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LETTER TO THE EDITOR

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ABOUT COVER

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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LETTER TO THE EDITOR

Case of COVID-19 presenting with gastrointestinal symptoms

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Abstract

Patients with coronavirus disease 2019 (COVID-19) predominantly present with the pulmonary symptoms such as fever, cough, and shortness of breath. We present a case of an 83 years old patient with COVID-19 who presented with only gastrointestinal symptoms without respiratory complaints. Our case raises the concern regarding our current lack of understanding of extrapulmonary manifestations of COVID-19. Given genetic homology between 2019 severe acute respiratory syndrome coronavirus (SARS-CoV) 2 and SARS-CoV, our case underscores the urgent need for further studies to understand the role of the gastrointestinal system in 2019 SARS-CoV-2 transmission and COVID-19 pathogenesis.

Key words: COVID-19; SARS-CoV-2; Coronavirus; Gastrointestinal; Diarrhea; Pandemic

Core tip: We present a case of an 83 years old patient with coronavirus disease 2019 (COVID-19) who presented with only gastrointestinal symptoms without respiratory complaints. Our case raises the concern regarding our current lack of understanding of extrapulmonary manifestations of COVID-19 and underscores the urgent need for further studies to understand the role of the gastrointestinal system in 2019 severe acute respiratory syndrome coronavirus 2 transmission and COVID-19 pathogenesis.

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TO THE EDITOR

Coronavirus disease 2019 (COVID-19) pandemic has resulted in a public health emergency unlike we have seen in generations. Patients with COVID-19 predominantly present with pulmonary symptoms such as fever, cough, and shortness of breath[1]. We present a case of COVID-19 who presented with only gastrointestinal (GI) symptoms without any respiratory complaints.

An 83 years old African American woman with a prior history of hypertension, type-2 diabetes mellitus and chronic kidney disease presented to the emergency department with diarrhea and nausea for 3 d. The patient reported 3-5 episodes/d of watery, non-mucoid, and non-bloody diarrhea. Her GI symptoms progressively worsened warranting a visit to the emergency department. The patient denied fever, cough, chills, rigors, night sweats, headache, sore throat, rhinorrhea, dyspnea, orthopnea, recent travel or sick contact. Patient was hemodynamically stable [blood pressure 116/56 mmHg, heart rate 63 beats per minute, respiratory rate 19 per minute, temperature 100.3 , and oxygen saturation (O_2 sat) 94%], but noted to have O_2 sat of 90%-91% on room air (RA) with ambulation. Labs showed normal white blood cells of 5.6 K/μL (Ref: 4.3 -11.1 K/μL) with differential showing normal lymphocyte of 25% (Ref: 13%-44%) and procalcitonin level of 0.10 ng/mL (Ref: ≤ 0.50 ng/mL). On further evaluation, chest X-ray revealed evidence of multifocal opacities suspicious for pneumonia. Computed tomography chest, abdomen and pelvis showed bilateral ground glass opacities (Figure 1). No acute pathologic findings were identified in the abdomen or pelvis including colitis, diverticulitis, appendicitis or small bowel obstruction.

Patient was admitted under droplet, contact and airborne isolation. Given high suspicion of COVID-19 with superimposed community bacterial pneumonia, she was treated with intravenous ceftriaxone (1 mg daily) and azithromycin (500 mg daily) for 7 d. Meanwhile, emergent disease panel (reverse transcription polymerase chain reaction on nasopharyngeal and oropharyngeal swab) performed by the Department of Health and Environmental Control tested positive for COVID-19. During the hospital stay, patient stayed afebrile without any respiratory distress. She had a benign hospital course and was discharged after 7 d of intravenous antibiotics. Her O_2 sat was 98% on RA at rest and 94%-96% on RA with ambulation, and the GI symptoms resolved by discharge.

Our case had a unique presentation with digestive symptoms and raises the concern regarding our current lack of understanding of extrapulmonary manifestations of COVID-19. Diagnosis of COVID-19 cases with predominant extrapulmonary symptoms may be delayed as these symptoms currently may not be on the clinician's radar. A recent report on 204 patients positive for COVID-19 in Hubei, China showed that approximately 18% of patients had digestive symptoms such as abdominal pain, diarrhea or vomiting[2]. In fact, 6 patients presented just with GI symptoms without respiratory complaints, similar to our patient. In addition, patients with GI symptoms had a longer time from illness onset to admission compared to patients without GI symptoms (8.95 ± 5.40 d *vs* 7.26 ± 4.20 d)[2].

Genome sequences of 2019 severe acute respiratory syndrome coronavirus 2 (2019 SARS-CoV-2) have demonstrated 79% sequence identity with the severe acute respiratory syndrome-like coronaviruses (SARS-CoV) and 50% sequence identity with the Middle East respiratory syndrome-like coronaviruses[3]. Patients infected with SARS-CoV and Middle East respiratory syndrome-like coronaviruses are known to have digestive symptoms[4-6]. Similar to SARS-CoV, the 2019 SARS-CoV-2 uses the angiotensin-converting enzyme 2 receptors[7]. Presence of angiotensin-converting enzyme 2 protein on small intestine erythrocytes along with epithelia of lung also supports the hypothesis that SARS-CoV-2 may shed through GI tract[8]. Moreover, SARS-CoV-2 was present on fecal swabs and blood samples from patients positive for COVID-19[9]. Intestinal biopsy specimens of patients with SARS have also shown active viral replication suggesting the role of fecal-oral route in SARS-CoV transmission[10].

Several antiviral and antimicrobial drugs such as hydroxychloroquine, remdesivir, lopinavir-ritonavir, and favipiravir are currently being studied for efficacy in patients with COVID-19 but at present, no drug is approved by United States Food and Drug Administration for COVID-19[11,12]. Supportive treatment is the mainstay of COVID-19 management. Azithromycin has been used in some protocols as adjunct therapy, and may benefit from its immunomodulatory properties and/or by preventing bacterial superinfection[11].

Given genetic homology and other similarities between 2019 SARS-CoV2 and SARS-CoV, our case underscores the urgent need for further studies to understand the role of the GI system in 2019 SARS-CoV-2 transmission and COVID-19 pathogenesis. During these unprecedented times, where the primary focus is to screen

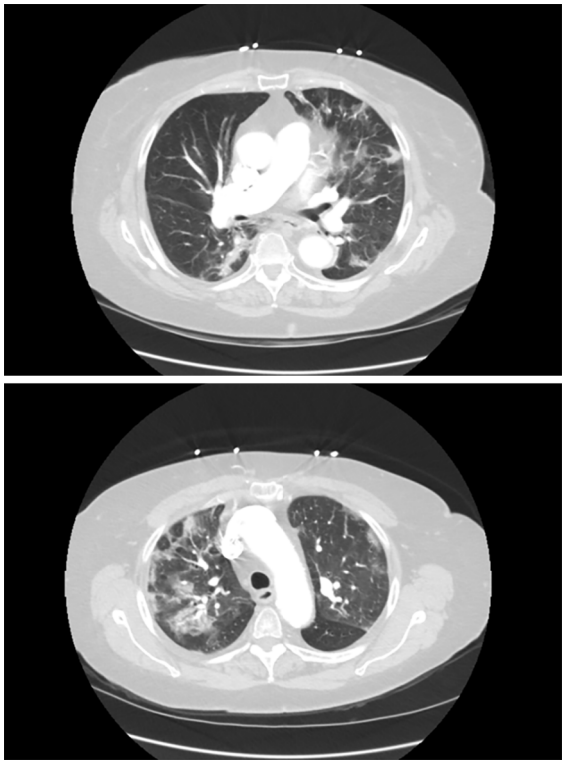

Figure 1 Computed tomography chest showing bilateral ground glass opacities.

patients with respiratory symptoms for COVID-19, our case also underscores the importance of maintaining a low threshold of suspicion for COVID-19 in patients presenting with GI symptoms which will not only help with early diagnosis and intervention but may also reduce its associated morbidity and mortality.

