of renal impairment. Data regarding infants and teenagers with CKD is scarce, therefore it is difficult to determine any reliable values for its incidence and prevalence in this population.

As a possible reflex of the rarity of severe COVID-19 cases in children, there are few studies related to the damages of the aforementioned disease in the lives of said individuals, and those who exist are not enough to build a solid evidence-based approach. One of them, an Italian national-scale study, attempted to determine the impact of the pandemic in children with CKD or immunosuppression related to kidney transplant but found no severe cases of COVID-19 among individuals under the age of 18. That same research, on the other hand, estimated that around 80% of children with CKD have a glomerular filtration rate ≤ 60 mL/min/1.73 m^2 and that 25% of this fraction are under dialysis treatment[69]. A Spanish retrospective study (n = 16) also concluded that there seems to be no difference in the actual clinical course of the disease between healthy children and children with CKD but reiterated that special attention should be brought upon fluid management and the adjustment of drug doses [70]. Other case reports have been encountered; however, due to the limited methodological design intrinsic to these types of studies, they do not provide any information that can be applied in a larger scenario[71,72].

There were no registries of COVID-related AKI cases among children without chronic renal pathologies. As a result of the relative absence of information or overall existence of clinically relevant COVID-19 cases in pediatric nephrological patients, the guidelines directed to them do not differ much when compared to the ones orientated towards the adult population[73].

CONCLUSION

In summary, dialysis patients are more vulnerable to develop severe COVID-19 and are at higher risk of a worst prognosis. Because of that, it is necessary to secure that the counteractive measures related to the pandemic are being thoroughly followed by dialysis units and HCP alike as well as ensuring that patients and visitors adhere to this public health commitment. However, even if all is correctly done, an outbreak can still occur in the dialysis unit setting. Until the vaccine against COVID-19 is widely available to dialysis patients worldwide, an evidence-based approach is required to avoid the spread of the virus and consequently the death of patients.

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META-ANALYSIS

New-onset diabetes in COVID-19 and clinical outcomes: A systematic review and meta-analysis

Dhan Bahadur Shrestha, Pravash Budhathoki, Sumit Raut, Sugat Adhikari, Prinska Ghimire, Sabin Thapaliya, Ali A Rabaan, Bibodh Jung Karki

ORCID number: Dhan Bahadur Shrestha 0000-0002-8121-083X; Pravash Budhathoki 0000-0001-8856-5417; Sumit Raut 0000-0001-6090-8027; Sugat Adhikari 0000-0002-5140-9653; Prinska Ghimire 0000-0003-0848-8322; Sabin Thapaliya 0000-0002-9110-1696; Ali A Rabaan 0000-0002-6774-9847; Bibodh Jung Karki 0000-0002-3203-9554.

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Dhan Bahadur Shrestha, Department of Internal Medicine, Mount Sinai Hospital, Chicago, IL 60608, United States

Pravash Budhathoki, Department of Internal Medicine, BronxCare Health System, Bronx, NY 10457, United States

Sumit Raut, Department of Emergency Medicine, Kathmandu Medical College, Kathmandu 44600, Nepal

Sugat Adhikari, Department of Internal Medicine, Nishtar Medical University, Multan 59330, Pakistan

Prinska Ghimire, Department of Internal Medicine, Tribhuvan University, Kathmandu 44600, Nepal

Sabin Thapaliya, Department of Internal Medicine, Tribhuvan University Teaching Hospital, Kathmandu 44600, Nepal

Ali A Rabaan, Molecular Diagnostic Laboratory, Johns Hopkins Aramco Healthcare, Dhahran 34465, Saudi Arabia

Ali A Rabaan, Department of Public Health & Nutrition, The University of Haripur, Haripur 22620, Pakistan

Bibodh Jung Karki, Division of Infectious Diseases, University of Louisville, Louisville, KY 40292, United States

Corresponding author: Dhan Bahadur Shrestha, MD Resident physician, Doctor, Department of Internal Medicine, Mount Sinai Hospital, Chicago, IL 60608, USA. medhan75@gmail.com

Abstract

BACKGROUND

Diabetes mellitus (DM) is associated with adverse clinical outcomes and high mortality in patients with coronavirus disease 2019 (COVID-19). The relationship between diabetes and COVID-19 is known to be bidirectional.

AIM

To analyze the rate of new-onset diabetes in COVID-19 patients and compare the clinical outcomes of new-onset diabetes, pre-existing diabetes, hyperglycemic,

and non-diabetes among COVID-19 patients.

METHODS

We used the Meta-analysis of Observational Studies in Epidemiology statement for the present meta-analysis. Online databases were searched for all peer-reviewed articles published until November 6, 2020. Articles were screened using Covidence and data extracted. Further analysis was done using comprehensive meta-analysis. Among the 128 studies detected after thorough database searching, seven were included in the quantitative analysis. The proportion was reported with 95% confidence interval (CI) and heterogeneity was assessed using I^2.

RESULTS

Analysis showed that 19.70% (CI: 10.93-32.91) of COVID-19 patients had associated DM, and 25.23% (CI: 19.07-32.58) had associated hyperglycemia. The overall mortality rate was 15.36% (CI: 12.57-18.68) of all COVID-19 cases, irrespective of their DM status. The mortality rate was 9.26% among non-diabetic patients, 10.59% among patients with COVID-19 associated hyperglycemia, 16.03% among known DM patients, and 24.96% among COVID-19 associated DM patients. The overall occurrence of adverse events was 20.52% (CI: 14.21-28.70) among COVID-19 patients in the included studies, 15.29% among non-diabetic patients, 20.41% among patients with COVID-19 associated hyperglycemia, 20.69% among known DM patients, and 45.85% among new-onset DM. Meta-regression showed an increasing rate of mortality among new hyperglycemic patients, known diabetics, and new-onset DM patients in comparison to those without diabetes.

CONCLUSION

A significantly higher rate of new onset DM and hyperglycemia was observed. Higher mortality rates and adverse events were seen in patients with new-onset DM and hyperglycemia than in the non-diabetic population.

Key Words: Acute respiratory distress syndrome; COVID-19; Diabetes mellitus; Hyperglycemia; Mortality

Core Tip: The relationship between diabetes and coronavirus disease 2019 (COVID-19) is known to be bidirectional. The rate of COVID-19 associated diabetes mellitus (DM) and hyperglycemia was significantly high. Higher mortality rates and adverse events were seen in patients with new-onset DM and hyperglycemia in comparison to the non-diabetic population.

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INTRODUCTION

The ongoing coronavirus disease 2019 (COVID-19) has infected 93 million patients and claimed the lives of 2.02 million people as of January 19, 2021[1]. Extensive research has been conducted to study the comorbidities associated with increased severity of disease and worse clinical outcomes. Diabetes has consistently been associated with adverse clinical outcomes and high mortality in COVID-19 patients independent of or in association with other comorbidities[2-4]. Such findings have been linked to the alteration of immune and inflammatory responses caused by hyperglycemia among diabetic patients suffering from COVID-19[5]. However, it is now known that the relationship between diabetes and COVID-19 is bidirectional[6]. Not only does having

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diabetes increase the risk of severe COVID-19, but severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is also known to have diabetogenic effects.

Multiple theories have been postulated to explain the increasing rate of new-onset diabetes in COVID-19 patients. One of the proposed mechanisms is that SARS-CoV-2 binds to the angiotensin-converting enzyme-2 (ACE-2) receptors expressed on adipose tissue, lungs, small intestine, kidneys, and pancreas. After endocytosis of the virus, downregulation of ACE-2 occurs, leading to overexpression of angiotensin II, which may impede insulin secretion. Similarly, it has been suggested that the direct entry of SARS-CoV-2 into the islet cells of the pancreas damages the beta cells, which normally secrete insulin[7,8].

In the light of new evidence and theories suggesting that there is increased susceptibility of worsening pancreas function and glucose homeostatic mechanisms in COVID-19 patients, the objective of this study is to analyze the rate of new-onset diabetes in COVID-19 patients and compare their clinical outcomes with those of other COVID-19 patients who had normal or increased blood sugar levels or a pre-existing diagnosis of diabetes.

MATERIALS AND METHODS

This study was conducted according to the Meta-analysis of Observational Studies in Epidemiology statement[9]. Our protocol was registered in the PROSPERO International Prospective Register of Systematic Reviews (PROSPERO ID: CRD42021219284).

Search strategy

Investigators independently searched databases such as PubMed, PubMed Central, Scopus, Embase, and Google Scholar for all peer-reviewed articles published until November 6, 2020. The terms "New onset diabetes mellitus (DM)", "DM", "hyperglycemia", "SARS-Cov-2" and "COVID-19" connected with "OR" and "AND". Boolean operators were searched under the medical subject headings terms. The reference section of each study shortlisted from this process was checked to identify further studies not found in the previous database searches. Additional studies collected from this method were included if they fulfilled the inclusion and exclusion criteria. Electronic search details are provided in Supplementary Material 1.

Selection of studies

The studies were selected based on the following criteria: Inclusion criteria: (1) Study type(s): Observational studies with a comparison of outcomes among individuals with new onset diabetes, pre-existing diabetes, hyperglycemic and non-diabetics with COVID-19 were included in this review; (2) Study participant(s): Individuals of any age, gender, or nationality diagnosed with COVID-19 and new-onset DM; and (3) Objective outcome(s): Mortality, mechanical ventilation/intubation, and intensive care unit (ICU) admission were defined as the primary outcomes of our study. Complications such as Acute Respiratory Distress Syndrome (ARDS), acute cardiac injury, acute liver injury, acute kidney injury, cerebrovascular accident, coagulopathy, and secondary infection were secondary outcomes. Exclusion criteria: (1) Inadequate or unclear descriptions; (2) Animal studies; (3) Review articles; (4) Full text unavailable; and (5) Studies published in a language other than English.

Data extraction

The titles and abstracts of studies retrieved in Covidence during the search were screened independently by two reviewers (PG and SR). The full-texts of potentially relevant studies were then reviewed by two reviewers (SA and SR) according to the eligibility criteria. Any conflict in the first phase of review was resolved by SA and in the second phase by PG. The included studies were then collated, and the three reviewers extracted the data using standardized data extraction formats. The extracted data included: First author, year of publication, country of study, study design, number of patients, age, sex, comorbidities, case definitions, inclusion and exclusion criteria, COVID-19 associated DM, COVID-19 associated hyperglycemia, outcomes, and follow-up duration. The outcomes were mortality and adverse events such as severe COVID-19, intubation, complications and ICU admission. All three reviewers matched their data with each other after extraction and revisited papers in case of disagreements. Discrepancies were resolved through consensus among the reviewers.

Table 1 JBI bias assessment for observational studies

Questions (Yes/No/Unclear/Not applicable)	Smith *et al* [19], 2021	Zhou *et al* [16], 2020	Wang *et al* [20], 2020	Fadini *et al* [17], 2020	Wang *et al* [21], 2020	Li *et al* [14], 2020
Were the two groups similar and recruited from the same population?	Yes	Yes	Yes	Yes	Yes	Yes
Were the exposures measured similarly to assign people to both exposed and unexposed groups?	Yes	Yes	Yes	Yes	Yes	Yes
Was the exposure measured in a valid and reliable way?	Yes	Yes	Yes	Yes	Yes	Yes
Were confounding factors identified?	Yes	Yes	Yes	Yes	Yes	Yes
Were strategies to deal with confounding factors stated?	Yes	No	No	Yes	No	Yes
Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	Yes	Yes	Yes	Yes	Yes	Yes
Were the outcomes measured in a valid and reliable way?	Yes	Yes	Yes	Yes	Yes	Yes
Was the follow-up time reported and sufficient to be long enough for outcomes to occur?	No	No	No	No	Yes	Yes
Was follow-up complete, and if not, were the reasons for loss to follow-up described and explored?	Yes	Yes	Yes	Yes	Yes	Yes
Were strategies to address incomplete follow-up utilized?	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Yes
Was appropriate statistical analysis used?	Yes	Yes	Yes	Yes	Yes	Yes
Overall appraisal	Include	Include	Include	Include	Include	Include

Data analysis: The data were analyzed using comprehensive meta-analysis, employing a random effect model. Proportions were presented appropriately using 95% confidence intervals (CI). Forest plots were derived for a visual representation of the analysis. Sensitivity analysis was performed, excluding individual studies to gauge the impact of those studies on the overall results. Meta-regression was undertaken for mortality, considering diabetes status as a moderator among patients with hyperglycemia, patients with new-onset DM, patients with known diabetes, and the non-diabetic population.

Risk of bias in individual studies: We assessed the risk of bias using the JBI tool to evaluate the quality of case reports, case series, and retrospective studies (Tables 1,2, 3) [10]. Publication bias across the included studies was evaluated using funnel plot.

RESULTS

We imported 128 studies after a thorough database search and removed 27 duplicates. The title and abstract of 101 studies were screened, and we excluded 76 irrelevant studies. We assessed the full text of 25 studies and excluded 15 studies with definite reasons (Figure 1). Finally, ten studies were included in our qualitative analysis (Table 4) and seven in our quantitative analysis.

Qualitative summary

A summary of the included studies including type of study, location, study population and the relevant outcomes is presented in Table 4.

Quantitative result

A total of 7 papers were included in the quantitative synthesis.

COVID-19 associated DM

Pooling data from six studies that reported new-onset diabetes among COVID-19 cases using a random effect model showed that 19.70% (CI: 10.93-32.91, I^2 = 96.71) of COVID-19 cases were associated with DM (Figure 2). Sensitivity analysis after

Table 2 JBI critical appraisal for case series

Question	Ref.		
	Suwanwongse and Shabarek [22], 2021	Kuchay *et al* [23], 2020	Yang *et al*[24], 2020
Were there clear criteria for inclusion in the case series?	Yes	Yes	Yes
Was the condition measured in a standard, reliable way for all participants included in the case series?	Yes	Yes	Yes
Were valid methods used for the identification of the condition for all participants included in the case series?	Yes	Yes	Yes
Did the case series have consecutive inclusion of participants?	No	No	Yes
Did the case series have complete inclusion of participants?	No	No	Yes
Was there clear reporting of the demographics of the participants in the study?	Yes	Yes	Yes
Was there clear reporting of clinical information of the participants?	Yes	Yes	Yes
Were the outcomes or follow-up results of cases clearly reported?	Yes	Yes	Yes
Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	No	No	Yes
Was statistical analysis appropriate?	Unclear	Unclear	Yes
Overall: (Include/Exclude/Seek Further Info)	Include	Include	Include

Table 3 JBI critical appraisal checklist for case reports

Ref.	JBI critical appraisal checklist for case reports	Remarks
Marchand *et al*[25], 2020	Were the patient's demographic characteristics clearly described?	Yes
	Was the patient's history clearly described and presented as a timeline?	Yes
	Was the current clinical condition of the patient on presentation clearly described?	Yes
	Were diagnostic tests or assessment methods and the results clearly described?	Yes
	Was the intervention(s) or treatment procedure(s) clearly described?	No
	Was the post-intervention clinical condition clearly described?	No
	Were adverse events (harms) or unanticipated events identified and described?	Yes
	Does the case report provide takeaway lessons?	Yes
	Overall: (Include/Exclude/Seek Further Info)	Include

excluding individual studies is shown in Supplementary Material 2 and Figure 1.

COVID-19 associated hyperglycemia

Pooling data from five studies that reported hyperglycemia among COVID-19 cases using a random effect model showed that 25.23% (CI: 19.07-32.58, I^2 = 86.6) of COVID-19 cases were associated with hyperglycemia (Figure 3). Sensitivity analysis after removing individual studies is shown in Supplementary Material 2, and Figures 2 and 3.

Mortality outcome

Pooling data among COVID-19 cases using a random effect model showed a 9.26% mortality rate among non-diabetic (CI: 6.28-13.46, I^2 = 50.69), 10.59% among those with COVID-19 associated hyperglycemia (CI: 4.92-21.33, I^2 = 77.49), 16.03% among known DM patients (CI: 10.95-22.88, I^2 = 54.35), and 24.96% among new-onset DM (CI: 18.10-33.37, I^2 = 55.88). The overall mortality rate was 15.36% (CI: 12.57-18.68, I^2 = 81.75) among all COVID-19 cases, irrespective of their DM status (Figure 4).