REFERENCES

1 **Arentz M**, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M. Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State. *JAMA* 2020 [PMID: 32191259 DOI: 10.1001/jama.2020.4326]

2 **Pan L**, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol* 2020; **115**: 766-773 [PMID: 32287140 DOI: 10.14309/ajg.0000000000000620]

3 **Lu R**, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; **395**: 565-574 [PMID: 32007145 DOI: 10.1016/S0140-6736(20)30251-8]

4 **Chiu YC**, Wu KL, Chou YP, Fong TV, Tsai TL, Kuo CM, Kuo CH, Chiu KW, Liu JW, Eng HL, Jawan B, Cheng YF, Chen CL. Diarrhea in medical care workers with severe acute respiratory syndrome. *J Clin Gastroenterol* 2004; **38**: 880-882 [PMID: 15492605 DOI: 10.1097/00004836-200411000-00009]

5 **Assiri A**, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, Flemban H, Al-Nassir WN, Balkhy HH, Al-Hakeem RF, Makhdoom HQ, Zumla AI, Memish ZA. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis* 2013; **13**: 752-761 [PMID: 23891402 DOI: 10.1016/S1473-3099(13)70204-4]

6 **Dawson P**, Malik MR, Parvez F, Morse SS. What Have We Learned About Middle East Respiratory Syndrome Coronavirus Emergence in Humans? A Systematic Literature Review. *Vector Borne Zoonotic Dis* 2019; **19**: 174-192 [PMID: 30676269 DOI: 10.1089/vbz.2017.2191]

7 **Guo YR**, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY, Yan Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res* 2020; **7**: 11 [PMID: 32169119 DOI: 10.1186/s40779-020-00240-0]

8 **Hamming I**, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631-637 [PMID: 15141377 DOI: 10.1002/path.1570]

9 **Zhang W**, Du RH, Li B, Zheng XS, Yang XL, Hu B, Wang YY, Xiao GF, Yan B, Shi ZL, Zhou P. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect* 2020; **9**: 386-389 [PMID: 32065057 DOI: 10.1080/22221751.2020.1729071]

10 **Leung WK**, To KF, Chan PK, Chan HL, Wu AK, Lee N, Yuen KY, Sung JJ. Enteric involvement of

severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterology* 2003; **125**: 1011-1017 [PMID: 14517783 DOI: 10.1016/s0016-5085(03)01215-0]

11 **Smith T**, Bushek J, LeClaire A, Prosser T. COVID-19 Drug Therapy – Potential Options. Elsevier ©, 2020. Available from: http://www.aesculapseguridaddelpaciente.org.mx/alianzapsqx/docs/informacion-cientifica/clinicadiagnostico/COVID-19-Drug-Therapy_Mar-2020.pdf

12 **Singhal T**. A Review of Coronavirus Disease-2019 (COVID-19). *Indian J Pediatr* 2020; **87**: 281-286 [PMID: 32166607 DOI: 10.1007/s12098-020-03263-6]

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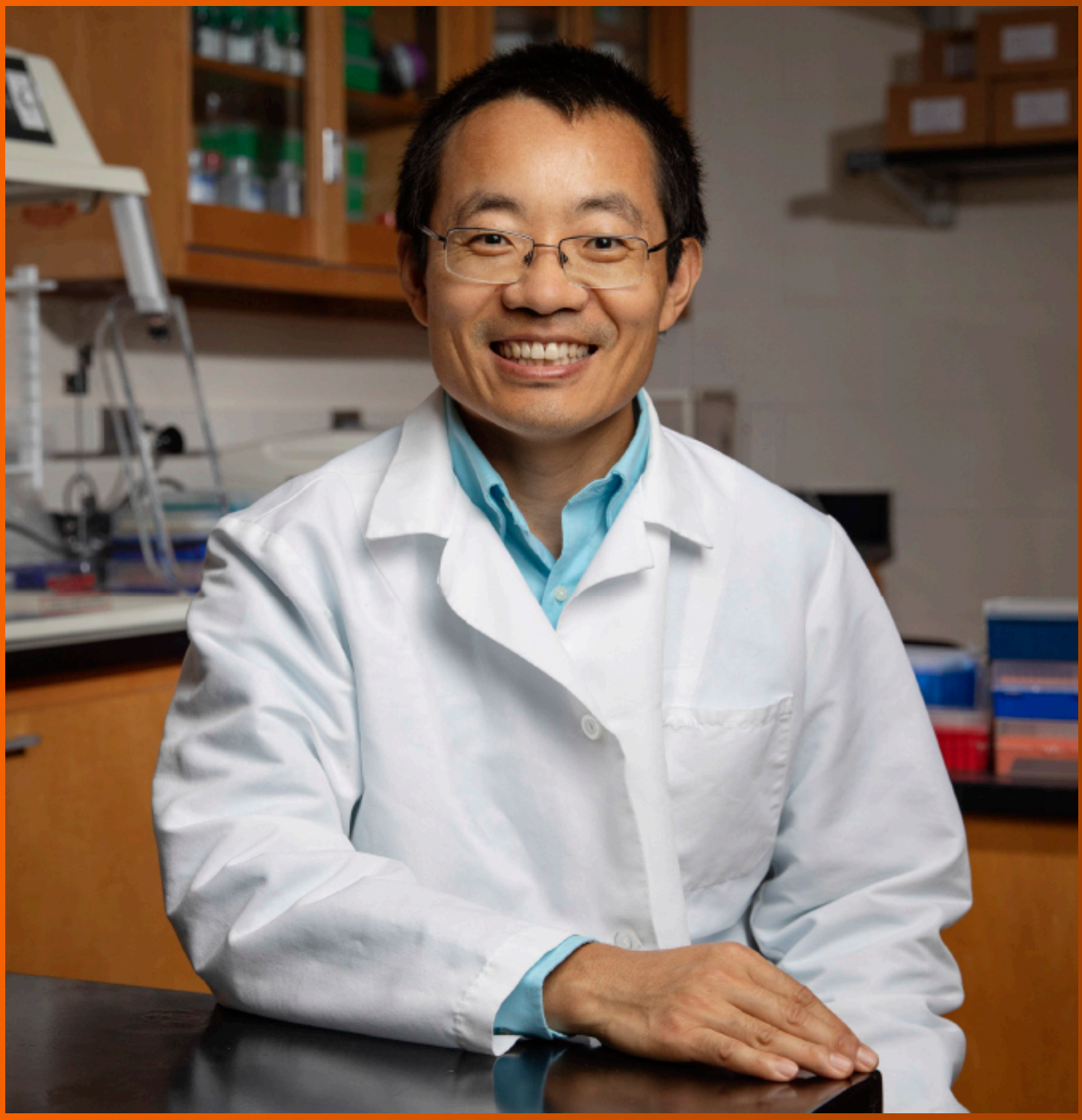