Table 4 Qualitative analysis of included studies

Ref.	Type of study	Country	Population	Outcome
Smith *et al*[19], 2021	Retrospective study, spanning over 7 wk	New Jersey, United States	*n* = 184, M/F = 98/86. Avg age = 64.4 yr (21-100). Below or equal to 60 yr = 75, Above 60 yr = 109. Mean BMI = 29.8 (17.5-61.4). COVID-19 diagnosis based on: 177 patients: Confirmed positive lab test for SARS-CoV-2. Remaining (7 patients): Clinical diagnosis. Case definitions used by the study: New-onset DM: Persistently elevated FBG > 125 mg/dL and requiring insulin therapy; Pre-DM: HbA1C of 5.7% to 6.4%; Non-diabetic patients: HbA1C < 5.7% and FBG ≤ 125 mg/dL	DM = 114/184 (New-onset DM= 29/184). Pre-DM = 44/184. Non-DM = 26/184. HbA1C levels: (1) ≥ 6.5% = 82/171; and (2) 5.7% to 6.4% = 64/171. Among intubated patients (44/184): (1) DM = 35/44 (Newly diagnosed DM = 7/44; New onset DM = 5/44); (2) Pre-DM with high FBG levels = 7/44; and (3) Non-DM = 1/44 (normal HbA1C and FBG levels at admission, but was clinically obese with a BMI > 30). Among intubated patients (44/184): (1) Mean BMI = 32.2 (*vs* 29.3 in non-intubated); (2) Mean HbA1C (%) = 8.0 (*vs* 7.2 in non-intubated); and (3) Mean FBG (mg/dL) = 238.0 (*vs* 163.7 in non-intubated). Death before intubation: 24/184: (1) DM = 17/24; (2) Pre-DM = 4/24; and (3) Non-DM = 3/24
Zhou *et al*[16], 2020	Retrospective study	Hefei, China	*n* = 80. Euglycemia group: (1) 44 (21 males and 23 females); and (2) Age range was 27-52 yr. Secondary hyperglycemia group: (1) 22 (17 males and 5 females); (2) Conditions of no past histories of diabetes, HbA1c < 6.5%, random blood glucose > 11.1 mmol/L during hospitalization, and normal blood glucose after discharge from the hospital; (3) Age range was 40-70 yr; and (4) 5 patients among them had elevated blood sugar after glucocorticoid therapy. Diabetes group: (1) 14 patients (10 males and 4 females); (2) All were T2DM patients; (3) Treated with oral antidiabetics or insulin before hospitalization and without glucocorticoid therapy during hospitalization; and (4) Ages ranged from 43 to 67 yr	Euglycemia group: 44/80. Secondary hyperglycemia group: 22/80. Diabetes group: 14/80. Non-severe COVID: (1) Euglycemia (*n* = 44): 34 (77.27); (2) Secondary hyperglycemia (*n* = 22): 15 (68.18); and (3) Diabetes (*n* = 14): 6 (42.86). Severe COVID: (1) Euglycemia (*n* = 44): 10 (22.73); (2) Secondary hyperglycemia (*n* = 22): 7 (31.82); and (3) Diabetes (*n* = 14): 8 (57.14). Evidence of pneumonia on CT = 78/80: (1) Euglycemia group = 42/44; (2) Secondary hyperglycemia group = 22/22; and (3) Diabetes group = 14/14
Wang *et al*[20], 2020	Retrospective study	Beijing, China	*n* = 132. Exclusion criteria: (1) If not tested positive for COVID-19; (2) Receiving glucocorticoids; (3) Hemolytic anemia; (4) Myelosuppression after leukemia chemotherapy; and (5) Median time from onset to admission was 14 (IQR 10.0–17.8) d. Three groups: A, B, and C-(1) Group A had no diabetes and their HbA1c level was 6.0; (2) Group B had no diabetes and their HbA1c level was > 6.0; (3) Group C were diabetic	41/132 patients in group A. 44/132 patients in group B. 47/132 patients in group C: (1) 31/47 = History of type 2 diabetes; and (2) 16/47 = Newly diagnosed with diabetes. Death = 22/132: (1) Deaths in group A = 4/41; (2) Deaths in group B = 5/44; and (3) Deaths in group C = 13/47
Suwanwongse and Shabarek [22], 2021	Case series	United States	*n* = 3 (18/M, 51/M , 64/F)	New-onset diabetes was diagnosed after infection with COVID-19. 2 out of 3 cases were diagnosed as Diabetic Ketoacidosis. All were discharged home after successful management of blood glucose levels. None of the cases developed any pulmonary, renal, hepatic or cardiac complications due to COVID-19. Invasive Mechanical Ventilation, ICU Admission, or Death did not occur in any of the three cases
Marchand *et al* [25], 2020	Short communication	France	*n* = 1	New-onset type-I DM after COVID-19. No information on severity or outcome of COVID-19
Kuchay *et al* [23], 2020	Case series	Haryana, India	*n* = 3 (30/M, 60/M, 34/M). Follow up duration: 14 wk. Three patients with newly diagnosed Diabetes Mellitus and Diabetic Ketoacidosis with positive SARS-CoV-2 laboratory report. Case Definition: Diabetic Ketoacidosis: DKA was defined as plasma glucose > 250 mg/dL, a positive test for urine or serum ketones, and arterial pH < 7.35 and/or a bicarbonate level less than 18 mmol/L	All three patients responded well to intravenous fluids, antibiotics, and insulin and were discharged after the third week. All three patients were given oral antihyperglycemic drugs after their requirement for exogenous insulin diminished after 4-6 wk. No mortality
Fadini *et al*[17], 2020	Retrospective study	Italy	COVID-19 positive hospitalized patients included: *n* (Total) = 413. Median observation time of 17 d	No diabetes = 306/413. Diabetes = 107/413 (Pre-existing diabetes = 86/413; Newly-diagnosed diabetes = 21/413). Primary Outcome (composite of ICU admission or death): 62/306 (20.3%); 7/86 (31.4%); 13/21 (61.9%). Death: 33/306 (10.8%); 12/86 (14.0%); 3/21 (14.3%). Discharged alive: 238/306 (77.8%); 51/86 (59.3%); 9/21 (42.9%). Mean time to discharge in alive pts: 10.1 ± 5.7 (*n* = 306); 11.6 ± 6.6 (*n* = 74); 17.4 ± 8.5 (*n* = 18). Mean days of hospitalization in survivors: 11.3 ± 7.1 (*n* = 306); 13.8 ± 8.0 (*n* = 74): 19.7 ± 9.3 (*n* = 18)
Wang *et al*[21], 2020	Multicenter retrospective study	China	Without previous diagnosis of diabetes. *n* = 605 among 1258. Non-survivor = 114. Survivor = 491. Median age: 59.0 yr (IQR 47.0, 68.0). M/F =	Major outcome studied: 28-d mortality. Admission FBG (Total Non-survivor Survivor): (1) < 6.1 mmol/L = 329/605, 35/114, 294/491; (2) 6.1–6.9

			322/283. Out of total patients included in analysis: (1) FBG < 6.1 mmol/L (*n*) = 329; (2) FBG 6.1-6.9 mmol/L (*n*) = 100; and (3) FBG ≥ 7.0 mmol/L (*n*) = 176	mmol/L = 100/605, 21/114, 79/491; (3) ≥ 7.0 mmol/L = 176/605, 58/114, 118/491; and (4) Complications 237/605, 114/114, 123/491. With complications: (1) < 6.1 mmol/L = 86/605, 35/114, 51/491; (2) 6.1–6.9 mmol/SL = 48/608, 21/114, 27/491; and (3) ≥ 7.0 mmol/L = 103/605, 58/114, 45/489. Without complications: (1) < 6.1 mmol/L = 243/605, 0/114, 243/491; (2) 6.1–6.9 mmol/L = 52/605, 0/114, 52/491; and (3) ≥ 7.0 mmol/L = 73/603, 0/114, 73/490
Yang *et al*[24], 2020	Retrospective case series	China	*n* = 69 among 120 evaluated. Exclusion Criteria: (1) Previously diagnosed Diabetes Mellitus; (2) Patients treated with Glucocorticoids; (3) Patients with heart disease (myocardial infarction and heart failure); (4) Patients with kidney disease (maintenance dialysis or renal 20 transplantation); and (5) Patients with liver disease (liver cirrhosis). Median age = 61 (IQR 52-67). M/F = 34/35	FBG ≥ 7.0 mmol/L for two times during hospitalization and without a history of diabetes in COVID-19 patients: 69/120. COVID-19 Severity: (1) Moderate = 23/69; (2) Severe = 20/69; and (3) Critical = 26/69. Mortality = 16/69
Li *et al*[14], 2020	Retrospective study	China	Inclusion: Laboratory confirmed SARS-CoV-2 Infection. Exclusion: Incomplete data available, cases without clinical results, patients with pneumonia due to other pathogens. *n* = 453. Non survivor (*n*) = 39. Recovered (*n*) = 414. Median age = 61 yr (IQR 49-68). Divided into four groups: (1) Normal glucose: FBG < 5.6 mmol/L, HBA1c: < 5.7% (*n* = 132); (2) Hyperglycemia: FBG 5.6-6.9 mmol/L HbA1c: 5.7%-6.4% (*n* = 129); (3) Newly diagnosed Diabetes: No history of previous Diabetes. FBG: ≥ 7 mmol/L and/or HbA1c ≥ 6.5% (*n* = 94); and (4) Known Diabetes: Previously diagnosed Diabetes Mellitus (*n* = 98)	Main clinical outcomes: (1) Invasive mechanical ventilation: 3/132; 6/129; 11/94; 9/98; (2) ICU admission: 2/132, 8/129, 11/94, 4/98; and (3) Death: 2/132, 6/129, 20/94, 11/98. Other outcomes: (1) ARDS: 1/132, 4/129, 10/94, 3/98; (2) Acute Cardiac Injury: 27/132, 26/129, 23/94, 32/98; (3) Coagulopathy: 12/132, 12/129, 15/94, 17/98; (4) Hypoalbuminemia: 14/132, 15/129, 37/94, 36/98; and (5) Length of hospital stay (days): 22.5 (1.19), 21.9 (1.16), 26.5 (1.37), 23.6 (1.37)

ARDS: Acute Respiratory Distress Syndrome; BMI: Body mass index; COVID-19: Coronavirus disease 2019; CT: Computed tomography; DKA: Diabetic ketoacidosis; DM: Diabetes mellitus; F: Female; FBG: Fasting blood glucose; HbA1C: Hemoglobin A1C; ICU: Intensive care unit; IQR: Inter quartile range; M: Male; N: Total participants; Non-DM: Non-diabetes mellitus; Pre-DM: Pre-diabetes mellitus; T2DM: Type 2 diabetes mellitus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Adverse events such as severe COVID-19, intubation, complications, and ICU admission

Pooling data for the occurrence of adverse events among COVID-19 cases using a random effect model showed 15.29% occurrence among non-diabetic patients (CI: 9.06-24.65, I^2= 84.47), 20.41% among those with COVID-19 associated hyperglycemia (CI: 6.20-49.86, I^2= 93.41), 20.69% among known DM patients (CI: 8.12-43.50, I^2 = 90.14), and 45.85% among those with new-onset DM (CI: 22.23-71.50, I^2 = 94.21). The overall occurrence of adverse events was 20.52% (CI: 14.21-28.70, I^2 = 93.53) among all COVID cases irrespective of their DM status (Figure 5).

Meta-regression for mortality outcome

Meta-regression showed an increasing rate of mortality among newly hyperglycemic patients, known diabetic patients, and new-onset DM compared to non-diabetic patients (Figure 6 and Table 5).

Publication bias

Publication bias across the included studies was evaluated using Egger's test to evaluate funnel plot asymmetry. Publication bias reporting new-onset DM showed some publication bias depicted by the asymmetry of the funnel plot (Supplementary Material 2 and Figure 4). Similarly, publication bias for mortality outcome is shown in Supplementary Material 2 and Figure 5.

DISCUSSION

Our meta-analysis is the first to pool the prevalence of new-onset DM and compare mortality and adverse events among patients with new-onset DM *vs* patients with hyperglycemia, pre-existing DM, or no DM. Prior meta-analyses have shown DM to be associated with mortality, severe COVID-19, ARDS, and disease progression[11-13]. However, there was a paucity of data to compare the outcomes among infected patients with pre-existing diabetes compared to new-onset DM. We found the pooled

Table 5 Main results for meta-regression model, random effects, Z-distribution, logit event rate

Covariate	Coefficient	SE	95% lower	95% upper	Z value	*P* value
Intercept: No DM	-2.3183	0.2504	-2.8091	-1.8276	-9.26	0
Hyperglycemia	0.2519	0.3788	-0.4905	0.9944	0.67	0.506
Known DM	0.6642	0.3552	-0.0319	1.3603	1.87	0.0615
New DM	1.1865	0.3552	0.4903	1.8827	3.34	0.0008

Test of the model: Simultaneous test that all coefficients (excluding intercept) are zero: Q = 12.51, df = 3, P = 0.0058. Goodness of fit: Test that unexplained variance is zero: Tau² = 0.1610, Tau = 0.4012, I^2 = 62.66%, Q = 34.81, df = 13, P = 0.0009. Total between-study variance (intercept only): Tau² = 0.3751, Tau = 0.6124, I^2 = 81.75%, Q = 87.66, df = 16, P = 0.0000. Proportion of total between-study variance explained by Model 1: R^2 analog = 0.57. DM: Diabetes mellitus.

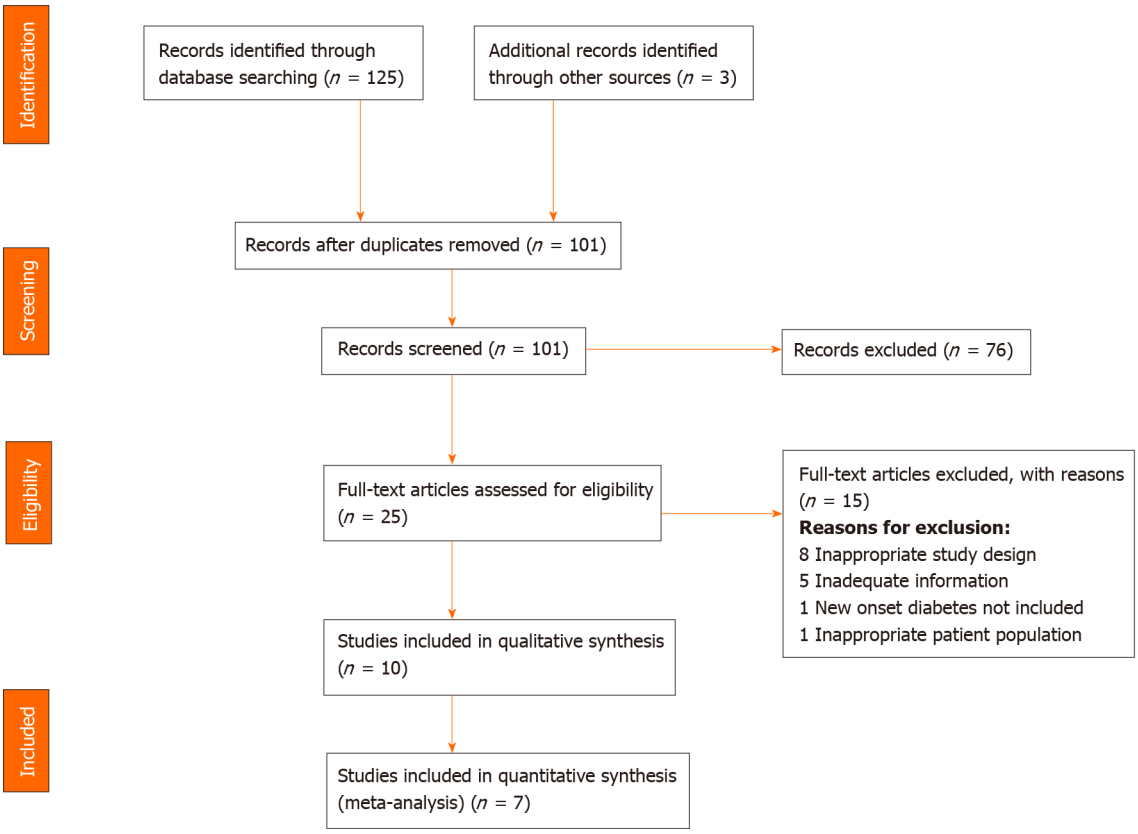

Figure 1 PRISMA flow diagram.

prevalence of COVID-19 associated DM (new-onset) to be 19.7%, while the prevalence of COVID-19 associated hyperglycemia was 25.23%. Angiotensin II has been shown to increase hepatic glucose production and decrease insulin sensitivity. A multitude of explanations have been proposed for impaired blood glucose levels among patients infected with COVID-19, including downregulation of ACE-2 receptors leading to increased angiotensin II and defective insulin secretion as well as direct damage to beta cells of islets of the pancreas[7,8]. Infection with the virus itself leads to oxidative stress, resulting in hypoxia and inflammation, which aggravates glucose homeostasis [14]. Additionally, damage to key organs involved in glucose metabolism such as the kidney and the liver resulting in abnormal blood glucose levels, has been observed in cases of COVID-19 infection. The use of corticosteroids is common among COVID-19 patients, especially those with severe COVID-19[15]. However, in our meta-analysis, only one study[16] included patients receiving steroids, which eliminates steroid use as a possible cause of hyperglycemia. The mortality rate was highest among patients with new-onset DM (24.96%), followed by known DM patients (16.03%), patients with COVID-19 associated hyperglycemia (10.59%), and non-diabetic patients (9.26%). The

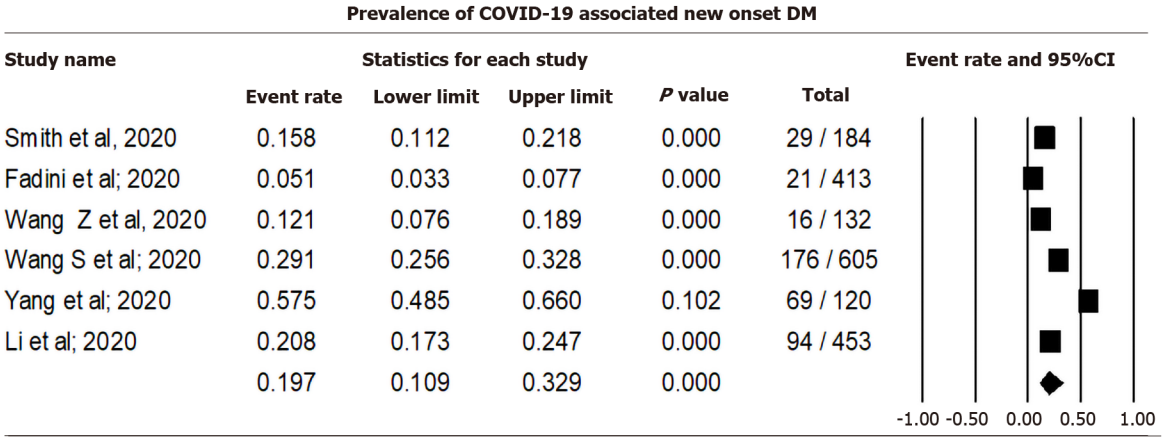

Figure 2 Prevalence of coronavirus disease 2019 associated new onset diabetes mellitus. COVID-19: Coronavirus disease 2019; DM: Diabetes mellitus.

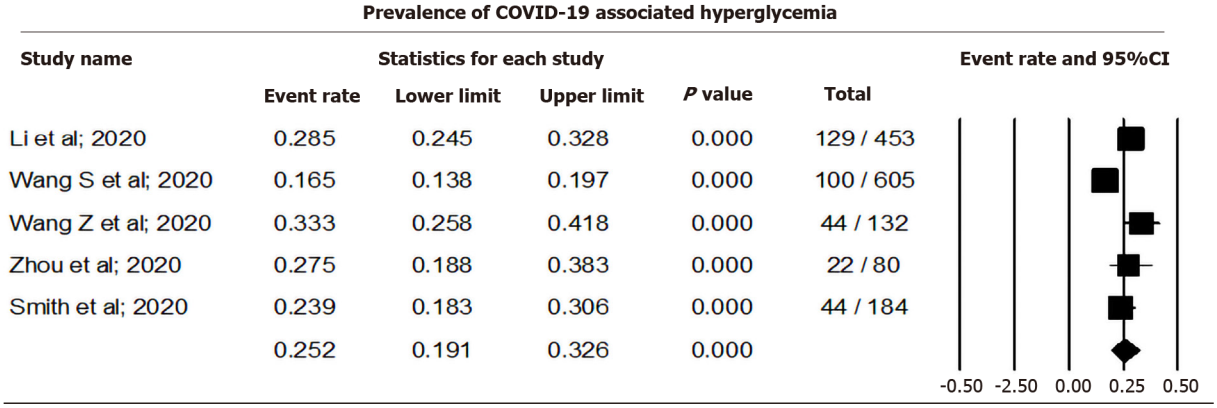

Figure 3 Prevalence of coronavirus disease 2019 associated hyperglycemia. COVID-19: Coronavirus disease 2019.

higher prevalence in patients with new-onset DM could be explained by the masked presence of organ damage due to ongoing diabetes, which cannot be accounted for during statistical analysis in contrast to cases of pre-existing diabetes in which organ damage is accounted for statistically[17]. Similarly, metabolic inflammation caused by high blood sugar levels affects the body's immune system and healing process prolonging recovery[14]. Hyperglycemia has been found to affect lung volume and diffusion capacity, causing respiratory deterioration and a decrease in PaO_2/FiO_2 ratio [17]. Chronic hyperglycemia causes down regulation of ACE-2, which has a protective effect against inflammation and in turn leads to inflammatory damage by the virus and potential cytokine storm. These are the reasons for increased mortality among patients with diabetes and hyperglycemia compared to non-diabetic patients. The pooled mortality of 16.03% among diabetic patients was lower than that shown in Shang's meta-analysis (21.4%) and higher than that in Miller *et al*[11] (9.9%). Adverse events such as severe COVID-19, intubation, complications, and ICU admissions were highest among new-onset DM (45.85%), followed by known DM patients (20.69%), patients with COVID-19 associated hyperglycemia (20.41%), and non-diabetic patients (15.29%). Our findings concurred with previous studies that have shown a strong association between DM and severe COVID-19, leading to increased complications, including multi-organ dysfunction and ICU admissions[18]. The need for intubation can be explained by the respiratory deterioration noted among patients with hyperglycemia.

Our study has several limitations. Due to the inadequate number of existing studies, we could not include controlled studies, instead using only observational studies, case reports, and case series. The included studies had small sample sizes and low power. Each study had its own limitations, such as the absence of data on body mass index, Hemoglobin A1C in all patients, the possibility of stress hyperglycemia, single-center study, retrospective study design, *etc.*

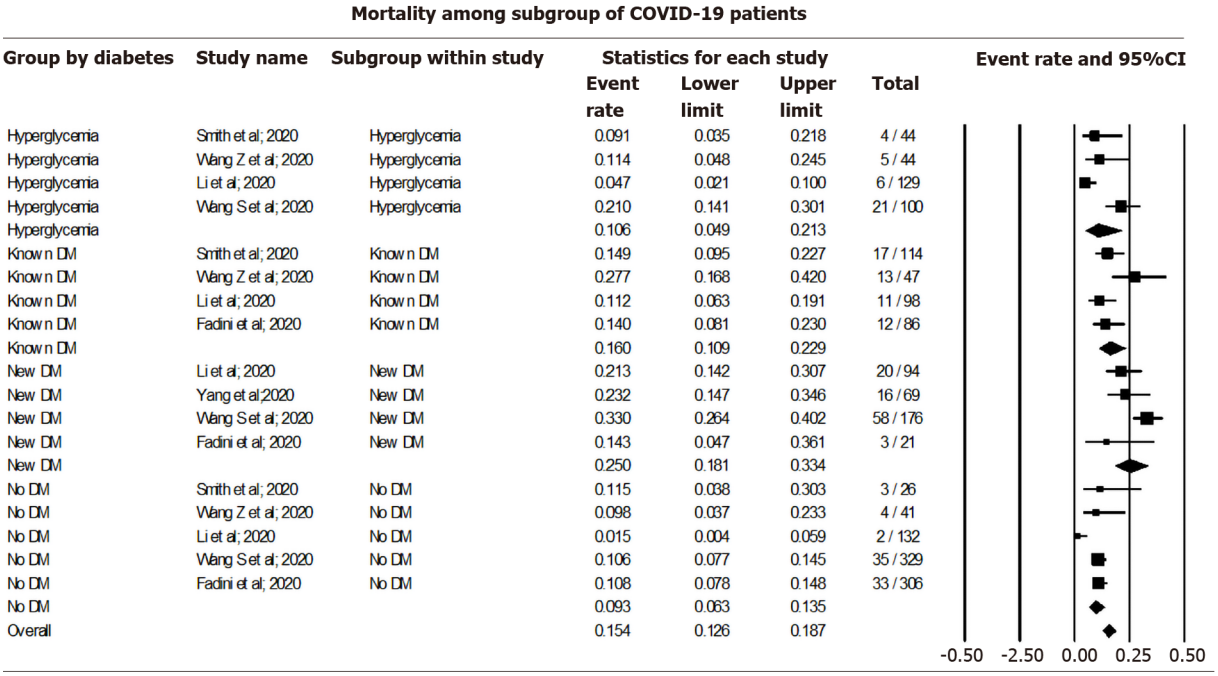

Figure 4 Mortality among coronavirus disease 2019 cases with subgroup analysis based on their diabetes status. COVID-19: Coronavirus disease 2019; DM: Diabetes mellitus.

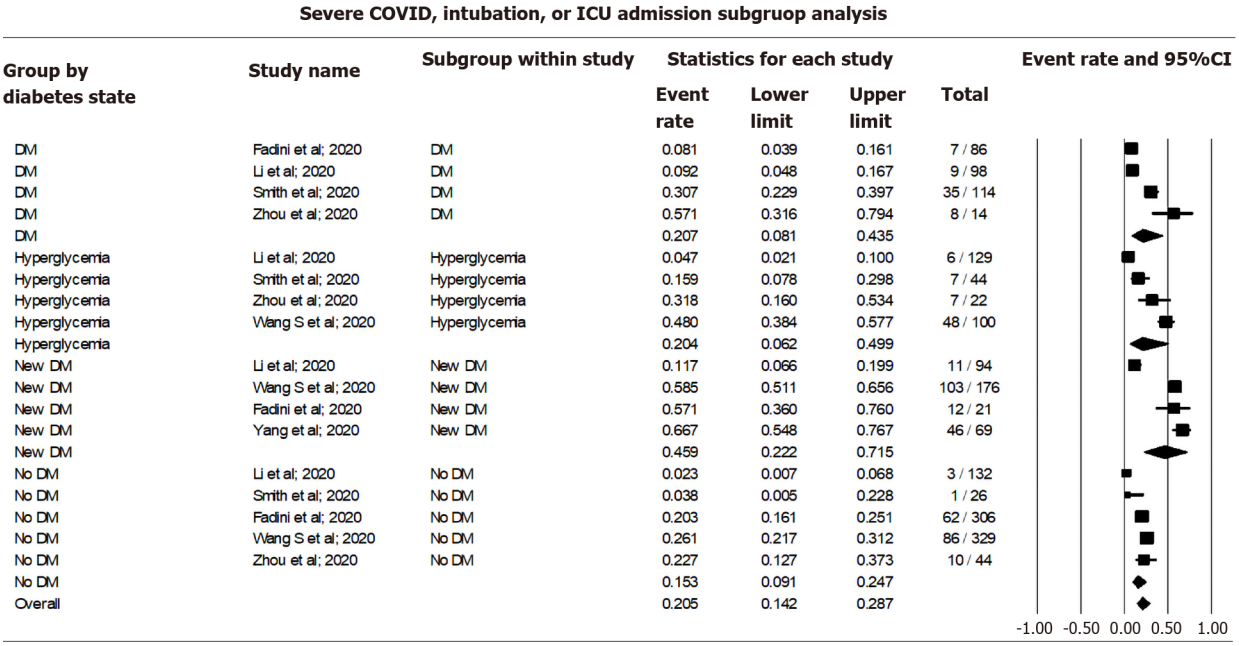

Figure 5 Occurrence of adverse events among coronavirus disease 2019 cases with subgroup analysis based on their diabetes status. COVID-19: Coronavirus disease 2019; DM: Diabetes mellitus; ICU: Intensive care unit.

CONCLUSION

The pooled prevalence of COVID-19 associated DM was 19.70%, and for COVID-19 associated hyperglycemia was 25.23%. Among COVID-19 patients, higher mortality rates and adverse events were seen in patients with new-onset DM compared to those with pre-existing diabetes, those with COVID-19 associated hyperglycemia, and those without diabetes.

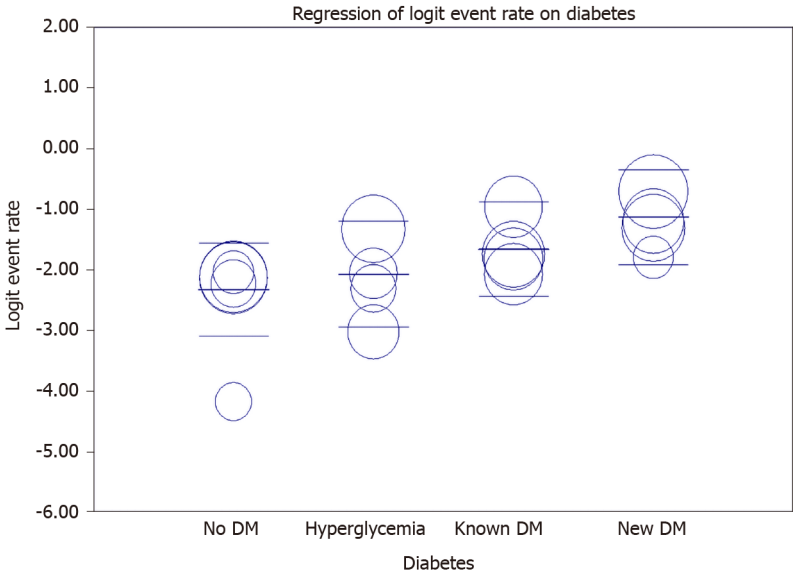

Figure 6 Meta regression of diabetes status and mortality.

ARTICLE HIGHLIGHTS

Research background

Diabetes has been shown to be associated with worsening severity of disease and poor prognosis in coronavirus disease 2019 (COVID-19). Interestingly, various cases of new onset diabetes mellitus (DM) were seen in patients with COVID-19. The virus is believed to bind to angiotensin-converting enzyme-2 receptors leading to increased angiotensin II and subsequent decreased insulin secretion.

Research motivation

In relation to various theories and proposed mechanisms of how COVID-19 may lead to abnormal glucose homeostasis, our study was conducted to evaluate new onset DM in COVID-19.

Research objectives

The study aimed to pool the prevalence of new onset DM and hyperglycemia in COVID-19 patients and compare various outcomes such as mortality, intubation and complications among infected patients who had hyperglycemia or preexisting DM or new onset DM or normal blood sugar levels.

Research methods

Meta-analysis of Observational Studies in Epidemiology was used for the meta-analysis. Studies were screened using Covidence after searching various databases including PubMed, PubMed Central, Embase and Scopus. Comprehensive meta-analysis software was used for data analysis.

Research results

The results showed that 19.70% and 25.23% of patients had COVID-19 associated DM and hyperglycemia, respectively. The mortality rate was highest among COVID-19 associated DM patients (24.96%) followed by patients with preexisting DM (16.03%), and was least in non-diabetic patients (9.29%). The occurrence of adverse events was highest among COVID-19 associated new-onset DM patients followed by patients with preexisting DM, COVID-19 associated hyperglycemia and non-diabetic patients.

Research conclusions

COVID-19 was associated with hyperglycemia and new-onset DM. Infected patients with new onset DM had worse prognosis in terms of mortality and adverse events.

Research perspectives

The findings of this study should alarm clinicians that new onset diabetes and

hyperglycemia is a bad prognostic factor for COVID-19.

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LETTER TO THE EDITOR

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MINIREVIEWS

Protein-protein interactions: Methods, databases, and applications in virus-host study

Qurat ul Ain Farooq, Zeeshan Shaukat, Sara Aiman, Chun-Hua Li

ORCID number: Qurat ul Ain Farooq 0000-0001-9951-2388; Zeeshan Shaukat 0000-0002-7901-6712; Sara Aiman 0000-0002-4115-4984; Chun-Hua Li 0000-0002-0895-3506.

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Qurat ul Ain Farooq, Sara Aiman, Chun-Hua Li, Faculty of Environmental and Life Sciences, Beijing University of Technology, Beijing 100124, China

Zeeshan Shaukat, Faculty of Information Technology, Beijing University of Technology, Beijing 100124, China

Corresponding author: Chun-Hua Li, PhD, Professor, Faculty of Environmental and Life Sciences, Beijing University of Technology, No. 100 Pingleyuan, Chaoyang District, Beijing 100124, China. chunhuali@bjut.edu.cn

Abstract

Almost all the cellular processes in a living system are controlled by proteins: They regulate gene expression, catalyze chemical reactions, transport small molecules across membranes, and transmit signal across membranes. Even, a viral infection is often initiated through virus-host protein interactions. Protein-protein interactions (PPIs) are the physical contacts between two or more proteins and they represent complex biological functions. Nowadays, PPIs have been used to construct PPI networks to study complex pathways for revealing the functions of unknown proteins. Scientists have used PPIs to find the molecular basis of certain diseases and also some potential drug targets. In this review, we will discuss how PPI networks are essential to understand the molecular basis of virus-host relationships and several databases which are dedicated to virus-host interaction studies. Here, we present a short but comprehensive review on PPIs, including the experimental and computational methods of finding PPIs, the databases dedicated to virus-host PPIs, and the associated various applications in protein interaction networks of some lethal viruses with their hosts.

Key Words: Protein-protein interactions; Experimental and computational methods; Protein-protein interaction networks; Protein-protein interaction databases; Disease pathways; Protein-protein interaction applications

Core Tip: This paper provides a comprehensive review on protein-protein interactions (PPIs), including the experimental and computational methods of finding PPIs, the

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databases dedicated to virus-host PPIs, and the associated applications in the studies of some lethal viruses with their hosts. PPIs can be mapped into networks and innumerable novel insights into the functional organization of proteomes can be gained by analyzing the networks. Many studies have used network biology to construct PPI networks of lethal pathogens with their host *Homo sapiens* to dig deep down into the molecular constitution of the disease pathways, and have successfully found multiple potential drug targets against the viruses.

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INTRODUCTION

Proteins have been declared as the chief representative of biological function[1]. It has been reported that more than 80% of proteins do not function alone[2], but instead often interact with each other or with other molecules like DNA or RNA to perform distinct cellular functions. Protein-protein interactions (PPIs) are thought to execute many biological processes including complex metabolic pathways and signaling cascades, and hence it is crucial to understand the particular nature of these associations[1,3].

De Las Rivas and Fontanillo[4] defined PPIs as "physical contacts with molecular docking between the proteins that occur in a cell or in a living organism *in vivo*". The physical contacts between the proteins should be specific and intentional, *i.e.*, evolved for a particular function. Protein interacting with other proteins can be in any form, *i.e.*, in binary, multi-protein complexes or in the form of long chains[1,4]. Proteins involved in a certain cellular pathway or biological process are often found to interact with each other repeatedly, suggesting that the proteins with associated functions are more likely to interact with each other[2,5]. Conversely, researchers can reveal the functions of unidentified or uncharacterized proteins if the proteins with which they are interacting are known[6,7]. The outcome of most of the cellular processes can be deciphered by protein interactions. The information about PPIs can help scientists find out potential drug targets by investigating the pathogen-host interaction network[8,9]. Therefore, it is significant to study PPIs for understanding the functions of proteins within a cell or a living organism.

EXPERIMENTAL METHODS TO DETECT PPIS

PPIs can be determined by different high-throughput experimental and computational methods which yield different types of PPI data. The high-throughput experimental techniques either identify the interactions directly or infer them indirectly based on different approaches[1,4]. In the following, the two main experimental methods, yeast two-hybrid (Y2H) and tandem affinity purification-mass spectrometry (MS), will be introduced.

Y2H

Y2H, also known as a binary method initially reported in 1989, is the most widely and commonly used interaction detection approach that identifies direct physical interactions between two proteins *in vivo*[10]. It detects the interactions between the query protein of interest and the protein library. In this approach, the former fused with the binding domain of a particular transcription factor is known as the bait and the latter fused with the activation domain of the transcription factor is referred to as the prey. If the bait and prey can interact with each other, they will bring together the two halves of the transcription factor to activate the transcription complex (shown in Figure 1), which transcribes the downstream reporter gene leading to the expression of the reporter gene[1,4,11]. The availability of many full genomes with the advancement of next-generation sequencing techniques allows us to use protein interactions to help

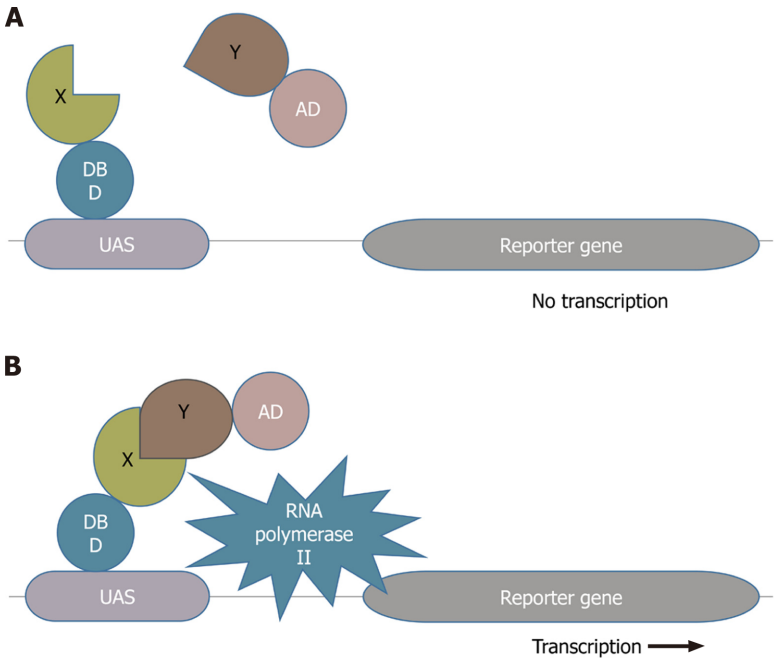

Figure 1 Yeast two-hybrid technique. A: There is no transcription of the reporter gene because the transcriptional factor is broken down into two halves; B: The reporter gene is being transcribed because the two halves of the transcription factor are brought together by the interaction between bait (X) and prey (Y) proteins[10]. DBD: DNA binding domain; AD: Activation domain; UAS: Upstream activation domain.

understand the functions of their gene products. Y2H has outranked the other experimental techniques and has become the system of choice for researchers in large-scale, high-throughput, and comprehensive investigations of PPIs. The complete proteomes of several pathogens including hepatitis C virus (HCV), bacteriophage T7, and vaccinia have been analyzed using the Y2H screen[12-14]. Several scientists have performed the comprehensive two-hybrid analysis of the yeast protein interactome, including the construction and analysis of PPI map of all possible associations between the yeast proteins[15-17].