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MINIREVIEWS

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ABOUT COVER

Editorial board member of *World Journal of Virology*, Dr. Leyi Wang is Clinical Assistant Professor of Veterinary Clinical Medicine and Virology Section Head of the Veterinary Diagnostic Laboratory, College of Veterinary Medicine at University of Illinois at Urbana-Champaign (United States). In addition, he is a certified Veterinary Virologist by the American College of Veterinary Microbiologists. He has co-authored 70 peer-reviewed articles and 5 book chapters. He serves as a grant reviewer for the National Institutes of Health (NIH) and National Pork Board. Dr. Wang's significant contributions to the field of veterinary virology include the first identifications of variant porcine endemic diarrheal virus (PEDV), deltacoronavirus in diarrheic pigs, novel sparrow deltacoronavirus, novel Bottlenose dolphin coronavirus, and SARS-CoV-2 in a tiger at the Bronx Zoo (NY, United States). (L-Editor: Filipodia)

AIMS AND SCOPE

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MINIREVIEWS

Geometric architecture of viruses

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Abstract

In the current SARS-CoV-2 disease (COVID-19) pandemic, the structural understanding of new emerging viruses in relation to developing effective treatment and interventions are very necessary. Viruses present remarkable differences in geometric shapes, sizes, molecular compositions and organizations. A detailed structural knowledge of a virion is essential for understanding the mechanisms of capsid assembly/disassembly, antigenicity, cell-receptor interaction, and designing therapeutic strategies. X-ray crystallography, cryo-electron microscopy and molecular simulations have elucidated atomic-level structure of several viruses. In view of this, a recently determined crystal structure of SARS-CoV-2 nucleocapsid has revealed its architecture and self-assembly very similar to that of the SARS-CoV-1 and the Middle-East respiratory syndrome virus (MERS-CoV). In structure determination, capsid symmetry is an important factor greatly contributing to its stability and balance between the packaged genome and envelope. Since the capsid protein subunits are asymmetrical, the maximum number of inter-subunit interactions can be established only when they are arranged symmetrically. Therefore, a stable capsid must be in a perfect symmetry and lowest possible free-energy. Isometric virions are spherical but geometrically icosahedrons as compared to complex virions that are both isometric and helical. Enveloped icosahedral or helical viruses are very common in animals but rare in plants and bacteria. Icosahedral capsids are defined by triangulation number (T = 1, 3, 4, 13, *etc.*), *i.e.*, the identical equilateral-triangles formed of subunits. Biologically significant defective capsids with or without nucleic acids are common in enveloped alpha-, flavi- and hepadnaviruses. The self-assembling, stable and non-infectious virus-like particles have been widely exploited as vaccine candidates and therapeutic molecules delivery vehicles.

Key words: Virus; Virion; Capsid structure; Icosahedron; Triangulation number

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Core tip: A detailed structural knowledge of a pathogenic virus is essential for understanding the mechanisms of capsid assembly, antigenicity, cell receptor interaction, and designing therapeutic strategies. X-ray crystallography, cryo-electron microscopy and molecular simulations have elucidated atomic-level structures of several viruses. Notably, a recently determined crystal structure of SARS-CoV-2 capsid has revealed its close similarity to that of SARS-CoV-1 and MERS-CoV. Capsid symmetry greatly contributes to virion stability and balance between genome. Enveloped icosahedral viruses are very common in animals, and rare in plants. Several of self-assembled, stable and non-infectious virus-like particles have been widely exploited as vaccine candidates and therapeutic molecules delivery vehicles.

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INTRODUCTION

In the current severe acute respiratory syndrome virus-2 (SARS-CoV-2) disease (COVID-19) pandemic, detailed structural knowledge of new emerging viruses in relation to developing effective treatment and interventions are very necessary[1]. Of the previous pandemics caused by emerging or re-emerging pathogenic viruses, the 1918 "Spanish Flu" pandemic had exerted a big toll on public health and world economy[2]. Viruses are complex "biomolecule capsules" with genomic nucleic acid (Deoxyribonucleic acid/Ribonucleic acid) and associated protein(s). Unlike most other microorganisms, viruses are obligate intracellular parasites that must invade live cells and hijack the host biochemical machinery to perpetuate. Though, viruses have been debated for being classified as living or non-living, they are the most populated life-forms following the prokaryotes. After the discovery of the three different ribosomes, the cellular organisms have been placed together in a universal "phylogenetic tree". Interestingly, all viruses that do not synthesize ribosomes are re-classified into the phylogenetic tree. The International Committee on Taxonomy of Viruses however, has established a unified taxonomy for all viruses that includes 3 orders, 56 families, 9 subfamilies, and 233 genera of about 1550 species[3].

Since the early 19th century, information on the biology of viruses and their structures has remarkably advanced with experimental and computational tools and techniques. Although viruses were defined as filterable infectious agents, knowledge on their shape, size and physiochemical properties remained unknown until the isolation and characterization of tobacco mosaic virus (TMV), using a polarizing light microscope in 1953[4]. The TMV particles were further examined using X-ray diffraction concluding viruses as homogenous substances with a "protein capsid" of a definite shape and size[5]. The capsid, also called as "core", "coat" or "nucleocapsid" protects the viral genome against a hostile environment and delivers it to the host cells. The continuation of the TMV work in different laboratories subsequently confirmed its capsid's structural subunits[6,7]. High-resolution crystallography of the self-assembled protein subunits further improved the structural knowledge of its virions[8]. Morphologically, the assembly units of a capsid seen under electron microscope (EM) are called "capsomers" that may or may not be equal to the number of protein subunits.

Fluorescence and interferometry based microscopy are the common approaches to track the virion's cell-surface/receptor attachment, entry, cytoplasmic motility, uncoating, genome delivery and host-protein interactions[9]. In recent decades, advancements in molecular and computational biology, high-resolution X-ray crystallography, cryo-EM and molecular dynamics simulation have elucidated atomic-level structures of several important viruses towards understanding of their virion compositions, capsid assembly or disassembly, cell-receptor interactions, antigenicity and developing antiviral strategies[9,10]. This article presents the basics of virus structures and principles underlying capsid formation as well as therapeutic implications.