Y2H has been used massively by scientists to infer physical interactions between macromolecules. It is advantageous because of its simple organization and easy detection for the transient interactions. However, despite its importance, there are certain disadvantages[10,18] which will be discussed in the section of experimental errors in PPI detection.

Tandem affinity purification-MS

MS is a powerful *in vitro* tool for the detection of macromolecular interactions. The principle of MS was explained extensively in one of our previous reviews[19]. MS allows us to identify polypeptide sequences by ionizing them and then detecting analyte ions based on their mass-to-charge ratios[20,21]. To interpret the mass spectra and detect PPIs, various MS-based methods have been developed so far. The MS-based detection of PPIs has become significant in the recent era especially for the large-scale investigations, through which high-throughput and high confidence PPIs can be identified[22,23]. These MS-based technologies include cross-linking MS (CLMS)[24], tandem affinity purification MS (TAP-MS)[25,26], and several others.

TAP-MS is a conventional MS-based qualitative method to study protein functions and interactions. Sinz[27] and Yugandhar *et al*[28] have extensively reviewed CLMS, which is a more recent and advanced MS technique for interpreting protein interaction networks. Many scientists have been working on the techniques using MS for finding potential interactors where true positives are segregated and prioritized from false positives. Gavin *et al*[29] and Collins *et al*[30] developed score-based methods to infer high-accuracy physical interactions.

According to the EMBL-EBI statistics (https://www.ebi.ac.uk/intact/about/statistics?conversationContext=2), TAP-MS has overtaken Y2H as a major source of generating PPI data.

Compared with Y2H which detects only binary interactions, TAP-MS is a co-complex method which determines both direct and indirect associations between proteins *in vitro*. In this technique, a TAP tag is fused at the C- or N-terminus of a

protein of interest (the bait), which has two independent binding regions, allowing two successive affinity purification steps. The most common TAP tag consists of two immunoglobulin G binding repeats of Protein A from *Staphylococcus aureus* (ProtA) and a calmodulin-binding peptide which are separated by a tobacco etch virus protease cleavage site. In TAP, a group of protein complexes can be caught by a tagged bait protein in a pull-down assay, which are called prey proteins[2,4,31]. The prey proteins interacting with the bait are separated *via* sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then identified by MS[18,32].

In addition to the tandem affinity purification, there is another co-complex method called co-immunoprecipitation (CoIP) for determining PPIs. The interaction data derived from co-complex methods cannot be used to infer binary interactions directly, and the related algorithms are needed to interpret the pairwise interactions from the experimental data[4].

Experimental errors in PPI detection

High-throughput experimental approaches for determining PPIs are very efficient, but they also have some limitations. They have a high possibility of false negative and false positive errors. False positives in an experimental system are those interactions that do not occur in the system naturally. One reason for the false positives in Y2H can be the auto-activation of transcription by the bait protein itself or sometimes the transient interactions that are not always specific, *i.e.*, the interactors can be the sticky prey proteins fused with the bait protein and chosen by Y2H analysis[4,10,33]. The precise percentage of the false-positive interactions in Y2H is not well known but the estimated rate of the inaccurate interactions is about 50%, which is quite a big percentage, yet still Y2H is one of the most powerful interaction determining methods [2,10]. Additionally, the experimental system for determining PPIs faces false negative errors too, *i.e.*, some interactions cannot be identified due to the flaws in the experimental system. In Y2H, most of the interactions between membrane proteins are undetectable. Hence, it is important to choose the Y2H design thoughtfully based on the type of cellular proteome. Sometimes in Y2H, very weak transient interactions escape from being identified by the method[10].

Co-complex methods also encounter errors in their interaction detection mechanisms. There can be sticky prey proteins in the TAP pull down assay that are detected by the method as interacting partners of the bait protein. The TAP is an *in vitro* technique, which means that it is not sure whether the interactions that occur *in vitro* will surely exist *in vivo*. Additionally in TAP, the very transient interactions often vanish due to the series of purification levels[1,2]. Another drawback of co-complex methods is that they might analyze all the elements of a protein complex which certainly may not have direct interactions with each other[10] (crossed links in Figure 2).

PPI studies do not just rely on Y2H or affinity purification methods, and due to the false positives and false negatives, several other methods have also been made into practice by researchers for PPI detection. Some of these *in vitro* techniques are CoIP [18], protein microarrays[34], protein-fragment complementation[35], X-ray crystallography, and nuclear magnetic resonance spectroscopy[36].

COMPUTATIONAL METHODS FOR PREDICTING PPIS

As discussed in the previous section, experimental methods for PPI detection have many limitations including a high percentage of false positives, high cost, and being significantly laborious and time-consuming. Besides, due to the completion of various genome sequencing projects, it is necessary to speed up to find the functional linkages between proteins. Thus, the computational prediction of PPIs seems to be very crucial. Now, computational methods are being practiced successfully to evaluate and analyze the interaction data generated by high-throughput experimental approaches as well as to predict novel PPIs by gaining insights from the already known interactions.

The computational methods are a quick and low-cost alternative to the traditional experimental techniques to predict PPIs. An important advantage of computational methods over the experimental ones is that we can study proteins by mapping the pairwise associations into a comprehensive network according to their distinct functional level[1,37]. Table 1 lists some of the important *in silico* methods of PPI prediction.

Table 1 List of some important computational methods of protein-protein interaction prediction along with their brief descriptions

Method	Description	Ref.
In silico two-hybrid (I2H)	The I2H method is based on the detection of direct physical associations between the interacting proteins and it relies on the presumption that in order to maintain the protein function reliable, the interacting proteins should go through coevolution	Pazos and Valencia[38]
Ortholog-based approach	It is a sequence-based approach that uses a pairwise local search algorithm to obtain the similarities between the query protein pairs and the known interaction pairs. It is dependent upon the homologous nature of the target proteins	Lee *et al*[39]
Gene fusion	Also known as Rosetta stone method. According to this method, some of the proteins with single domains fuse together in one organism and form a multi-domain protein in another organism	Enright *et al*[40]
Domain-pairs-based approach	This method predicts the interactions between proteins by the domain-domain interactions	Wojcik and Schächter[41]
Gene expression	An indirect way to predict PPIs. Based on the concept that the proteins translated from the genes that belong to the common expression profiling clusters more likely interact with each other than the proteins translated from the genes that belong to different clusters	Grigoriev[42]
Structure-based approaches	It predicts protein-protein interactions based on the structural similarity	Zhang *et al*[43]
Phylogenetic tree	This method predicts protein-protein interactions based on the concept that the interacting proteins show similarity in their evolution history	Sato *et al*[44]

PPI: Protein-protein interaction.

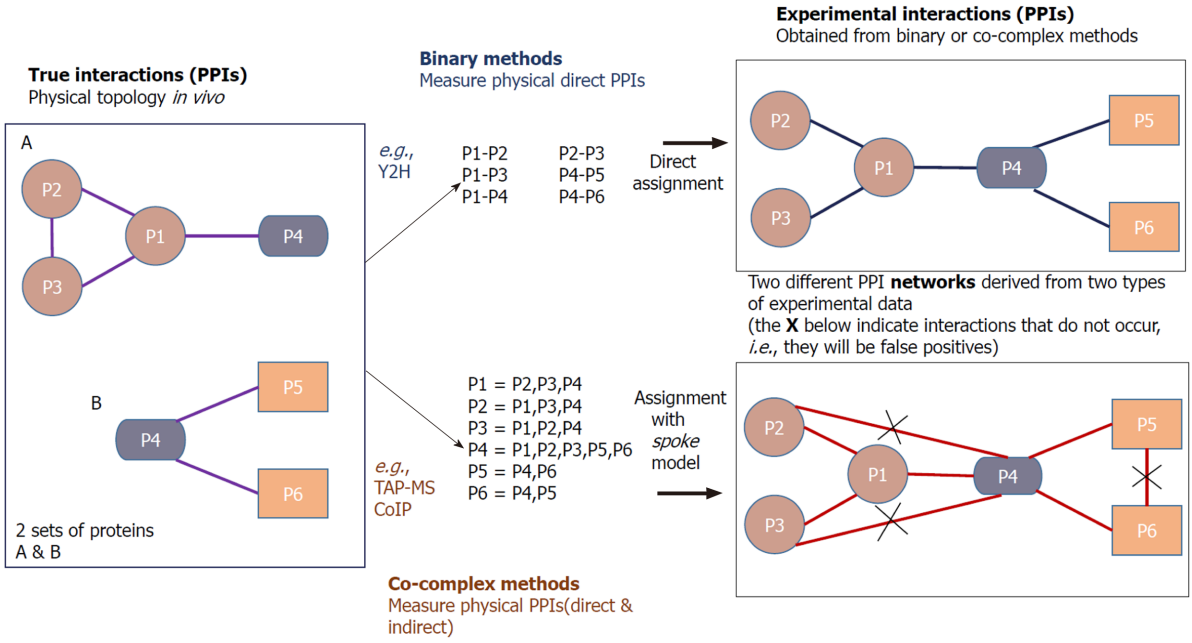

Figure 2 Binary and co-complex methods to determine protein-protein interactions. Yeast two-hybrid (Y2H) and tandem affinity purification mass spectrometry are the two most extensively used approaches for detecting protein-protein interactions. Given here are the two sets of proteins (4 proteins in set A while 3 proteins in set B) in the left panel and the connections show the genuine interactions between them. The right side shows the experimentally determined interaction network among the six proteins. The network in the upper right shows the interactions derived from Y2H, and the network in the lower right shows the interactions got from co-complex method, in which three of the interactions inferred do not exist[4]. PPI: Protein-protein interaction; TAP-MS: Tandem affinity purification mass spectrometry; CoIP: Co-immunoprecipitation.

PPI DATABASES

The continuous increase in PPI data produced by high-throughput technologies needs the formation of biological repositories where these data should be stored in an effective and organized way. The data in the publicly available PPI databases makes it much easier to analyze different types of interactions according to our concerns[37]. There are more than 100 repositories accessible online related to PPI data[45]. Here we will discuss the most popular databases (see Table 2) of PPI information that have

Table 2 List of popular protein-protein interaction databases with total numbers of interactions and last updated time

PPI database	URL	Total interactions	Last updated	Ref.
STRING	http://string-db.org/	> 2000 mio	2020	Szklarczyk *et al*[50]
BioGrid	http://thebiogrid.org/	1746922	2021	Oughtred *et al*[47]
HPIDB	https://hpidb.igbb.msstate.edu/index.html	69787	2019	Ammari *et al*[51]
MINT	https://mint.bio.uniroma2.it/	131695	2012	Zahiri *et al*[3] and Licata *et al*[55]
DIP	https://dip.doe-mbi.ucla.edu/dip/Main.cgi	81923	2017	Zahiri *et al*[3] and Salwinski *et al*[56]
IntAct	http://www.ebi.ac.uk/intact/	1130596	2020	Orchard *et al*[52]
HPRD	http://www.hprd.org/	41327	2010	Zahiri *et al*[3] and Keshava Prasad *et al*[57]

PPI: Protein-protein interaction; URL: Uniform resource locator.

been used by most of the researchers worldwide and contain experimentally verified virus-host PPIs.

Biological General Repository for Interaction Datasets

The Biological General Repository for Interaction Datasets (BioGRID) is a publicly retrievable and comprehensive database which stores experimentally determined PPI data of almost all important model organisms[3,46]. It has constantly being updated and according to the February 2021 release, it carries 1740000 non-redundant protein and genetic interactions collected from 70000+ publications[47]. The current version of BioGrid (v 4.3.194) themed curation projects focuses on curated interactions of different diseases including coronavirus disease 2019 (COVID-19), ubiquitin-proteosome system, fanconi anemia, glioblastoma, and autophagy.

Search Tool for Retrieval of Interacting Genes

Search Tool for Retrieval of Interacting Genes (STRING) is equipped with the complete information about the functional relationships between proteins. The current version STRING v11.0 contains interaction data of 5090 organisms that is the highest number of organisms covered by any PPI database. The major assets of STRING database are its exhaustive coverage, confidence scoring of the interactions, and its intuitive user interface[48,49]. Currently, the database covers 3123056667 PPIs which are the sum of high-confidence and low-confidence interactions. An important new feature in the current version of STRING is that users can perform Gene Ontology and KEGG analysis of their input which has provided ease in gene-set enrichment analysis[50].

HPIDB

HPIDB is a curated database that contains host-pathogen interaction data. Developed in 2010, it is updated yearly and presents new versions. Currently, it contains protein interaction data between 66 hosts and 668 infectious pathogen species. The number of unique interactions is 69787 according to the last update (July 29, 2019). The pathogenic species that can be found superabundantly in HPIDB are influenza virus, herpes virus, papillomaviruses, *Saccharomyces cerevisiae*, and several others[51].

IntAct

Developed in 2002, IntAct is a freely available molecular interaction data source and contains the data obtained from literature curation or deposited directly by the researchers. In 2013, IntAct and MINT joined their efforts and started the MINTACT project to maximize the coverage and curation output[52].

International Molecular Exchange Consortium databases

The International Molecular Exchange Consortium (IMEx) is an international consortium established by the joint efforts of prime public interaction databases including DIP, IntAct, HPIDB, MINT, BioGRID, MatrixDB, I2D, and some others. BIND and MPIDB which used to be large PPI databases are also members of IMEx but they no longer are active anymore. The data in IMEx is a comprehensive and integrated consortium of databases recording meta data for PPIs in a standard PSI-MS format and is available for all the researchers to re-use and re-analyze. Over the last two decades, there has been a massive increase in protein interaction data and out of

all the resources, IMEx is the only source which is providing up to the minute information regarding protein interactions and annotations[45,53,54].

Some protein interaction databases are dedicated to a specific viral pathogen for example HCVPro[58] containing the data on PPIs between HCV and human. VirHostNet[59] covers an extensive range of human specific viruses and contains nearly 22000 virus-human PPIs.

APPLICATIONS OF PPIS IN DISEASE NETWORKS AND IN VIRUS-HOST RELATIONSHIP

Bacteria and viruses are the major pathogens affecting humans on earth. Bacterial infections can be eradicated by using antibiotics, and viruses not easy to be eliminated can only be inhibited in their growth. Viruses depend entirely on their hosts and infect hosts often by virus-host protein interactions[54]. PPIs can be mapped into networks and innumerable novel insights into the functional organization of proteomes can be gained by analyzing the networks. Several protein interaction network construction and visualization tools are available, including Cytoscape[60], BioLayout[61], and VisANT[62]. Analyzed by these tools, PPI networks can provide the differences between normal and the diseased states, and thus the fundamental knowledge about the disease can be obtained based on the related pathways revealed through the analyses of PPI network, *i.e.*, by looking into the subnetworks constructed by the proteins involved in the disease[1,63]. Protein interaction networks can help find new disease-related genes by the presumption that the neighboring genes of the disease-causing gene are expected to be causing the same disease or involved in causing some similar diseases (Figure 3)[64]. Various researchers have been using network biology to study pathogen-host relationship at the molecular level, which ultimately helps in identifying key viral proteins and their human targets and helps scientists in further biological investigations.

The quickly developing knowledge of human interactome map and the availability of different host-pathogen networks have paved us the way for a better understanding of diseases. Viral genomes code for a very small number of proteins, which makes it easy to understand the mechanisms of the infections by viruses[64,65]. The network-based study on the infection of host with viral pathogenesis is progressing over time. In one of our previous studies, we constructed a comprehensive protein interaction network of HCV with its host *Homo sapiens*[66] and found out many crucial insights into finding potential targets against HCV and some other disease pathways, such as cancer pathways (Figure 4). In fact, certain viruses such as papilloma and herpesvirus have been reported to be causing up to 20% of the cancers[67]. Additionally, virus-host relationship was also studied by us for human papillomavirus[68], influenza A virus (IAV)[69], and dengue virus with *Homo sapiens*. Interestingly, in a study performed by Navratil *et al*[70], they compared a set of virus targets with a list of 1729 human genetic disease-related proteins, and found that 13% of human virus targets are also linked with at least one human genetic disease. In short, there are so many types of viruses causing a wide variety of infections worldwide. From Ebola virus outbreak in Africa to Middle East respiratory syndrome coronavirus outbreak, viruses have killed thousands of people with no specific effective treatment. Every viral infection involves PPIs between the virus and its host including the viral entry to the host cell and hijacking the host transcription machinery. Identification of PPIs between the viruses and their hosts lets us understand the infection mechanisms of the viruses and find a way to combat the infections using antiviral drugs or vaccines[71].