VIRION ARCHITECTURE

Structurally, viruses present remarkable differences in their shapes, sizes, molecular compositions and organizations. Their geometric shapes may be spherical, polyhedral, elliptical, rod-like, and pleomorphic ranging between 20-400 nm sizes (Figure 1). While the simplest known capsids are composed of one oligomeric protein, complex capsids also contain different proteins organized in sub-structural assemblies (*e.g.*, portals, tails, fibers or spikes *etc.*). A key structural basis of classification of a virus is whether the virion has a lipid "envelope" or "shell" (enveloped viruses) or not (non-enveloped viruses). Further, in some "enveloped viruses", the shell may have further complex structures made of other glycoproteins and/or nucleoproteins. Enveloped viruses have a further level of classification by describing the morphology of their nucleocapsids- either isometric or helical. In non-enveloped viruses, capsids are defined as isometric, filamentous, and complex. An isometric virion is morphologically spherical but geometrically an icosahedron or icosadeltahedron, a universally adopted structure in human art and culture since antiquity (Figure 2). Filamentous or rod-like virions are relatively simple and helical. The complex virions are on the other hand, neither isometric nor helical, but an intricate combination of both (*e.g.*, T2 bacteriophage)[11]. Enveloped icosahedral and helical viruses are very common in animals and rare in plants and bacteria. Comparatively, while there are relatively few purely icosahedral bacteriophages, plants almost exclusively have non-enveloped helical viruses.

In virion structure determination, capsid symmetry is an important factor. Inherently, a capsid's geometric symmetry greatly contributes to its stability and balance between the packaged genome (deoxyribonucleic acid/DNA or ribonucleic acid/RNA) structure and/or the labile envelope that should melt out in a cytoplasm at a precise location and time. The physical condition for any geometrical structure's stability is the necessity of minimum free-energy state. In view of this, the maximum number of strong interactions formed between the capsid subunits is required to attain minimum free-energy and to hold its structural integrity[12]. The MDS and Cryo-EM approaches appear to predict near experimental results on capsid stability and the structural role of packaged genome. Following the first atomic-resolution structure of TMV, a number of computational studies, such as highly coarse grained simulations and long timescale assessments on a range of capsids structures and stability have been performed[13]. As compared to non-enveloped capsids, there have been fewer simulation studies on enveloped capsids. In the relatively large sized enveloped viruses, greater structural complexity and lack of symmetry in the envelope bilayer, simulation of all components becomes relatively very complex. Nonetheless, structure of the mature enveloped human immunodeficiency virus native capsid has been recently determined using Cryo-EM and MDS[14].

CAPSID TRIANGULATION NUMBER

Viral capsid is described as empty and symmetric oligomers of one or polymers of different types of protein subunits in which viral DNA/RNA is packaged[15]. In a given capsid, the minimum number of protein subunits is determined by the symmetry of the face (*i.e.* triangle, square, tetrahedron *etc.*), and multiplying it by the number of all faces gives the total number of subunits. The triangulation number (T) is the smaller, identical equilateral-triangles that compose each triangular face, and is calculated using the law of solid geometry ($T = Pf^2$; where P is a positive integer *i.e.*, 1, 3 and 7; and f is face number *i.e.*, 1, 2, 3, 4, *etc.*). The minimum number of subunits (n) is thus 3 for triangle, 4 for square, 12 for tetrahedron, 24 for octahedron, and 60 for dodecahedron or icosahedron. For instance, in an icosahedral capsid, the triangulation number allows to determine the number of subunits as $n = 60T$. A capsid volume can be increased by either increasing T value or adding equatorial capsomers. Generally, spherical viruses with capsids $T > 1$ tend to be of larger sizes.

CANONICAL CUBIC SYMMETRY

In solid geometry, the cubic symmetry is the characteristic of canonical polyhedral structures like, tetrahedron, cube, octahedron, icosahedron and dodecahedron formed of three or more identical faces, identical vertices and identical edges (Figure 3A). For example, a cube has 6 identical square faces, 8 identical vertices and 24 edges. Further,

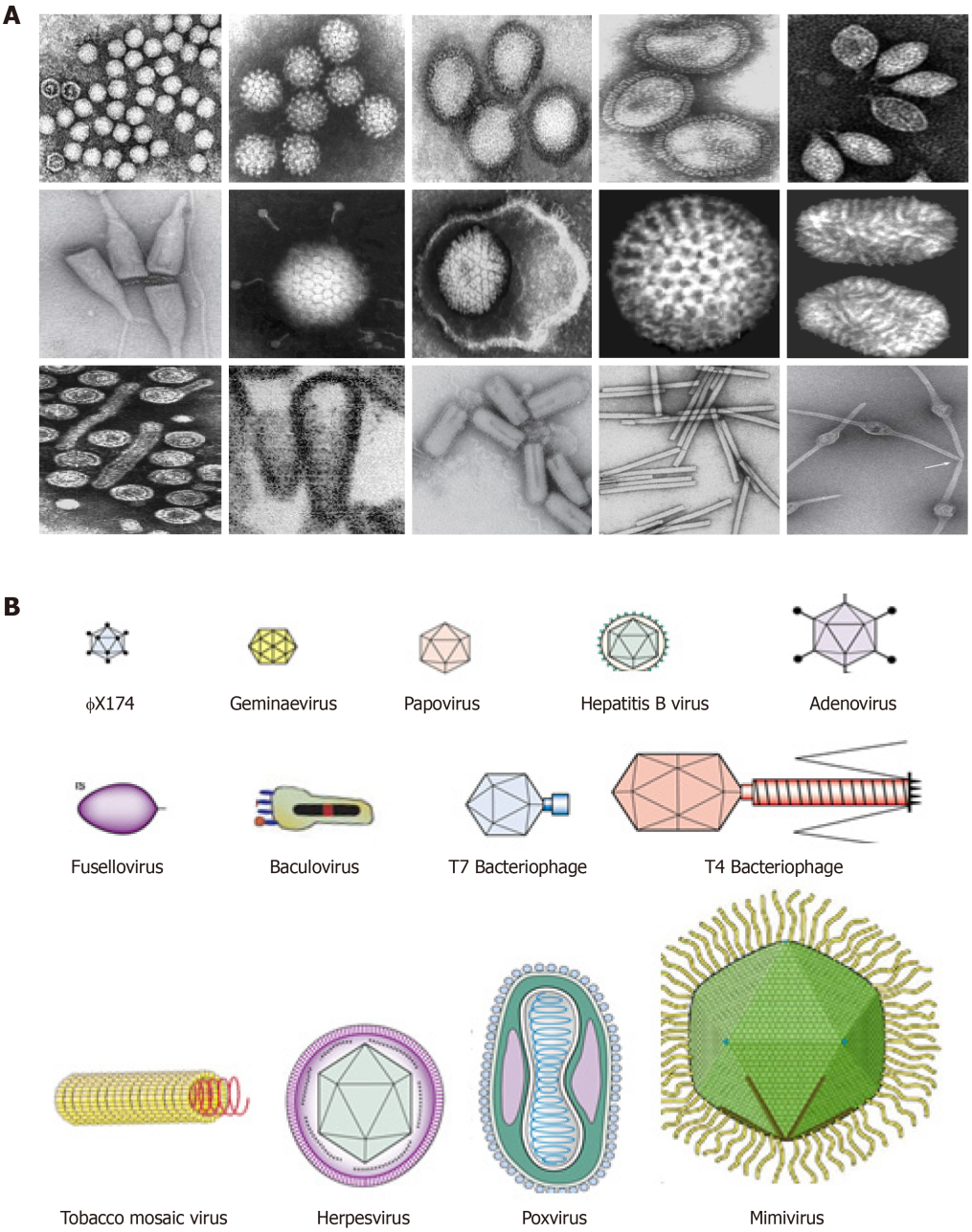

Figure 1 The structural and morphological diversity of viruses. A: Electron micrograph of some representative animal, plant, fungal and bacterial viruses; B: Cartoon representation of virions showing their sizes (20-400 nm) and shapes (spherical, polyhedral, elliptical, rod-like *etc.*).

a cube has twofold rotational symmetry axes passing through the centers of the opposite faces and threefold axes along the diagonals passing through each of the vertices. The combination of these two rotational symmetry elements gives rise to additional 3 fourfold symmetry axes going through centers of the opposite faces and 6 twofold symmetry axes going through the midpoints of opposite edges. A cube with four-, three-, and twofold symmetry axes thus, allows placement of 12 identical units. In viruses, the polyhedral capsids with inherent cubic symmetry have at least 4 threefold rotational axes.

ICOSAHEDRAL SYMMETRY

Icosahedral virions follow exclusive pathways of capsid assembly and maturation regulated by symmetry principles having three axes of symmetry: Fivefold, threefold, and twofold or 5-3-2 symmetry (Figure 3B). For example, a T = 1 icosahedron has 5-3-2 symmetry with n = 60. Notably, though most of the plant satellite viruses icosahedral capsids have n = 60, many spherical viruses have n > 60 produced by one or more

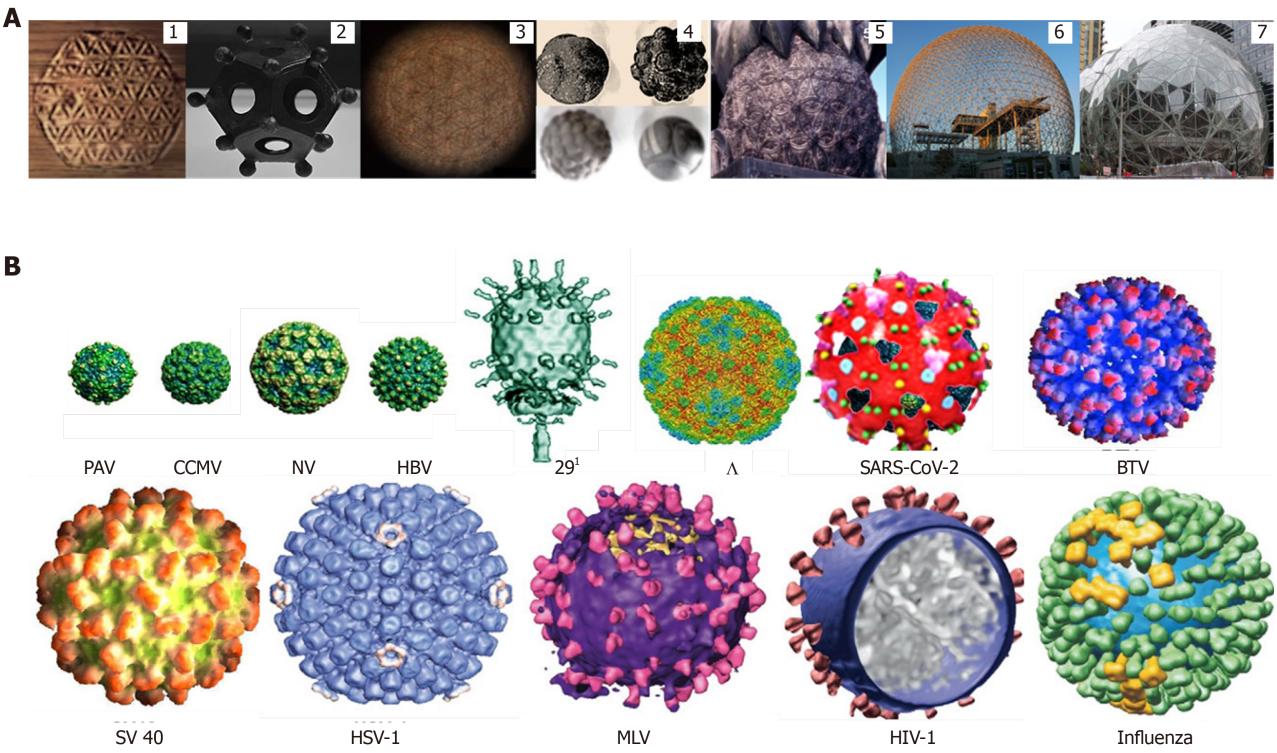

Figure 2 Morphologically spherical but geometrically polyhedral formations. A: The virion-like universally adopted structures in human art and culture: 1. Ancient wood carving, Ephesus, Turkey; 2. Ancient Roman dodecahedron, Gallo-Roman Museum of Tongeren, Belgium; 3. Ancient petroglyph, Osirian Temple, Abydos, Egypt; 4. Neolithic stone spheres, Scotland, United Kingdom; 5. Guardian lion or Shishi, Yonghe Temple, Beijing, China; 6. Amazon Sphere, Seattle, United States; and 7. Tianjin Binhai Library, Tianjin, China; B: Spherical viruses with icosahedral capsids. PAV: Parvovirus; CMV: Cytomegalovirus; NV: Norovirus; HBV: Hepatitis B virus; 29[1]: bacteriophage; SARS-CoV-2: Severe acute respiratory syndrome virus-2; BTV: Bluetongue virus; SV40: Simian virus 40; HSV-1: Herpes simplex virus; MLV: Murine leukemia virus; HIV-1: Human immunodeficiency virus-1; Influenza: Influenza virus.

genes. Moreover, an icosahedral capsid consists of f = 20 (5 on top, 5 at bottom and 10 in middle) and 12 vertices. While there are rings of five subunits (pentamers) at the vertices of each of the original faces, there are rings of six subunits (hexamers) at all the new vertices generated (Figure 4). The icosahedral capsids with pentamer and hexamer subunits are called "quasi-equivalent" that however, remains in the minimum free-energy state. Following tomato bushy stunt virus (TBSV)[16], turnip yellow mosaic virus was the second spherical virus whose icosahedral capsid was determined, using X-ray crystallography[17].

Further, not only icosahedrally symmetric capsids have > 60 identical subunits, in several cases it is formed by subunits of different gene products. Therefore, based on their *T* numbers, icosahedral capsids are categorized into different classes (Figure 5).

T = 1 icosahedron

The smallest and simplest known viruses have T = 1 capsids made of a single symmetrical protein. The small plant satellite viruses, like satellite tobacco necrosis virus (STNV) icosahedron is T = 1, n = 60[18].

T = 3 icosahedron

Some virus capsids have T = 3, n = 180 structure where in each triangular face (n = 3), the subunits are asymmetrical (*e.g.*, pentamers or hexamers). For example, in TBSV (T = 3, n = 180), each triangle is made of three identical subunits but in different conformations to accommodate the quasi-equivalent assembly[16]. In contrast, picornavirus icosahedral capsids are made of 60 copies of each of four subunits (VP1 = 60, VP2 = 60, VP3 = 60 and VP4 = 60)[19].

T = 7 icosahedron

Bacteriophage T7 icosahedron is composed of 12 pentamer and 60 hexamers with a T = 7 symmetry[8,20].