When we talk about human interactome, more than 645000 PPIs are reported to be disease-associated while only 2% of these proteins are targeted by drugs[72]. The reason for most of the proteins considered to be undruggable is because of the absence of detectable pockets for binding ligands[73]. Researchers have been significantly investigating PPI inhibitors and stabilizers and have succeeded in developing new technologies that have enabled the systematic discovery of drugs focused on PPIs[74, 75]. Zhang *et al*[76] and Robertson and Spring[77] have extensively explained the use of peptidomimetics to find the 'hot spots' on the protein surfaces for drug design. Targeting PPIs for designing therapeutics was once considered a difficult and impossible task. However, during the past two decades, the concept has changed and PPI drug targets have gained considerable interest from the scientific community. Some researchers have been conducting drug target studies in both wet and dry labs, hoping to find potential hot spot regions in PPIs' binding interfaces for designing therapeutic drugs. The discovery of small molecule PPI modulators by the emergence

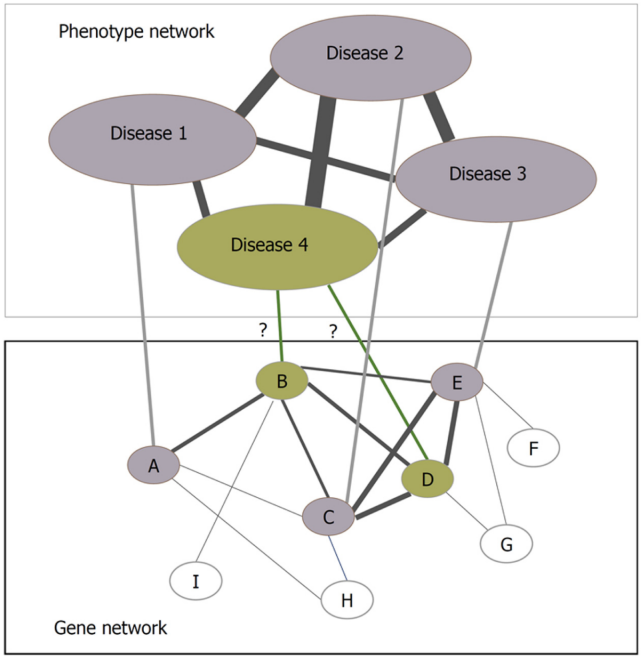

Figure 3 Protein interaction networks can help find new disease-related genes. The concept depicts that diseases 1, 2, and 3 are caused subsequently by genes A, C, and E, and the genes causing disease 4 are unknown but disease 4 is phenotypically associated with diseases 1, 2, and 3. If the known genes, *i.e.*, A, C, and E are closely associated functionally, it can be hypothesized that genes B and D are the cause of disease 4[86].

of new technologies has made the PPIs significant drug targets[72,78]. Until now, three databases have been dedicated to modulators of PPIs: (1) 2P2I database[79]; (2) TIMBAL[80]; and (3) iPPI-DB[81], and more than 40 PPIs have been targeted successfully[82]. To our knowledge, some of the druggable hotspots for well-studied PPI targets identified by various studies are: MDM2/p53, IL-2/IL-2Ra, HPV-11 E2/HPV-11 E1, TNF-α/TNFR1, and several others[83].

Currently, much focus has been diverted towards the recent COVID-19 pandemic, and many studies have been carried out to combat the deadly virus experimentally and computationally. Gordon *et al*[84] performed affinity purification-MS and identified 332 physical interactions between human proteins and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins. The study helped researchers to dig deep down into the host molecular machineries and identify potential hotspots for developing therapeutic compounds to treat COVID-19. PPI identification will also help in predicting the behavior of the virus and the biological processes targeted by the virus. Khorsand *et al*[85] developed a three-layered network model to predict SARS-CoV-2-human PPIs and reported the most central human proteins in the network by investigating host proteins that are targeted by the viral proteins.

In summary, network biology has become the focus of attention in the recent era by scientists for understanding diseases and the biological processes targeted by the disease. Interaction networks are playing a significant role in understanding virus-host relationship and drug discovery.

CONCLUSION

The study on PPIs is not just a new field, but a new era in study of virus-host relationships, and we can say that PPIs are at the core of any viral infection. Scientists can use PPIs to gain innumerable novel insights into the functional constitution of a proteome by analyzing all kinds of network parameters. Network biology can help scientists find many potential drug targets that might be involved in certain viral pathways. Many studies have used network biology to construct protein interaction networks of lethal pathogens such as HCV, IAV, dengue virus, and human papilloma virus with their host *Homo sapiens* to dig deep down into the molecular constitution of the disease pathways, and have successfully found multiple potential drug targets against the viruses. In short, the future of PPI-induced network biology is quite clear and scientists can perform plenty of useful studies against any disease or pathway.

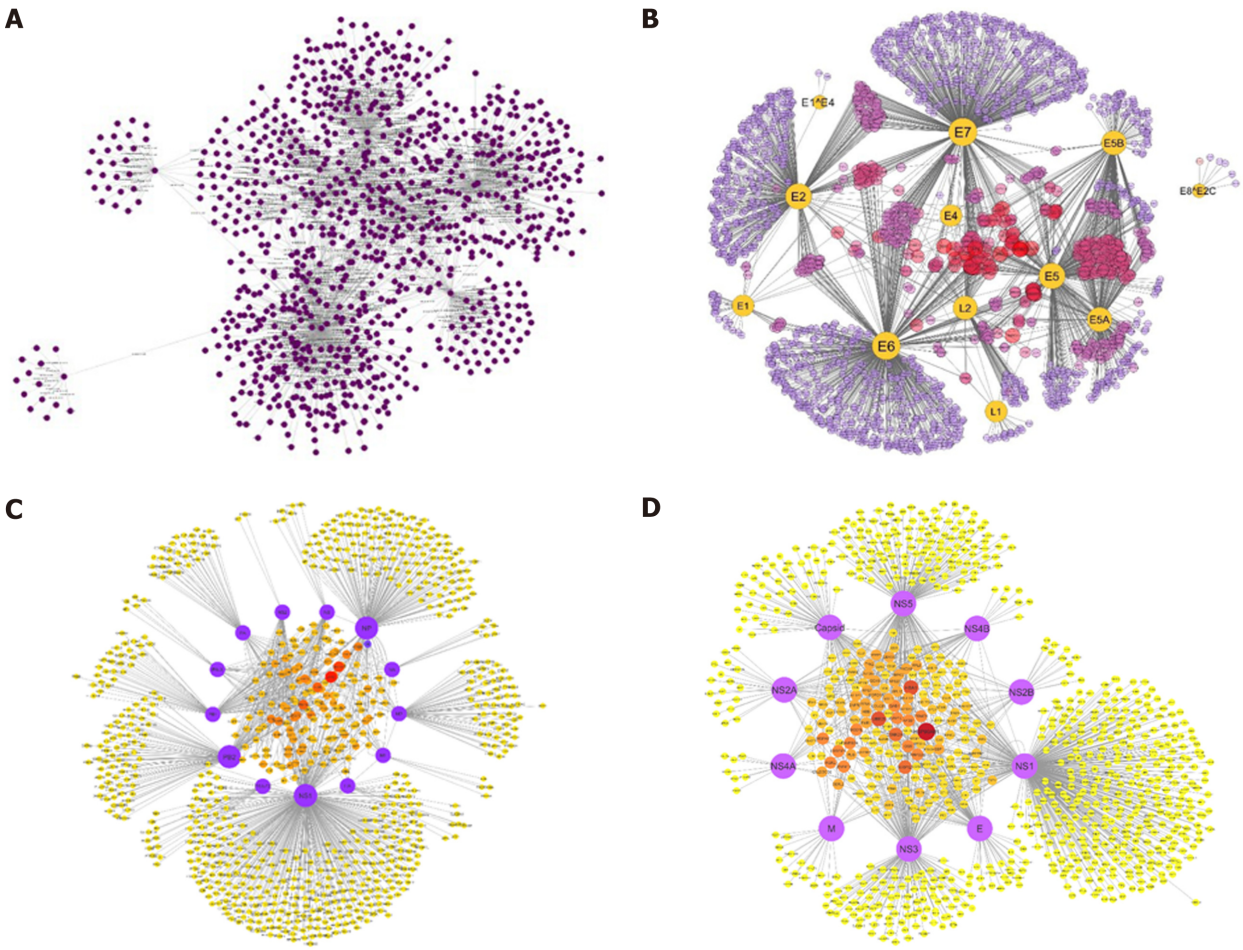

Figure 4 Comprehensive protein interaction networks of hepatitis C virus, human papillomavirus, influenza A virus, and dengue virus with host *Homo sapiens* constructed in Cytoscape by literature curated experimentally verified and computationally predicted protein-protein interactions. The network explains virus-host relationship between the infectious agents and host factors which contribute to disease pathways in human body. A: Hepatitis C virus; B: Human papillomavirus; C: Influenza A virus; D: Dengue virus.

Computational prediction of PPIs has become a mandatory tool for finding out the functionalities of unknown proteins.

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MINIREVIEWS

Impact of COVID-19 on liver disease: From the experimental to the clinic perspective

Sheila Gato, Ana Lucena-Valera, Rocío Muñoz-Hernández, José Manuel Sousa, Manuel Romero-Gómez, Javier Ampuero

ORCID number: Sheila Gato 0000-0002-4141-4897; Ana Lucena-Valera 0000-0003-1460-545X; Rocío Muñoz-Hernández 0000-0003-3765-6276; José Manuel Sousa 0000-0002-8158-273X; Manuel Romero-Gómez 0000-0001-8494-8947; Javier Ampuero 0000-0002-8332-2122.

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Sheila Gato, Rocío Muñoz-Hernández, Manuel Romero-Gómez, Javier Ampuero, SeLiver Group, Instituto de Biomedicina de Sevilla, Sevilla 41013, Spain

Ana Lucena-Valera, José Manuel Sousa, Manuel Romero-Gómez, Javier Ampuero, Digestive Department, Hospital Universitario Virgen del Rocio, Sevilla 41013, Spain

Rocío Muñoz-Hernández, Manuel Romero-Gómez, Javier Ampuero, University of Seville, Sevilla 41013, Spain

Corresponding author: Javier Ampuero, MD, MSc, PhD, Doctor, Professor, Senior Scientist, Digestive Department, Hospital Universitario Virgen del Rocio, Avda. Manuel Siurot s/n, Sevilla 41013, Spain. javi.ampuero@gmail.com

Abstract

Coronavirus disease 2019 (COVID-19) has caused a global pandemic unprecedented in over a century. Although severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a predominantly respiratory infection, various degrees of liver function abnormalities have been reported. Pre-existing liver disease in patients with SARS-CoV-2 infection has not been comprehensively evaluated in most studies, but it can critically compromise survival and trigger hepatic decompensation. The collapse of the healthcare services has negatively impacted the diagnosis, monitoring, and treatment of liver diseases in non-COVID-19 patients. In this review, we aim to discuss the impact of COVID-19 on liver disease from the experimental to the clinic perspective.

Key Words: SARS-CoV-2; COVID 19; Liver disease; Transaminases

Core Tip: The outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has posed a critical threat to global public health. Beyond the respiratory symptoms, some patients with COVID-19 show liver damage. In this scenario, it has been suggested that there might be a specific relationship between SARS-CoV-2 infection and liver injury.

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INTRODUCTION

Coronaviruses are enveloped single-stranded RNA viruses belonging to the Coronaviridae family and Orthocoronavirinae subfamily[1]. They cause zoonotic infections in humans, predominantly associated with the upper respiratory tract[2]. Two coronaviruses caused relatively recent epidemics: severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012[3].

The outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first reported in China in December 2019, has posed a critical threat to global public health[4,5]. Therefore, COVID-19 has been declared an international public health emergency by the World Health Organization (WHO). As of 21st March 2021, more than 122 million confirmed cases and over 2.7 million deaths have been reported[6] (Figure 1).

In most cases, the infection is followed by a benign course with usual characteristics of viral pneumonia, such as fever, dry cough, and lymphopenia. A relatively low percentage of patients require hospitalization and intensive care for acute respiratory failure secondary to diffuse alveolar damage. There is also an important incidence of extrapulmonary manifestations, such as acute kidney injury, cardiovascular disease, neurological disorders, or hypercoagulation[7].

On the other hand, some patients with COVID-19 show different degrees of liver injury, showing mainly elevated serum transaminase and lactate dehydrogenase levels and hypoalbuminemia[8-10]. In this scenario, it has been suggested that there might be a specific relationship between SARS-CoV-2 infection and liver injury. Thus, this article reviews the impact of COVID-19 on liver disease from the experimental to the clinic perspective.

MECHANISMS OF LIVER DAMAGE IN COVID-19

Direct cytopathic effect of SARS-CoV-2

As with SARS-CoV, angiotensin-converting enzyme 2 (ACE2) appears to be the susceptible receptor for SARS-CoV-2 and is expressed in more than 80% of lung alveolar cells. *In vitro* studies from the SARS epidemic identified ACE2 as the host receptor for viral entry[11], but in this new coronavirus, a recent study showed a 10-20-fold higher receptor binding affinity[12].

The hepatic distribution of ACE2 is quirky; it is highly expressed in the endothelial layer of small blood vessels but not in the sinusoidal endothelium. Indeed, a study revealed that the ACE2 cell surface receptor was more highly expressed in cholangiocytes (59.7%) than hepatocytes (2.6%). Both the level of ACE2 expression in cholangiocytes and lung alveolar type 2 cells are similar, indicating that the liver could be a potential target for SARS-CoV-2[13].

SARS-CoV-2 exerts a cytopathic effect by directly binding to ACE2 positive cholangiocytes. They are involved in liver physiology functions, including regeneration and adaptive immune response mechanisms; thus, their disruption can cause hepatobiliary damage. This is supported by cholestatic markers, including gamma-glutamyl transferase, which can be found in some case reports of COVID-19[14-16]. Permissiveness to SARS-CoV-2 infection was observed in a human organoid model of liver ductal organoids. In this experiment, the viral infection damaged the barrier and bile acid transporting functions of cholangiocytes through the dysregulation of genes implicated in tight junction formation and bile acid transportation, supporting the susceptibility of cholangiocytes in SARS-CoV-2 infection[17]. On the other hand, a significant increase in mitotic cells and ballooned hepatocytes was observed in liver biopsies of patients with SARS-associated coronavirus infection, suggesting that it may induce apoptosis of liver cells[18]. Moreover, the virus was detected in liver tissue, although the viral load was relatively low.

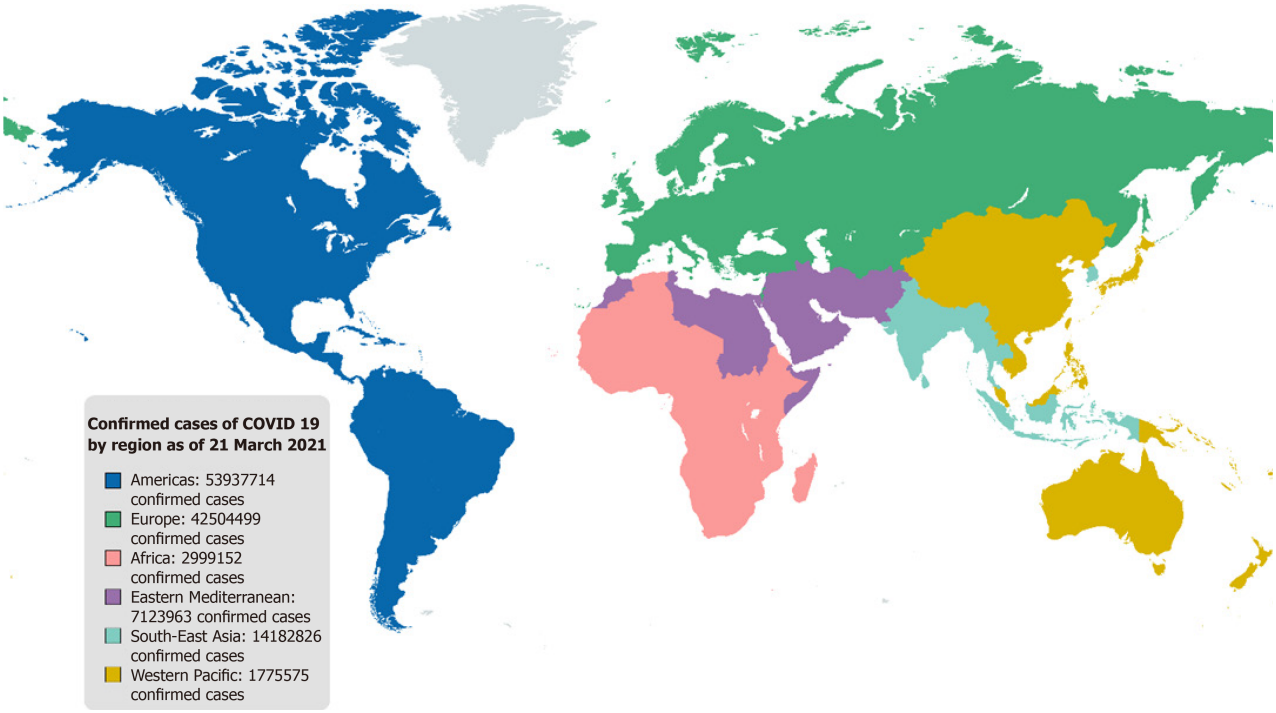

Figure 1 Coronavirus outbreak: World map of confirmed cases (updated March 21st, 2021). COVID-19: Coronavirus disease 2019.

A recent study demonstrated that SARS-CoV-specific protein 7a induces apoptosis *via* a caspase-dependent pathway in cell lines of different organs, including the liver, further confirming the supposition that SARS-CoV-2 directly affects the liver tissue [19]. Nevertheless, some authors have refuted this hypothesis since the disorder of liver function is usually mild, and there is no evidence that late-onset symptoms are associated with greater liver damage[20].

Host inflammatory response to SARS-CoV-2

As we have described previously, liver injury in patients with COVID-19 might be due to the viral infection in liver cells. However, it might also be due to other causes such as drug-induced liver injury and systemic inflammation induced by cytokine storm or pneumonia-associated hypoxia[15].

A well-established driver of liver injury is hepatic inflammation, involving the activation of innate immune cells and the release of cytokines[21] (Figure 2). A possible cause of liver injury in COVID-19 can be the dysregulation of the innate immune response. Noticeable activation of inflammatory markers, including abnormal levels of C-reactive protein (CRP), lymphocytes, neutrophils, and cytokines - particularly interleukin-6 (IL-6) - are found in patients with COVID-19[15,22-24]. In some of the available case series of COVID-19, a correlation between lymphopenia and liver injury was observed. Moreover, high levels of CRP and a low lymphocyte count were independent risk factors for liver injury. Notably, lymphopenia in COVID-19 studies was observed in 63% to 70.3% of patients, and those with lower lymphocyte counts were more susceptible to fatal outcomes[22]. These impairments have also been reported in some systemic viral infections, such as cytomegalovirus, herpes simplex virus, Epstein-Barr virus, parvovirus, and adenovirus, in which we can also observe the immune activation and inflammation caused by circulating cytokines[25]. Furthermore, some studies have reported higher serum pro-inflammatory cytokines and chemokine levels in patients with abnormal liver function than those with normal liver function[22]. Hence, these data point to a relationship between liver damage and the inflammatory response induced by SARS-CoV-2 infection.