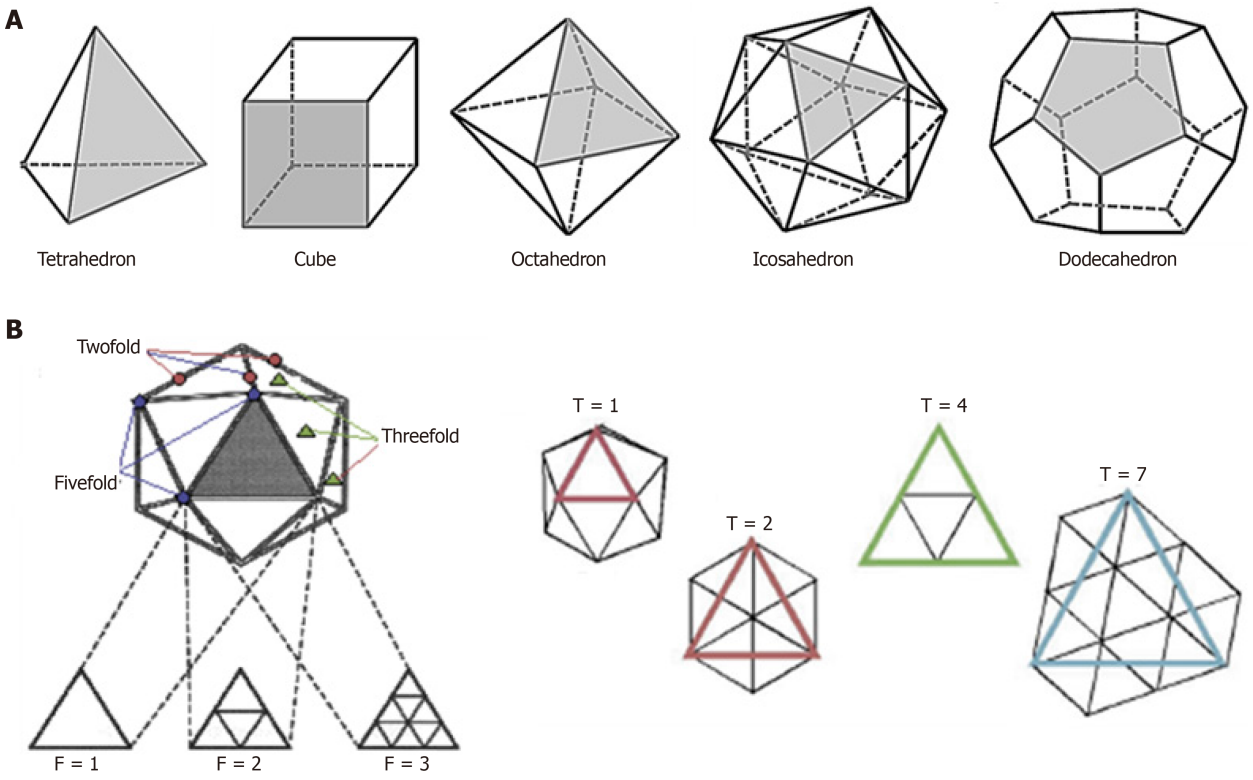

Figure 3 Cubic symmetry, the inherent characteristic of polyhedrons. A: Tetrahedron, cube, octahedron, icosahedron and dodecahedron formed of three or more identical faces, identical vertices and identical edges; B: Icosahedral capsid (*e.g.,* T = 1, 3, 4) with principle of axes of symmetry (*e.g.*, 2-3-5 symmetry).

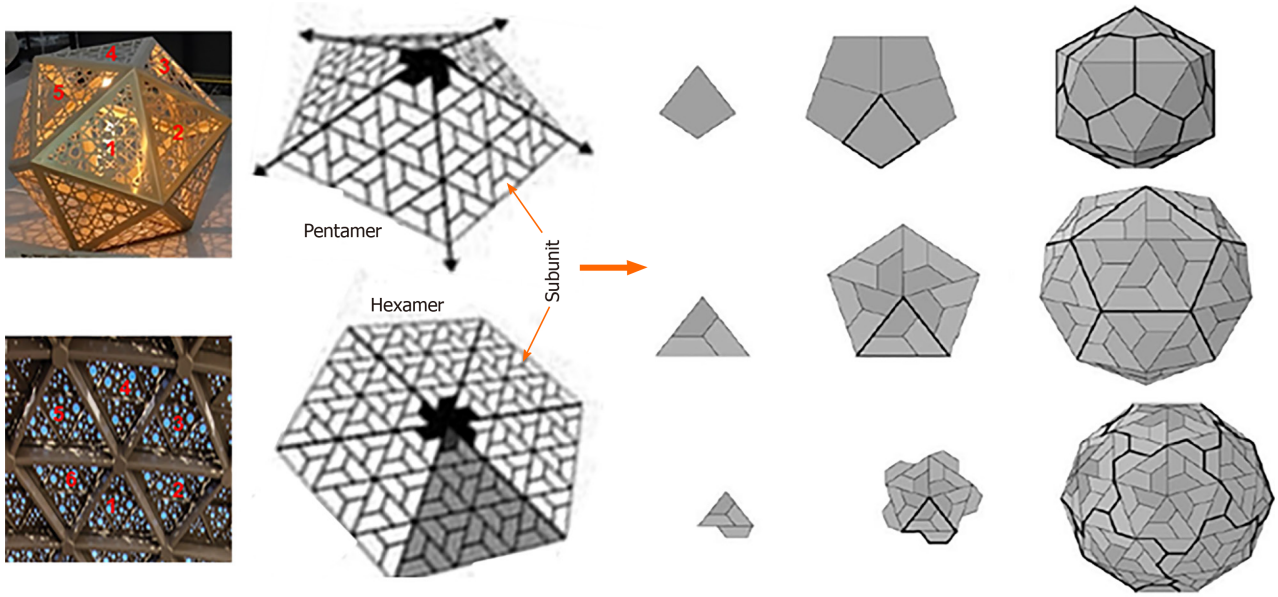

Figure 4 Icosahedral capsid formation. Capsid formation with rings of five subunits *i.e.*, pentamers or six subunits *i.e.* hexamers at the vertices of each of the faces (Left panel; Penta-/hexameric artifacts, Gallery Mall, Riyadh, Saudi Arabia).

T = 13 icosahedron

The reoviruses have double-shelled isometric capsids *i.e.* a capsid within a capsid. The outer capsid has a T = 13, n = 780 symmetry while the inner capsid has a T = 2, n = 120 symmetry[21].

T = 16 icosahedron

The herpes simplex virus (HSV) capsid has a T = 16, n = 960 symmetry[22].

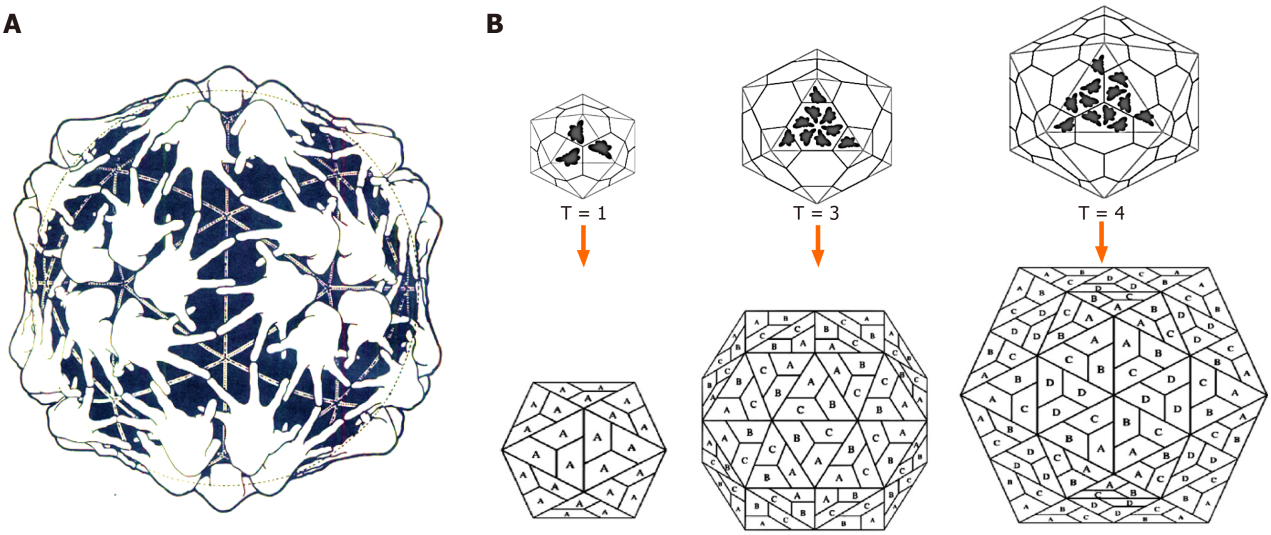

Figure 5 Schematic presentation of icosahedral capsids. A: A Clug model of icosahedral capsid assembly; B: Formation of icosahedral T = 1 (subunit $_A$), T = 3 (subunits $_A$, $_B$ and $_C$) and T = 4 (subunits $_A$, $_B$, $_C$ and $_D$) capsids.

T = 25 icosahedron

In highly complex adenovirus icosahedrons, the T = 25, *n* = 1500 structure is made of 12 pentameric and 240 hexameric subunits, including a fiber of different proteins[23].

T = 147 icosahedron

The insect chilo iridescent virus icosahedral capsid consists of 12 pentamericand 1460 hexamericcapsomers, arranged with T = 147 symmetry[24].

T = 219 icosahedron

The marine algae *Phaeocystis Pouchetii* virus (PpV01) has the largest capsid diameter and *T* number of any icosahedral DNA virus studied[25]. PpV01 capsid consists of 2180 trimeric and 12 pentameric capsomers arranged with T = 219 quasisymmetry. Above the capsid 60 fiber-like structures project, having nearly uniform distribution on the surface.

Further, based on T numbers, three classes of icosahedron are also proposed. Of these, the two classes that follow T = Pf^2, *n* = 60 are also referred to as P = 1 and P = 3 classes. The third one is "prolate" class of icosahedron found in T7[20] and 29[26] phages and as well as aberrant flock house virus[27]. Geometrically, prolate icosahedra are stretched along one of the axes and therefore, defined as *n* = 30 (T + Q) where Q is "elongation number" and Q > T. On the other hand, while an icosahedron is "obate" when Q < T and "isometric" when T = Q.

HELICAL FILAMENT OR ROD SYMMETRY

Majority of helical filamentous or rod-shaped capsid structures and assemblies belong to either plant viruses or bacteriophages[7]. In TMV, the rod-shaped capsid is made of asymmetrical subunits or capsomers in a high-aspect-ratio geometry. The subunits (*n* = approximately 2130) are joined in a helical circle to form symmetrical discs that are stacked on top of another, resulting in a hollow tube or rod.

HEAD-TAILED SYMMETRY

In the head-tail architecture, an isometric "icosahedral" head is attached with a "helical" tail. Though the head-tail is an inherent feature of bacteriophages *e.g.* T7 phage[8], many have other morphologies, too. The tails can be short, long and non-contractile or complex and contractile, and may have additional appendages such as "base-plates" and "collars".

ENVELOPED-ISOMETRIC SYMMETRY

The Sindbis virus is the only known enveloped virus to have a geometrically symmetric virion[28]. Its single protein, isometric icosahedral core (T = 3, *n* = 180) is covered by an isometric glycolipid envelope (T = 4, *n* = 240) from which transmembrane glycoprotein "spikes" protrude.

ENVELOPED-HELICAL SYMMETRY

In animal enveloped viruses, like retroviruses (*e.g.*, human immunodeficiency virus, HIV), paramyxoviruses (measles and mumps viruses), orthomyxoviruses (influenza viruses), and rhabdoviruses (*e.g.*, vesicular stomatitis virus, VSV; rabies virus, RBV) as well as plant viruses, the segmented genome is packaged in multiple compact helical/coiled filamentous nucleocapsids[29]. In influenza virus, its eight rod-shaped RNA individually encapsidated in separate cores made of matrix protein and nucleoprotein, are all contained within a spherical envelope dotted with hemagglutinin and neuraminidase spikes. In contrast, rhabdoviruses are non-isometric with bullet-shaped helical core made of nucleoprotein only. However, the structural geometry involved in the formation of rhabdovirus core still remains elusive.

ENVELOPED-SPINDLE SYMMETRY

Hyperthermophilic archaeal fuselloviruses have spindle/lemon-shaped virions with short tail-fibers attached to one end of the envelope. In sulfolobus spindle-shaped virus, for example, the capsid is made of three core proteins VP1, VP3 and VP4, including a nucleoprotein VP2[30].

VIROIDS, VIRUSOIDS OR SATELLITES

Owing to their similarities with conventional viruses, viroids, virusoids or satellites are often referred to as sub-viral particles. Viroids are the smallest phytopathogens with rod or dumb-bell shaped unencapsidated infectious RNA that however, do not synthesize any proteins[31]. Viriods do not have a capsid or outer envelope, but, as with viruses, can reproduce only within a host cell. The potato spindle tuber viroid was the first viroid discovered in 1971[32]. The hepatitis D virus is a viroid or satellite virus that requires hepatitis B virus (HBV) co-infected cells to replicate its RNA[33]. Its only synthesized core utilizes HBV envelope protein for infectious virion maturation.

CAPSID ASSEMBLY

In a capsid assembly, protein subunits are joined by maximal hydrophobic contacts and/or non-covalent interactions, and sometimes by covalent bonds. Structurally, most capsid proteins can be ascribed to a very limited number of conformational motifs *i.e.*, "jelly-roll/antiparallel β barrel" and "HK97" leading to their perfect oligo/polymerization, stability and dynamics, formation of assembly intermediates, genome packaging and maturation[34]. While many viruses from the small STNV to the largest known *Acanthamoeba polyphaga* mimivirus utilize the jelly-roll motif, some mammalian DNA viruses like, HSV use HK97 motif in their capsids[35]. Evolutionarily, a capsid structure is less dynamic than the proteins of its specific motif. Also, the preference of HK97 in prokaryotic capsids and jelly-roll in eukaryotic capsids might suggest its early existence.