Drug-induced liver injury

The liver is involved in the metabolism of many drugs, and some therapeutic agents used to treat SARS-CoV-2 show potential hepatotoxicity. For example, alanine transaminase (ALT) and aspartate aminotransferase (AST) elevations were reported in 4%-

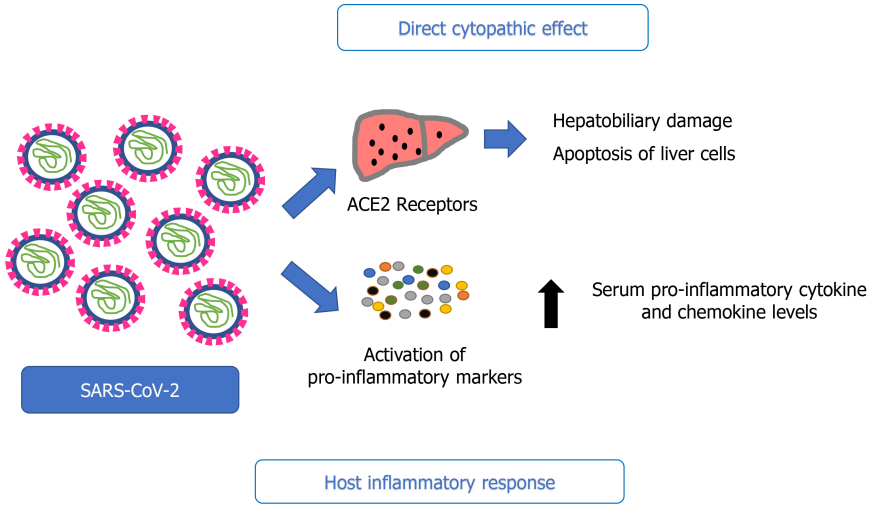

Figure 2 Proposed mechanisms of liver injury related to severe acute respiratory syndrome coronavirus 2 infection. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

6% of patients treated with remdesivir[26], and tocilizumab can also cause mild elevations in liver transaminases[27]. However, it seems unlikely that the leading cause of liver injury is the treatment as alterations in liver transaminases are usually reported at the time of hospital admission.

FREQUENCY OF LIVER IMPAIRMENT IN COVID-19

The prevalence of elevated liver enzymes occurs between 15% and 53% of patients with COVID-19[28,29]. The difference in the prevalence may be related to the exclusion of patients with a previous liver disease[30]. The most common disorder includes elevated aminotransferases (AST and ALT) up to 1-2 times the upper limit of normal, while the elevation of total bilirubin (TB) and alkaline phosphatase is less common. A recent study of 2073 patients with SARS-CoV-2 infection documented liver abnormalities in 1282 (61.8%) of these patients. This study observed liver impairment more frequently in patients with severe COVID-19. Besides, they described cholestasis and mixed types of liver abnormalities as independent variables associated with death [31]. Another recent meta-analysis that included more than 5000 patients from 26 studies also demonstrated that liver function (AST, ALT, and TB) was related to intensive care unit (ICU) admission and non-fatal severe complications[32]. The findings of these studies make us consider incorporating the liver profile to the routine inflammatory markers at the time of hospital admission in patients with SARS-CoV-2 infection to improve their management and anticipate the prognosis.

ROLE OF PRE-EXISTING CHRONIC LIVER DISEASE

Several studies have analyzed the impact of chronic liver disease on SARS-CoV-2 infection. First, the prevalence of underlying liver disease in hospitalized patients for COVID-19 ranges between 0.6% to 1.4%[33]. A recent international registry of 745 patients with chronic liver disease (CLD) and SARS-CoV-2 has observed an increased risk of major adverse outcomes and death in cirrhotic patients according to the Child-Pugh class[34]. In this study, a significant increase in ICU requirement, renal replacement therapy and rates of death according to Child Pugh class [A (19%), B (35%), C (51%)] has been observed. These findings have been demonstrated by other studies, proving an increase in complications and mortality with cirrhosis and Child Pugh score of 9 or more[35]. The mortality rate was 32%-34% for cirrhotic patients compared with CLD without cirrhosis, who had a similar risk of mortality than patients without any liver disease[36,37]. Although lung disease remained the predominant cause of death, SARS-CoV-2 infection appeared to precipitate acute hepatic decompensation in patients with cirrhosis[35,38].

The preexisting liver disease most often associated with COVID-19 is metabolic-associated fatty liver disease (MAFLD)[39]. A multicenter retrospective study by Zheng *et al*[40] demonstrated that the severity of SARS-CoV-2 infection was greater in patients with MAFLD and obesity[40,41].

There is disparity in the data on chronic hepatitis infection prevalence in COVID-19, with percentages ranging from 0.1% to more than 10% in relation to the prevalence of hepatotropic viruses in the area[42,43]. In China, a country with an intermediate-to-high prevalence of chronic hepatitis B (HBV) infection, a surprisingly low prevalence of chronic HBV in COVID-19 patients has been observed. Anugwom *et al*[44] have reported an incidence of HBV of 1.36%, while the corresponding rates of HBV ranged from 7% to 11% in patients without SARS-CoV-2. This may be explained by "immune exhaustion", as HBV infection provides an inadequate immune response during SARS-CoV-2 infection. Furthermore, chronic hepatitis infection does not appear to lead to a worse prognosis in patients with COVID-19[45]. This fact could be explained by the potential *in vitro* antiviral effect of the drugs used for chronic infection with hepatotropic viruses (inhibitors of the NS5A protein or nucleotide analogs)[46-48]. However, this has not been demonstrated in patients under active *in vivo* treatment[49,50].

Finally, the role of SARS-CoV-2 on autoimmune liver diseases has not been adequately evaluated. However, some studies have not observed a higher incidence of SARS-CoV-2 infection and severe complications than in the general population[51,52]. To date, there is no evidence to support or recommend a decrease or change in the immunosuppressive therapy in these patients.

SARS-COV-2 INFECTION AND LIVER TRANSPLANT PATIENTS

In liver transplant (LT) recipients, immunosuppression following LT may increase the likelihood of SARS-CoV-2 infection[53,54]. Once a transplant recipient is infected with SARS-CoV-2, the virus may remain to infect for a longer duration due to higher viral titers and a prolonged replication period[55]. On the other hand, immunosuppressive agents could ameliorate the systemic inflammation induced by the cytokine storm[56].

Some of the available case series in LT patients with COVID-19 show a higher hospitalization rate (40%-86.5%)[54,57-60], as well as an increase in ICU admission requirements and invasive ventilation[59,61] in these patients. Despite the fact that mortality in LT recipients by COVID-19 is approximately 20% (8%-30.6%)[54,57-60], several studies have not shown that COVID-19-related mortality could be greater in hospitalized LT patients than in the general population[59,61]. Risk factors associated with poor prognosis in LT patients with COVID-19 are older age[53,57,60,62], diabetes mellitus[57-60], chronic kidney disease[60], and liver injury (ALT > 2 times ULN)[58].

On the other hand, it has not been clearly established how the immunosuppressive treatment influences the prognosis of LT patients with COVID-19. For instance, a study showed that mycophenolate might increase the risk of severe COVID-19 in a dose-dependent manner[54], while tacrolimus use has had a positive independent effect on survival[60]. Therefore, it could be concluded that increased disease severity and mortality in LT patients with COVID-19 is caused by the higher prevalence associated with comorbidities than by the effect of immunosuppressive treatment. In fact, in LT recipients without COVID-19, international guidelines recommend against reducing immunosuppression. However, in patients diagnosed with COVID-19, a reduction of immunosuppression should be considered.

IMPACT OF THE PANDEMIC IN THE HEPATOLOGY UNITS

Since the beginning of the SARS-CoV-2 pandemic, the healthcare system has supported a substantial impact, and the hepatology units have suffered notable changes in the organization. The access to medical consultations has been limited due to the hospital overload and strict orders to stay at home, the resources and staff reallocation have caused a decrease in the care of non-COVID pathologies. After a year of pandemic, the epidemiology of COVID-19 has proven to be unpredictable, however, it is urgent to anticipate and plan to mitigate the consequences of the pandemic and achieve a dynamic balance of resources.

Screening of hepatocellular carcinoma

The prevalence of hepatocellular carcinoma (HCC) has increased globally in the last

few years. Significant efforts have been made to decrease HCC-related mortality. For this reason, HCC screening using imaging tests at regular intervals has been implemented and standardized, and is strongly recommended by the international clinical guidelines[63,64].

A recent retrospective study comprising 127 hospitals showed a significant diminution of HCC control during the pandemic, showing screening rates below 50% compared to 2019[65]. Other studies have also found similar results with a decreased HCC surveillance by ultrasound and, more important than this, a decrease in diagnostic tests such as computed tomography or magnetic resonance imaging[66]. Thus, a significant increase in HCC-related mortality could be observed in the next months.

On the other hand, the COVID-19 pandemic has also impacted the management of HCC patients. A recent French multicenter study of 670 patients described a significant decrease in the rate of patients with HCC referred for specific treatment. The rate of patients with a treatment delay of more than one month was higher in 2020 compared to 2019 (21.5% *vs* 9.5%, $P < 0.001$)[67].

Screening of hepatitis C virus

There were 1.7 million incident cases and 400000 deaths attributable to hepatitis C virus (HCV) in 2015; thus, this viral hepatitis has been recognized as a major cause of death[68]. A breakthrough in HCV treatment occurred in 2013 with the introduction of direct-acting antivirals. For this reason, the WHO approved some ambitious aims to eliminate HCV by 2030, including the reduction of new HCV cases by 80% and HCV-related deaths by 65% for 2030.

The pandemic has caused a slowing or even the halt of HCV elimination programs. The impact of COVID-19 on viral hepatitis in a recent survey has shown that only 47 (36%) of 132 responders could access viral hepatitis testing, and 28 people on treatment for hepatitis were unable to access their medication at this time[69]. Although the real impact is far from being seen, different studies have been carried out to measure the future consequences. Blach *et al*[70] using a previously validated Markov model, compared a "no delay" *vs* "one-year delay" scenario in elimination programs and evaluated changes in HCV liver-related deaths and liver cancer. Over the next ten years, the authors estimated that a single-year delay scenario could result in over 72300 liver-related deaths and 44800 excess cases of HCC[71].

To avoid the delay in HCV elimination programs, integrated circuits for massive and combined HCV, HBV, and SARS-CoV-2 diagnosis have been proposed[72]. Giacomelli *et al*[73] have developed a screening program using rapid immunochromatographic testing (RICT) for SARS-CoV-2 antibodies and a rapid HCV test in a single visit in three Italian cities. The results demonstrated that 2.9% of the tests were positive for HCV antibodies, and 54% of them did not know their serological status.

LT programs

During the SARS-CoV-2 pandemic, there has been an initial worldwide decline in the number of LTs for several reasons. Firstly, there has been a drastic decrease in liver donors, as well as in the availability of ICU beds for both donors and recipients. Secondly, testing organ donors for the presence of the virus is recommended, and those that are positive should be ineligible for donation. Thirdly, the evaluation of potential candidates for LT has been temporarily limited due to the lower availability of hospital resources, as well as to prevent exposure to SARS-CoV-2 in patients with advanced CLD. Finally, at the beginning of the pandemic, there was a temporary decrease in LT recommendations for patients at greater risk of worsening and mortality due to transplant delay: patients with acute liver failure, high MELD score, and HCC at upper limits of the Milan criteria[74-76].

In the United States (US), the impact on LT between March and August 2020 was evaluated using historical trends between 2016 and 2020. Within the first ten weeks of the pandemic, a dramatic decrease in new listings for LT (11%-21%), deceased donor LT (9%-13%), and living donor LT (42%-49%) was found. Besides, there was a reduction of 59% in patients included in the waiting list for LT. Despite these initial data, the mortality risk of LT waitlist candidates was not significantly different before and after COVID-19[77]. On the other hand, a national survey conducted in the US between March 24th and 31st 2020 showed that 67.7% of LT centers had stopped performing live donor LT[78]. A similar evolution in LT was observed in Italy. Considering the period of the first outbreak (March 1st–March 31st), a decrease of around 35% in LT was recorded due to the decrease in the number of donations[79]. In France, there was a 28% decrease in the number of organ donations in 2020 (543 in 2020 *vs* 752 organ donations in 2019) and a 22% decrease in the number of liver

transplantations (435 in 2020 *vs* 556 in 2019)[80], comparing two similar periods (January 1st-May 31st 2019 *vs*. January 1st-May 31st 2020). In Spain, during the first COVID-19 wave (between March 13th-April 23rd), the mean number of donors decreased from 7.2 to 1.2 per day, and the weekly mean number of LTs decreased from 23.6 to 5.7[81]. Throughout the year 2020, the number of donors and LTs reduced by 22.8% and 15.7% (1034 *vs* 1227), respectively, compared to 2019[82].

CONCLUSION

It is accepted that SARS-CoV-2 infection can cause liver damage, representing a relevant outcome that affects the prognosis of COVID-19. A direct pathogenic effect on the liver, systemic inflammation, and immune dysfunction appear to play a relevant role in this association. In this scenario, liver function tests such as AST, ALT, and bilirubin levels at admission have been related to a poor COVID-19-related prognosis, including more ICU admission requirements and deaths. Finally, we must pay attention to maintaining an adequate monitoring and follow-up of patients with liver diseases, focusing on the risk of cirrhosis decompensation and HCC screening.

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MINIREVIEWS

COVID-19 (SARS-CoV-2 infection) in lymphoma patients: A review

Valentina Bonuomo, Isacco Ferrarini, Michele Dell'Eva, Eugenio Sbisà, Mauro Krampera, Carlo Visco

ORCID number: Valentina Bonuomo 0000-0001-6491-8337; Isacco Ferrarini 0000-0001-9867-8335; Michele Dell'Eva 0000-0003-4965-3216; Eugenio Sbisà 0000-0003-2762-2208; Mauro Krampera 0000-0002-7280-2040; Carlo Visco 0000-0003-2863-0883.

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Valentina Bonuomo, Isacco Ferrarini, Michele Dell'Eva, Eugenio Sbisà, Mauro Krampera, Carlo Visco, Section of Haematology, Department of Medicine, University of Verona, Verona 37134, Italy

Corresponding author: Carlo Visco, MD, Professor, Section of Haematology, Department of Medicine, University of Verona, P. Le L.A. Scuro 10, Verona 37134, Italy. carlo.visco@univr.it

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection now has a global resonance and represents a major threat for several patient populations. Observations from initial case series suggested that cancer patients in general might have an unfavorable outcome following coronavirus disease 2019 (COVID-19), due to their underlying conditions and cytotoxic treatments. More recently, data regarding the incidence and clinical evolution of COVID-19 in lymphomas have been reported with the aim to identify those more frequently associated with severe complications and death. Patients with lymphoma appear particularly vulnerable to SARS-CoV-2 infection, only partly because of the detrimental effects of the anti-neoplastic regimens (chemotherapy, pathway inhibitors, monoclonal antibodies) on the immune system. Here, we systematically reviewed the current literature on COVID-19 in adult patients with lymphoma, with particular emphasis on disease course and prognostic factors. We also highlighted the potential differences in COVID-19 clinical picture according to lymphoma subtype, delivered treatment for the hematological disease and its relationship on how these patients have been managed thus far.

Key Words: Lymphoma; SARS-CoV-2 infection; Hematological malignancies; COVID-19; Rituximab; Bendamustine

Core Tip: Recently, the scientific literature has been widely occupied by reports on severe acute respiratory syndrome coronavirus 2 infection. However, patients with cancer have been under-represented, and patients with lymphoma have rarely been described. The real impact of this tremendous pandemic on the life expectancy of patients with different subtypes of lymphoma is still unknown, especially in relation to chemo-, chemo-immunotherapy and/or biologic treatments. Furthermore, the

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relationship between lymphoma patients' characteristics and the infection behavior is undescribed. With this review we pointed out what literature clarifies in the prognosis and management of patients with lymphoma during the coronavirus disease 2019 pandemic.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is a worldwide medical emergency impacting virtually all aspects of medical care. The clinical spectrum of individuals who are infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is remarkably heterogeneous, ranging from mild flu-like symptoms to life-threatening respiratory failure[1]. Mortality due to the infection is largely dependent on patients age, and the infection fatality ratio is lowest among 5–9-year-old children, with a log-linear increase by age among individuals older than 30 years. Estimated age-specific infection fatality ratios range from 0.001% in those aged 5–9-years-old to 8.29% in those aged 80+. Population age structures, heterogeneous inclusion criteria in terms of comorbidities and burdens in nursing explain some of the heterogeneity between countries in infection fatality ratio[2]. The leading cause of mortality is the acute respiratory distress syndrome. Indeed, after infecting the pneumocytes, SARS-CoV-2 triggers intracellular signaling pathways that promote the release of several proinflammatory mediators, leading to the recruitment of neutrophil and monocyte-macrophages[3-5].

Subgroups of patients with COVID-19 have been identified to be at increased risk of morbidity and mortality, including patients of older age, male sex (*vs* female) and those with comorbidities, such as hypertension, chronic lung disease, diabetes, immunodeficiency and cancer[6]. In particular, cancer patients often follow a more severe and rapid disease course, with requirement of high-level intensive care and an increased risk of COVID-19-related death[7-10]. In the first published report from the COVID-19 and Cancer Consortium, mortality among 928 analyzed adult patients with any malignancy was 13%, with 23% mortality for any admission to the hospital and 38% mortality for admission to the intensive care unit (ICU)[11]. Among 800 patients with cancer included in the United Kingdom Coronavirus Cancer Monitoring Project, reported mortality in the overall cohort was 28%[12]. A multicenter study in China of 205 patients with cancer reported mortality of 20%[13]. In the latter series, 22 patients with hematologic malignancies (HM) were included and had a mortality rate of 41%. In cancer series, hematologic patients account for 20%-25% of the total including a variable distribution of pathologies. Heterogeneous series addressing SARS-CoV-2 infection in patients with HM have been published, reflecting mortality rates ranging from 30% to 40%; however, these reports offer limited information on the characteristics of the various hematological diseases and their relationship with anticancer treatments. Patients with HM are immunocompromised, which makes them highly susceptible to severe infections. On the other hand, some authors have suggested that some patients with HM might be "protected" from severe COVID-19 morbidity due to an attenuated inflammatory response. In this review, we synthesized the current literature to illustrate the demographic, immunological and clinical features of COVID-19 infection in the specific setting of patients affected by lymphoma, a heterogeneous group of cancers arising from B or T lymphocytes and often associated with various degrees of immune dysfunction.

Lymphoma patients are at high risk of infections: patients with HM (and lymphomas) tend to carry more comorbidities than age and sex matched population, have more frequent contacts with medical systems and are often treated with immunosuppressive medications potentially blunting the antiviral immune responses. Hematologic malignancies affect the production and function of blood cells in fighting off infections[14]. Affected patients often have multiple immune dysfunctions of the innate and adaptive immune system including low immunoglobulin G serum levels (

i.e. chronic lymphocytic leukemia or other B cell neoplasms) or functionally impaired granulocytes (*i.e.* myeloid neoplasms)[15,16]. Crippled cellular and humoral immunity places these patients at risk of a diverse array of infections including COVID-19[17].

Lymphomas are a heterogeneous group of cancers broadly divided into two main histological subtypes: Hodgkin lymphoma (HL) and non-HL (NHL). HL tends to spread in a fairly orderly way from one group of lymph nodes to the next group and it affects young adults aged 20–40 years more frequently, while NHL can spread to extra nodal organs, bone marrow and spleen. The World Health Organization has recognized several forms of NHL, with diffuse large B-cell lymphoma being the most common subtype in adults[18].

Chemotherapy treatment combined with rituximab (widely available immunotherapy against B-lymphocytes) is the current standard upfront treatment for most histologies[19]. Together with lymphodepleting therapies, several intrinsic factors contribute to the typical immunosuppressive status of patients with lymphoma. Among them hypogammaglobulinemia, neutropenia and lymphopenia (both B- and T-cell related) are frequently observed features at disease presentation[20,21]. Furthermore, lymphomas are more likely to develop in patients with underlying immunosuppressive conditions, such as the human immunodeficiency virus infection, rheumatological chronic disorders, autoimmune disease or inherited congenital immune-deficiency states[22]. Lymphoma therapy has historically been based on chemotherapy variably associated with immunotherapy and radiotherapy. Moreover, in recent years, the approval of new molecules with different mechanisms of action (monoclonal antibody, small molecules, biologic agents, cell therapy) has allowed us to expand the therapeutic arsenal available for the treatment of these diseases. Among chemotherapy regimens, bendamustine is a strong inducer of T-cell immune deficiency[23]. Anti-CD20 monoclonal antibodies, such as rituximab or obinutuzumab, induce rapid depletion of more than 95% of CD20-positive mature B-cells, impairing cellular and humoral response towards new pathogens[24-26].

LITERATURE REVIEW

A review of the literature reporting on SARS-CoV-2 infection in lymphoma patients was conducted. In particular, we focused on the relationship with lymphoma characteristics and the clinical course of COVID-19 infection. An electronic search was performed to identify all studies reporting on the management of lymphoma patients during the SARS-CoV-2 pandemic. The PubMed/MEDLINE database was searched on February 6th, 2021. The search strategy was "SARS-CoV-2" OR "COVID-19" AND "lymphoma." Potential case duplicates were ruled out by analysis of demographic characteristics of the included patients and institution of origin of the reports.

PREVALENCE OF CANCER AND HM AMONG SARS-COV-2 INFECTED PEOPLE

Human infections with SARS-CoV-2 were first reported in late 2019. At the end of February 2021, the global cumulative numbers were 110.7 million cases and over 2.4 million deaths since the start of the pandemic[27]. The prevalence of cancer in patients with COVID-19 is uncertain. Studies from China reported that 1% to 2% of COVID-19 patients had cancer, and a study from the United States reported that 6% of hospitalized patients with COVID-19 had cancer. In Lombardy, Italy, they observed that 8% of the patients admitted to the ICU for COVID-19 had cancer. In a meta-analysis, the prevalence of cancer was 2% among COVID- 19 patients[28].

Reports about the prevalence of HM among COVID-19 patients are very limited. In a study from Turkey[29], 0.39% of the laboratory-confirmed COVID-19 patients had underlying blood cancer. Patients with HM were reported to be at increased risk for developing COVID-19 as compared to general population, after adjusting for age, gender, race and known COVID-19 risk factors. It has been reported that patients with cancer with different tumor types have differing susceptibility to SARS-CoV-2 infection and COVID-19 phenotypes[30]. Individualized risk tables have been generated for patients with cancer, considering age, sex and tumor subtype, reporting an increased susceptibility to SARS-CoV-2 in patients with HM.

CLINICAL MANAGEMENT AND FATALITY RATES OF PATIENTS WITH COVID-19 AND HM (INCLUDING LYMPHOMAS)

Among papers investigating the characteristics of COVID-19 infection in cancer patients, only some stratified the population by type of malignancy (reported in Table 1). He *et al*[31] conducted a cohort study at two centers in Wuhan, China, involving 128 hospitalized subjects with HM, 13 (10%) of whom developed COVID-19. There were no significant differences in baseline covariates between subjects with HM developing COVID-19 or not. Case rates for COVID-19 were similar between the two groups, but hospitalized subjects with HM were reported to suffer from more severe disease and higher case fatality rate (CFR). In a study conducted by Mehta *et al*[32] the CFR in COVID-19 patients with HM was 37%. A study from Spain[33] reported a CFR of 32% among 34 hospitalized COVID-19 patients with HM. Authors concluded that the status of underlying malignancy at the time of COVID-19 correlated with mortality, with disease activity that was directly associated with worse outcomes. Aries *et al*[34] reported a CFR as high as 40% in a small cohort including 35 patients with HM. In a study conducted by Yang *et al*[13] among 52 COVID-19 patients with solid tumors or HM, the rate of severe/critical disease was 36.5% and CFR of severe/critical patients was 57.8%. Wood *et al*[35] described 250 cases of patients with HM and COVID-19 that were enrolled into the ASH Research Collaborative COVID-19 Registry. Consistent with previous reports, patients with HM had poor outcomes, with an overall mortality rate of 28%, which increased to 42% for those patients requiring hospital-level care.

In Rüthrich *et al*[36] retrospective analysis of LEOSS study a total of 435 cancer patients with SARS-CoV-2 were included. The majority of patients were hospitalized (98%). Lymphoma and leukemia were documented for 76 (17.5%) and 48 (11%) patients, respectively. The commonest HM was NHL (16.5%). In solid tumors and HM, mortality appeared somewhat comparable, but HM were overrepresented compared to a non-COVID-19 cancer cohort from the United Kingdom, reporting a prevalence of 9.5%[30].

In the study by Passamonti *et al*[37], 536 HM patients were described. A high frequency of severe infections was reported: dyspnea occurred in 51% of patients and fever in 75% of patients. This was also evidenced by the high proportion (18%) of patients admitted to the ICU and the high number of deaths (198, 37%). Mortality of patients with HM and COVID-19 was nearly four times higher than that of the general population with COVID-19.

Similar conclusions have been reached by the Turkish study conducted by Yigenoglu *et al*[29] where COVID-19 patients with HM (n = 740) and an age, sex and comorbidity-matched cohort of COVID-19 patients without cancer (n = 740) were enrolled. NHL (30.1%), myelodysplastic syndrome (19.7%) and myeloproliferative neoplasm (15.7%) were the most common HM. The rates of severe and critical disease, hospital and ICU admission and mechanical ventilation support were significantly higher in patients with HM compared with patients without cancer. The length of hospital stay and ICU stay was similar between groups. The CFR was 13.8% in patients with HM and 6.8% in the control group. The lower CFR in this study compared with the other studies may be attributed to a high number of myeloproliferative neoplasm patients who were thought to be less immunocompromised compared with leukemia, multiple myeloma or lymphoma patients. Interestingly, they described higher use of antiviral drugs such as lopinavir/ritonavir in patients with HM.

Finally, recipients of autologous and allogeneic stem cell transplantation (HSCT) who develop COVID-19 have also been reported to have poor survival rates. The Center for International Blood and Marrow Transplant Research reported 318 HSCT recipients diagnosed with COVID-19. Disease severity was mild in 155 (49%) of 318 patients, while severe disease requiring mechanical ventilation occurred in 45 (14%), *i.e.* 28 (15%) of 184 allogeneic HSCT recipients and 17 (13%) of 134 autologous HSCT recipients. At 30 d after COVID-19 diagnosis, overall survival was 68% (95% confidence interval: 58%–77%) for recipients of allogeneic HSCT and 67% (55-78) for recipients of autologous HSCT[38]. Age 50 years or older, male sex and development of COVID-19 within 12 mo of transplantation were associated with a higher risk of mortality among allogeneic HSCT recipients.

When cancer patients are compared with control groups it appeared evident that the cancer itself constituted an independent prognostic factor in the case of COVID-19 infection. Studies investigating clinical factors associated with worse outcome in HM are summarized in Table 2.

Table 1 Characteristics of included studies

Ref.	Location	Type of malignancy included	Duration of study	Total No. of pts with HM included	Matched COVID-19 control	No. of lymphoma pts	No. of NHL pts	No. of HL pts	Mortality rate attributed to COVID-19 (Global)	Mortality rate attributed to COVID-19 (Lymphoma)	Mortality rate attributed to COVID-19 (NHL)	Mortality rate attributed to COVID-19 (HL)
Cancer studies including lymphoma pts												
Rüthrich *et al*[36], 2020	Europe	All	5 mo	435	2636	76	71	5	96/435 (22%)	20/76 (26%)	NR	NR
Lee *et al* [12], 2020	UK	All	1 mo	1044	282878	79	NR	NR	319/1044 (31%)	25/79 (31%)	NR	NR
Tian *et al* [50], 2020	China	All	9 wk	232	519	6	6	0	46/232 (20%)	2/6 (33%)	2/6 (33%)	NR
HM studies including lymphoma pts												
Aries *et al* [34], 2020	UK	HM	2 mo	35	No	8	8	0	14/35 (40%)	NR	NR	/
Biernat *et al*[51], 2020	Poland	HM	1 mo	10	No	3	3	0	7/10 (70%)	NR	NR	/
Booth *et al* [52], 2020	UK	HM	2 mo	66	No	15	15	0	34/66 (52%)	6/15 (40%)	6/15 (40%)	/
Cattaneo *et al*[42], 2021	Italy	HM	1 mo	102	101	42	40	2	40/102 (39%)	17/42 (40%)	16/40 (40%)	1/2 (50%)
Fox *et al* [53], 2020	UK	HM	1 mo	55	No	17	17	0	19/55 (35%)	7/17 (41%)	7/17 (41%)	/
Garcìa-Suàrez *et al* [54], 2020	Spain	HM	8 wk	697	No	220	187	33	230/697 (33%)	68/220 (31%)	59/187 (32%)	9/33 (27%)
Infante *et al*[55], 2020	Spain	HM	1 mo	41	No	15	14	1	15/41 (37%)	NR	NR	NR
Lattenist *et al*[56], 2021	Belgium	HM	2 mo	12	No	2	2	0	6/12 (50%)	2/2 (100%)	2/2 (100%)	/
Malard *et al*[57], 2020	France	HM	1 mo	25	No	7	7	0	10/25 (40%)	0/7 (0%)	0/7 (0%)	/
Martín-Moro *et al* [33], 2020	Spain	HM	5 wk	34	No	6	5	1	11/34 (32%)	0/6 (0%)	0/5 (0%)	0/1 (0%)
Mehta *et al* [32], 2020	USA	HM	3 wk	54	No	20	15	5	20/54 (37%)	8/20 (40%)	5/15 (33%)	3/5 (60%)
Passamonti *et al*[37], 2020	Italy	HM	12 wk	536	No	170	153	17	198/536 (37%)	65/170 (38%)	62/153 (40%)	3/17 (18%)
Sanchez-Pina *et al* [58], 2020	Spain	HM	1 mo	39	53	12	NR	NR	14/39 (36%)	2/12 (14%)	NR	NR
van Doesum *et al*[59], 2020	Europe	HM	9 wk	59	No	17	15	2	NR	NR	NR	NR
Yigenoglu *et al*[29], 2021	Turkey	HM	15 wk	740	188897	250	223	27	103/740 (14%)	28/250 (11%)	24/223 (11%)	4/27 (14%)
Wood *et al* [35], 2020	Worldwide	HM	3 mo	250	No	79	68	11	70/250 (28%)	20/79 (25%)	16/68 (24%)	4/11 (36%)
Lymphoma studies												
Regalado-Artamendi	Spain	Lymphoma	12 wk	177	No	177	158	9	61/177 (29%)	61/177 (29%)	NR	NR

et al[40], 2021												
Lamure *et al*[39], 2020	France	Lymphoma	8 wk	89	No	89	84	5	30/85 (34%)	30/85 (35%)	29/84 (34%)	1/5 (20%)
Laurenge *et al*[60], 2021	France	PCNSL	2 mo	13	No	13	13	/	3/13 (23%)	3/13 (23%)	3/13 (23%)	

COVID-19: Coronavirus disease 2019; HM: Hematologic malignancy; HL: Hodgkin lymphoma; NHL: Non-Hodgkin lymphoma; NR: Not reported; PCNSL: Primary central nervous system lymphoma; pts: Patients; UK: United Kingdom; USA: United States of America.

Table 2 Prognostic factors associated with survival in lymphoma series

Ref.	Details on study cohort	Univariate analysis for predictors of death	Multivariate analysis for predictors of death
Regalado-Artamendi *et al*[40], 2021	Lymphoma patients	Age ≥ 70 yr	Age ≥ 70 yr
		Comorbidities	Comorbidities
		CURB65 ≥ 3	CURB ≥ 2
		Low platelet count	Active disease
		Low hemoglobin level	
		High D-dimer	
		C-reactive protein >10 mg/dL	
		LDH > 300 U/L	
		Active disease[1] (reference to CR)	
		DLBCL histology (reference to FL)	
		High-risk lymphoma[2] (reference to low risk)	
Lamure *et al*[39], 2020	Hospitalized lymphoma patients	Age ≥ 70 yr	Age ≥ 70 yr
		Hypertension	Active disease
		Previous cancer	
		Bendamustine treatment	
		Active disease	

[1]Partial response or progression.

[2]High risk according to prognostic index at diagnosis. CR: Complete response; CURB65: Confusion, urea concentration, respiratory rate, blood pressure and age > 65; DLBCL: Diffuse large B cell lymphoma; FL: Follicular lymphoma; LDH: Lactate dehydrogenase.

LYMPHOMA SERIES AND CASE REPORTS, CLINICAL FEATURES AND FATALITY RATES

Lymphoma patients represented a small proportion of the entire cancer series, also reflecting the relative prevalence of this disease compared to solid tumors. Figure 1 resumed the number of lymphoma patients described all over the world in the largest HM studies. However, subset data from and disease-specific cohorts are emerging. Two recently published series focused specifically on patients with lymphoma. The first report was from France where Lamure *et al*[39] described clinical characteristics and outcomes of 89 adult patients with lymphoma hospitalized for COVID-19 in 12 hospitals during the first pandemic wave. Overall, reported 1 mo overall survival was 71%. The most common symptoms at presentation were dyspnea (65%), cough (60%), fever (48%) and diarrhea (24%). The median duration of symptoms before admission was 6 d. Lymphopenia was observed in 66% of patients. During hospitalization, 25 patients (28%) were admitted to the ICU. This CFR was documented despite a significant fraction of patients had received the best available cures against SARS-CoV-

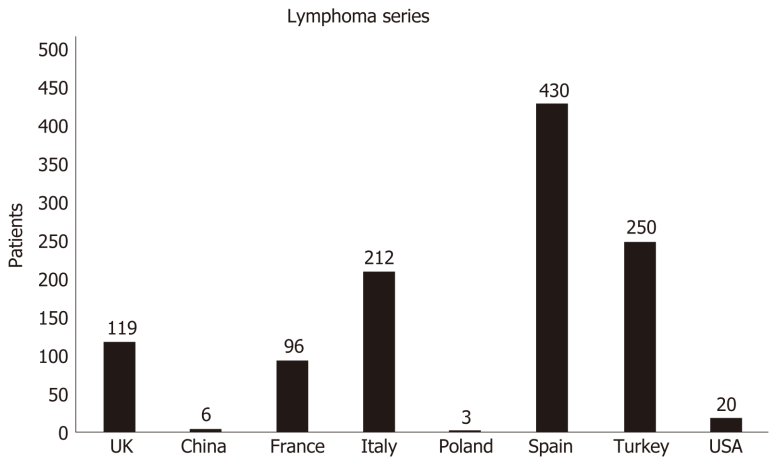

Figure 1 Number of lymphoma patients described all over the world in largest hematologic malignancy studies. UK: United Kingdom; USA: United States of America.

2: chloroquine and hydroxychloroquine (11 patients) or antiviral drugs combinations (10 patients). Six patients had received treatment for cytokine shock (tocilizumab, anakinra and eculizumab for two patients each). Seventeen patients (19%) developed a documented coinfection and three an (3%) acute pulmonary embolism.

The second series from Regalado-Artamendi *et al*[40] collected 177 cases affected by COVID-19 in Spain. The median incubation time was again 5 d, with fever and cough as the most frequent symptoms at presentation; the presence of dyspnea at presentation was related to CFR. More than 85% of patients required hospital admission, with 9% admitted to the ICU and an overall mortality rate of 34.5%.

Numerous case reports of patients affected by lymphoma and COVID-19 have been reported and summarized in Table 3. These cases have been published over the last 12 mo, witnessing the widespread interest of the scientific community and the difficulties encountered in the management of these patients. Several lymphoma histotypes are described, with disparate outcomes.

PROGNOSTIC FACTORS ASSOCIATED WITH SURVIVAL IN PATIENTS WITH LYMPHOMA

As previously mentioned, in most of the cancer series including HM, male sex, active disease and advanced age were associated with higher CFR attributed to COVID-19 [30,36,41,42]. Passamonti *et al*[37] observed that overall survival in patients affected by HM and COVID-19 was independently predicted by age, type of malignancy, disease status and the severity of COVID-19. NHL (with no mention of histological subtype), acute myeloid leukemia and plasma cell neoplasms, together with progressive disease status, were independently predictive of poor outcomes. Among patients with NHLs, 4 (31%) of 13 patients on rituximab maintenance, 27 (47%) of 57 on active treatment with rituximab–chemotherapy and 8 (44%) of 18 on chemotherapy alone died. No association between overall survival and time since HM diagnosis or last treatment was described. In Lamure *et al*[39] series from France, which specifically focused on hospital admitted lymphoma patients with a median follow-up of 33 d from admission, 30 d overall survival was 71%, (95% confidence interval: 62%-81%). Factors independently associated with death were advanced age (> 70 years) and relapsed/refractory lymphoma. Interestingly, treatment with bendamustine (n = 9) was associated with a higher risk of death. No significant difference in the rate of death was described for patients with different lymphoma histology.

In the Regalado-Artamendi *et al*[40] series from Spain, also specifically addressing lymphoma patients, the overall mortality rate was 34.5%. Age > 70 years, heart disease, chronic kidney disease and confusion, urea concentration, respiratory rate, blood pressure and age > 65 score ≥ 2 were statistically significant mortality predictors, resembling previous reports in cancer patients. Among the variables related to lymphoma, the presence of active disease was a strong predictor of death. However, active treatment, the number of previous lines or type of treatment did not modify mortality risk. Quite surprisingly but confirming previous reports, the use of

Table 3 Case reports and case series of coronavirus disease 2019 infection in lymphoma patients

Ref.	No. of patients described	Sex	Age	Details on lymphoma diagnosis	Details on lymphoma treatment	Outcome of COVID-19 infection	Global outcome
Li *et al*[61], 2020	1	M	26 yr	PMLBCL	R-DA-EPOCH	Recovered	Alive
Tepasse *et al*[62], 2020	2	M	65 yr	DLBCL with CNS relapse	R-DeVIC	Not recovered	Dead
		M	66 yr	MCL in CR	Rituximab maintenance	Not recovered	Dead
O'Kelly *et al*[63], 2020	1			cHL second relapse	Pembrolizumab	Recovered	Alive
Baang *et al*[64], 2021	1	M	60 yr	Relapsed/Refractory MCL	R-CHOP	Recovered	Alive
Moore *et al*[65], 2020	1	F	63 yr	NHL	Obinotuzumab maintenance	Recovered	Alive
Alsuliman *et al* [66], 2020	2	M	71 yr	MCL relapsed	Ibrutinib	Recovered	Alive
		M	NR	MCL relapsed	Ibrutinib	Recovered	Alive
Hoffmann *et al* [67], 2021	3	F	68 yr	DLBCL, FL	R-CHOP	Recovered	Alive
		M	60 yr	DLBCL, FL	R-ICE	Not recovered	Dead
		M	75 yr	DLBCL	R-CHOP	Not recovered	Dead
Yonal-hindilerden *et al*[68], 2021	1	F	55 yr	Relapsed/Refractory cHL	Brentuximab	Not recovered	Dead
Fujii *et al*[69], 2021	1	M	43 yr	cHL	A + AVD	Recovered	Alive
Kamel, 2021	1	M	58 yr	ALCL	None	Not recovered	Dead
Santana *et al*[70], 2021	1	F	47 yr	FL	Rituximab maintenance	Recovered	Alive
Velier *et al*[71], 2021	1	F	61 yr	WM	None	Recovered	Dead
Pelcovits *et al*[72], 2021	1	M	43 yr	High Grade B Cell Lymphoma, NOS	R-CODOX-M/IVAC	Recovered	Alive
Otsuka *et al*[73], 2020	1	M	56 yr	MCL	R-hyper CVAD/MA	Not recovered	Dead

A + AVD: Brentuximab vedotin, dacarbazine, doxorubicin, vinblastine; ALCL: Anaplastic large-cell lymphoma; cHL: Classic Hodgkin lymphoma; CNS: Cerebral nervous system; COVID-19: Coronavirus disease 2019; CR: Complete remission; DLBCL: Diffuse large B-cell lymphoma; F: Female; FL: Follicular lymphoma; M: Male; MCL: Mantle cell lymphoma; NHL: Non-Hodgkin lymphoma; NOS: Not otherwise specified; PMLBCL: Primary mediastinal large B-cell lymphoma; R-CHOP: Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; R-CODOX-M/IVAC: Rituximab, cyclophosphamide, vincristine, doxorubicin and methotrexate alternating with ifosfamide, etoposide and cytarabine; R-DA-EPOCH: Dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab; R-DeVIC: Rituximab, dexamethasone, etoposide, ifosfamide carboplatin; R-hyper CVAD/MA: Rituximab/cyclophosphamide/vincristine sulfate/doxorubicin and hydrochloride/dexamethasone/methotrexate/cytarabine; R-ICE: Rituximab, ifosfamide, carboplatin and etoposide; WM: Waldenstrom macroglobulinemia.

monoclonal antibodies (*i.e.* rituximab) was not associated with impaired survival for lymphoma patients. The detrimental effect of therapy based on bendamustine was not independently confirmed in this study.

A subanalysis in regard to lymphoma histology observed that aggressive tumors (*i.e.* diffuse large B-cell lymphoma) were associated with significantly worse overall survival compared with indolent forms (*i.e.* follicular lymphoma; 50% *vs* 80%, P = 0.0028). However, the study was not able to demonstrate clear differences between the various lymphoma histologies and therapeutic schemes; these variables were grouped into categories that could have limited the statistical power of this subanalysis. Finally, the persistence of SARS-CoV-2-positive PCR after week 6 was significantly associated with mortality. In the previously cited series describing the outcome of transplanted patients, the subgroup of patients with lymphoma (among other HM) was associated with a higher risk of death compared with plasma cell disorder or myeloma in

autologous HSCT recipients[38].

REPORTS OF SPONTANEOUS REMISSIONS IN PATIENTS WITH LYMPHOMAS

Few cases along the literature indicate that some patients may benefit of lymphoma remission when infected by COVID-19. In one case, a dramatic transient reduction in plasmatic Epstein–Barr virus (EBV)-DNA viral copies during COVID-19 pneumonia and resolution of lymphoma relapse were reported[43]. In another report, a 61-year-old man with EBV-positive classical HL with progressive lymphadenopathy and weight loss was admitted with breathlessness and wheezing and was diagnosed with PCR-positive SARS-CoV-2 pneumonia. No corticosteroid or immunochemotherapy was administered. Four months later, palpable lymphadenopathy had reduced, and an interim positron emission tomography–computed tomography scan revealed widespread resolution of the lymphadenopathy. The EBV viral PCR had also fallen [44]. The authors hypothesized that the SARS-CoV-2 infection triggered an antitumor immune response, as it has been described with other infections in the context of high-grade NHL. It is noteworthy that in both cases EBV reactivation was present.

A 61-year-old patient affected from follicular lymphoma also noted a shrinkage of a para-aortic lymph nodal lesion compared to baseline during SARS-CoV-2 infection [45]. Finally, complete spontaneous remission of diffuse large B-cell lymphoma of the maxillary sinus after concurrent SARS-CoV-2 infection was reported, with the patient's facial swelling resolving during the hospitalization[46].

Since these reports represent anecdotal observations, further data are needed to address or confirm the relationship between the virus and lymphoma subtypes as well its behavior in parallel to anti-neoplastic response.

CONCLUSION

In our opinion, our search for lymphoma patients among other cancer in the recent COVID-19 literature may deliver some important messages for the scientific community. The analysis we performed reveals that there is an increased risk of COVID-19 related serious events (ICU admission, mechanical ventilation support or death) in patients with lymphomas as compared to COVID-19 patients without cancer and confirms the high vulnerability of such patients in the current pandemic. Overall, among the HM series, lymphoma represented the commonest malignancy. In lymphoma patients COVID-19 presentation symptoms occurred a median of 5 to 6 d before hospitalization, being represented by fever, cough and dyspnea. The mortality rate, taking into account the different characteristics of the populations studied, and different lymphoma subtypes was relatively high, attesting at approximately 30% after 1-2 mo of follow-up, at least in hospitalized patients.

In a meta-analysis of hematologic malignancies and COVID-19 that incorporated data from more than 3000 patients, pooled risk of death for lymphomas was 32%[28].

Active disease at COVID-19 infection presentation or lymphoma status as progressive disease appeared to be among the strongest predictors of early death. Among histotypes, no definitive conclusions can be drawn, while the use of bendamustine (but not anti-CD20 antibodies) has been associated with increased risk of death in at least one study. Published results indicate that the start of treatment should not be delayed given that active treatment has not been associated to increased risk of mortality. Instead, achieving disease remission could lead to better outcomes. Currently, little is known about specific phenotypic and/or functional T cell changes associated with symptomatic and asymptomatic SARS-CoV-2 infection, as in patients treated with immune checkpoint inhibitors. In cancer patients[47,48], treatment with immune checkpoint inhibitors did not increase risk of adverse events compared to standard chemotherapy and did not seem to increase COVID-19 susceptibility. However, no data are reported on patients with lymphoma.

With several vaccines available, it would be extremely important to protect frail categories as soon as possible. The humoral response of patients with lymphoma to COVID-19 vaccines has been investigated by several groups[49]. Altogether, these data suggest that the humoral response in lymphoma patients is impaired as compared to other HM, especially after treatment with anti-CD20 containing therapies. Different vaccination strategies are therefore warranted for lymphoma patients. Longer term

clinical follow-up and biological monitoring of immune responses is warranted to explore the impact of lymphoma and its treatment on the immunity and prolonged outcome of patients with COVID-19 infection.

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LETTER TO THE EDITOR

Evaluation of an asymptomatic COVID-19 patient post-surgery with chest radiography: A surgeon's dilemma

Gaurav Govil, Lavindra Tomar, Pawan Dhawan

ORCID number: Gaurav Govil 0000-0002-2960-4372; Lavindra Tomar 0000-0001-8025-2723; Pawan Dhawan 0000-0001-8308-8228.

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Gaurav Govil, Lavindra Tomar, Pawan Dhawan, Department of Orthopaedics, Max Super Speciality Hospital, Patparganj, Delhi 110092, India

Corresponding author: Gaurav Govil, MBBS, MS, Surgeon, Department of Orthopaedics, Max Super Speciality Hospital, Patparganj, 108 A, I.P. Extension, Delhi 110092, India. gauravgovil@yahoo.co.in

Abstract

Routine chest radiography is not a requirement in post-surgery cardiac bypass patients. However, the safety of abandoning routine chest radiographs in critically ill patients remains uncertain. Surgery in an asymptomatic coronavirus disease 2019 (COVID-19) patient presents additional challenges in postoperative management. Chest radiography remains a valuable tool for assessment of all patients, even a stable one. Management of surgical patients as an emergency in an asymptomatic COVID-19 case remains a surgeon's dilemma.

Key Words: COVID-19; Cardiac surgery; Radiography; Critical care; Chest radiography; Intensive care; Postoperative

Core Tip: Spallanzani guidelines consider chest radiographs as a valuable tool for initial assessment and follow-up of coronavirus disease 2019 patients, even in stable asymptomatic patients. A high index of suspicion will reduce the risk of high fatal postoperative outcomes.

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TO THE EDITOR

We enjoyed reading the recently published article by Omar *et al*[1] about their observation on the necessity of chest radiographs (CXRs) in postoperative cardiac bypass graft cases in coronavirus disease 2019 (COVID-19)-positive patients. Although their series of patients with favourable post-surgery outcomes was small, their courage and willingness to help in the hour of need with the required COVID-19 protocols was commendable.

We agree with most of the content of the article. However, we would like to put forth more insights on the use of CXRs when dealing with surgical patients, especially an asymptomatic COVID-19 patient.

Omar *et al*[1] rightly indicated that routine CXRs are not a requirement in post-surgery cardiac bypass patients. This aspect has been researched and concluded by other authors in larger study groups. Rao *et al*[2] recommended performing CXRs only when clinically indicated, according to their finding from a study of 300 adult cardiac surgical patients showing satisfactory recovery. The systematic review and meta-analysis by Ganapathy *et al*[3] concluded that a restrictive CXR strategy in the intensive care unit does not cause harm; however, they cautioned that the safety of abandoning routine CXRs in critically ill patients remains uncertain. Tolsma *et al*[4] studied 1102 patients and concluded that selective CXR was an effective and safe approach once clear indications are defined. Porter *et al*[5] studied thoracic surgery patients and concluded that routine postoperative CXR in immediate intensive care management and later after final chest tube removal had a limited impact on clinical care.

Barkhordari *et al*[6] studied 25 asymptomatic COVID-19 patients undergoing emergent or urgent cardiac surgery, of which 84% received a cardiac bypass graft. They concluded that the majority of the patients had comparable early postoperative respiratory outcomes to their matched cohort of pre-COVID-19 patients. However, an intensive care unit readmission fared extremely poorly. They emphasised a lung-protective strategy during anaesthesia by maintaining appropriate tidal volumes with adjustments of ventilatory parameters based on perioperative acid-base and hemodynamic analyses.

Omar *et al*[1] reported on three asymptomatic cases with a mild grade of COVID-19 infection. Surgeries during the COVID-19 pandemic represent significant challenges for the patient and health care workers. There is a need for close monitoring of evaluation parameters or alarm signs in immediate postoperative management. The CXR utility for initial assessment and follow-up of COVID-19 patients is a valuable tool, even in stable patients as highlighted by the Spallanzani guidelines[7]. In COVID-19 infection, chest computed tomography in the postoperative period also needs judicious consideration based on the clinical distress symptoms to alert the surgeon of the possibility of the progression of respiratory involvement. A high index of suspicion will reduce the risk of fatal outcomes[8]. Abate *et al*[9], in their systematic review and meta-analysis on 2947 patients, revealed that perioperative mortality was 29% amongst the patients posted for emergency surgery. They also analysed hypertension as one of the most common comorbidities and pulmonary complications as one of the most common perioperative complications among surgical patients.

The developing strategies for management of asymptomatic COVID-19 patients during emergency surgery remains a surgeon's dilemma. An asymptomatic COVID-19 patient may deteriorate abruptly and collapse quickly. A surgeon should maintain focus on decreasing perioperative mortality, preventing transmission of infection to health care workers, avoiding undertreatment, and adopting a less risky approach by undertaking routine CXR evaluation for immediate postoperative management. Of note, dyspnoea may present with COVID-19 pneumonia as well as myocardial infarction or acute decompensated heart failure. The surgeon needs to adapt constantly to the challenges of evolving clinical presentations, developing virus mutations and changing transmissibility of the COVID-19 virus to ensure patient safety.

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LETTER TO THE EDITOR

Effects of COVID-19 in lymphoid malignancies

Öner Özdemir

ORCID number: Öner Özdemir 0000-0002-5338-9561.

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Öner Özdemir, Division of Pediatric Allergy and Immunology, Sakarya University Medical Faculty, Adapazarı 54100, Sakarya, Turkey

Corresponding author: Öner Özdemir, MD, Professor, Division of Pediatric Allergy and Immunology, Sakarya University Medical Faculty, Adnan Menderes Cad., Adapazarı 54100, Sakarya, Turkey. ozdemir_oner@hotmail.com

Abstract

I will have a couple of comments on the issues elaborated in the article titled as 'Impact of COVID-19 in patients with lymphoid malignancies'. First, the author did not emphasize and overlook the prolonged persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in coronavirus disease 2019 (COVID-19) patients with hematological malignancies. Second, the rise of a chronic lymphoid leukemia clone in COVID-19 was not mentioned by the authors. Third, achieving a complete remission in asymptomatic COVID-19 patients with follicular lymphoma in partial remission after bendamustine-based therapy is not specific to this lymphoma subtype. Fourth, follicular lymphoma does not always undergo complete remission with SARS-CoV-2 infection. Our aim is to help the authors to discuss and clarify these issues a little more in COVID-19 patients with hematological malignancies.

Key Words: COVID-19; Tumor; SARS-CoV-2; Lymphoid malignancy

Core Tip: I have several comments on the article titled as 'Impact of COVID-19 in patients with lymphoid malignancies'. The author did not emphasize a couple of issues related to the effects of severe acute respiratory syndrome coronavirus 2 infection in various lymphoid malignancies. This letter helps to clarify these issues more in coronavirus disease 2019 (COVID-19) patients with hematological malignancies.

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TO THE EDITOR

I have read the original article by Riches[1] entitled 'Impact of COVID-19 in patients with lymphoid malignancies' with great interest[1].

I will have a couple of comments on the issues elaborated in their article.

First, the author did not emphasize and overlook the prolonged persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in coronavirus disease 2019 (COVID-19) patients with hematological malignancies. The author just slightly touched upon within a sentence consisting of a couple of words (the persistence of a positive polymerase chain reaction for SARS-CoV-2) under the section of 'Impact of COVID-19 by Lymphoma Subtype'. However, I think that this is a huge and important problem itself and its management needs to be discussed especially in this kind of article. Here, I give some exemplary articles from the recent literature such as in King's College Hospital experience[2], Karataş *et al*[3]'s, and Perini *et al*[4]'s studies.

Second, Largeaud *et al*[5] reported 'major rise of a chronic lymphoid leukemia clone during the course of COVID-19'. This aspect of CLL and COVID-19 disease should also be discussed by the author.

Third, the author discusses achieving a complete remission in asymptomatic COVID-19 patients with follicular lymphoma in partial remission after bendamustine-based therapy. When we look at the literature, this is not just specific to follicular lymphoma, but other hematological malignancies as well, such as in diffuse large B-cell lymphoma and Hodgkin lymphoma after concurrent other and SARS-CoV-2 infections, respectively[6]. Also, just a perfect article titled as 'complete remission of follicular lymphoma after SARS-CoV-2 infection: From the "flare phenomenon" to the "abscopal effect"' is reported by Sollini *et al*[7]. This issue should also further be elucidated.

Fourth, follicular lymphoma does not always undergo complete remission with SARS-CoV-2 infection, reported by Tafti *et al*[8] and Wright *et al*[9]. Indeed, in some malignancy patients, SARS-CoV-2 infection persisted, and COVID-19 pneumonia and the multimicrobial superinfection developed. Even, convalescent plasma needed to be utilized in the patient[9].

The authors did not emphasize a couple of issues related to the effects of SARS-CoV-2 infection in various lymphoid malignancies. Our aim is to help to clarify these issues a little more in COVID-19 patients with hematological malignancies.

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