Since the capsid subunits are asymmetrical, the maximum number of inter-subunit interactions can be established only when they are arranged symmetrically. Therefore, in an ideally stable geometry, the capsid must be in a perfect symmetry and lowest possible free-energy. Watson and Crick first suggested the capsid formation by the association of multiple copies of the capsid protein(s), and the spherical viruses with cubic symmetry involving at least 4 threefold rotational symmetry axes[36]. The simplest helical capsid is assembled by first encircling the asymmetrical protein subunits to form symmetrical discs or rings, and lying one on the top of another

resulting in a hollow filament or rod-like structure. In complex arrangements, the smallest number of protein subunits are placed around the vertices of a cube *i.e.* 12 regular pentagonal subunits to form a tetrahedron, octahedron and dodecahedron while 20 equilateral triangular subunits to form an icosahedron (Figure 6). Computationally, a non-symmetric spherical capsid can be formed using at least 12 protein subunits with substantial energy difference, favoring the symmetrical 12-mer over near/complete structure. Conversely, the relatively larger subunits *i.e.*, 40-mer because of minor difference between their uniform sized complexes may not necessarily satisfy an icosahedral symmetry[37,38]. While there is limited knowledge on complex quaternary capsid structures, most viruses have icosahedral or helical symmetry. Notably, while nearly 50% of the virus families have icosahedral capsids, about 10% have helical capsids.

Notably, the X-ray precession image of TBSV was the first experimental evidence of icosahedral symmetry in a spherical virus[16]. Further advancements in Cryo-EM assisted structure determination of icosahedral capsids became a turning point in structural biology. The first reported MDS study on capsid self-assembly employed simple triangular shaped subunits (T = 3) with only two types of spherical virions[39]. Generally, the icosahedral capsid assembly is described by two timescales- nucleation and elongation[40]. Icosahedral reconstruction is a type of single particle three dimensional (3D) image reconstruction. Using Cryo-EM and image reconstruction, the HBV icosahedral core secondary structure has been deduced, revealing a new fold for a viral protein where *in vitro* expressed HBV capsid assembled to yield T = 3 as well T = 4 icosahedrons[41,42].

DEFECTIVE VIRIONS OR PARTICLES

Correct and strong interactions between protein-subunits as well as other macromolecules allow assembly of stable capsids whereas weak interactions lead to unstable or defective capsids. Formations of defective capsids are mainly reported from enveloped icosahedral alphaviruses, flaviviruses and hepadnaviruses, including irregular non-icosahedral capsids of some immature retroviruses[43]. Such defects may arise as scars at the beginning of capsid assembly to the completion where it may be incorporated stochastically during self-assembly or imposed by interactions with viral or host factors. Nonetheless, defective virions are not necessarily replication-incompetent or infectious. In HBV, for example, the newly assembled pleomorphic capsids (T = 3 and T = 4) may or may not contain viral DNA, and therefore, may or may not be infectious. In contrast, though the alphavirus capsids (T = 4) and envelope proteins appear to be well structured, a substantial fraction of Ross river virus capsid is shown to have defects[44].

Though icosahedral viruses are inherently symmetric, the imposed asymmetry can be regular, irregular or dynamic. The asymmetric or symmetric capsid modifications have both structural and biological advantages in many viruses. In regular asymmetry, the well-defined modification incapsids symmetry strengthens polymerase activities of HBV and cytoplasmic polyhedrosis virus[45,46], whereas enhances canine parvovirus and MS2 phage binding to their host cell-receptors[47,48]. Conversely, irregular asymmetry is stochastic, caused by defects trapped during capsid assemblies as observed in HBV and Ross river virus[49,50].

Biologically, identification of selective advantage of structural defects in a symmetric capsid allows the virions to better respond to their environment and exposure to internal components[4]. In both cases, such defects may facilitate capsid structural transitions, uncoating, regulated genome release, intracellular trafficking or accessibility of cellular factors. The dynamic asymmetry in capsid intermediates arises due to Brownian dynamics when internal components are exposed to the surface[49-51]. Notably, the inter-subunit hydrophobic interactions represent the primary driving force behind the thermodynamics of capsid self-assembly akin to surfactant micelle formation[52]. Analogously, while the inter-subunit electrostatic interactions can oppose hepadnavirus capsid assembly where its stability increases with ionic strength, the alphavirus capsids that appear uniformly assembled are extremely sensitivity to solution conditions[53].

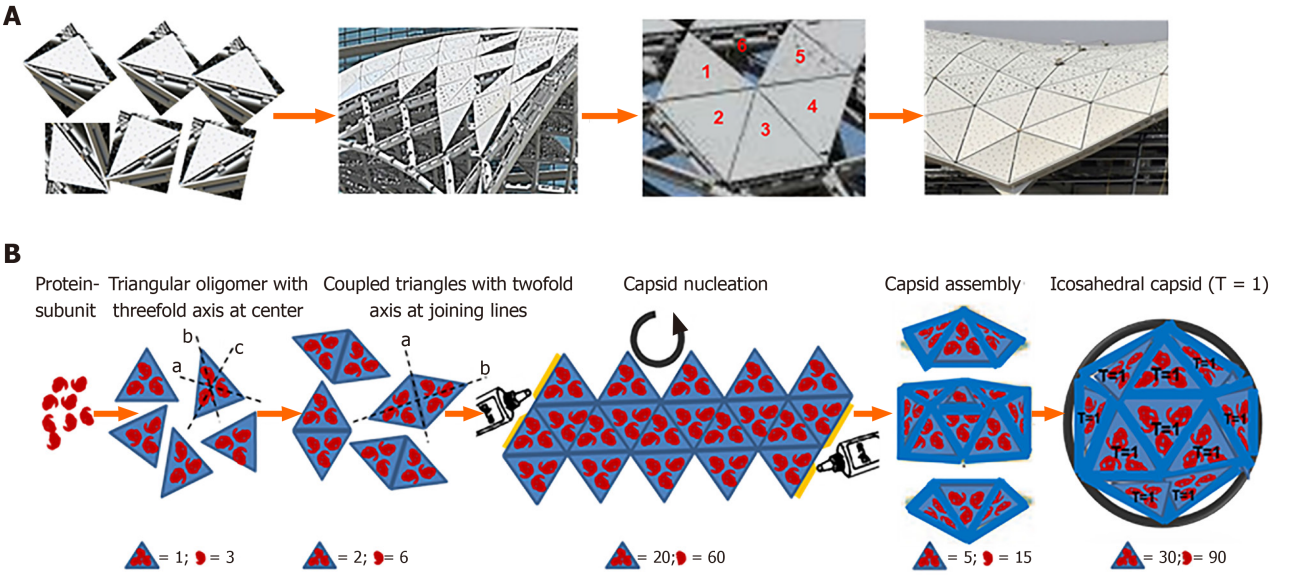

Figure 6 A cartoon presentation of an icosahedral capsid assembly. A: Construction of a polyhedral shade with equilaterally triangular glass panes (Metro Rail Project, Riyadh, Saudi Arabia); B: Assembly of an icosahedral (T = 1) capsid of a spherical virus.

VIRUS-LIKE PARTICLES

Though a nucleocapsid stability also depends on its strong interaction with the viral genome, several stable native capsidsor sub-viral particles are also formed without it, and are called virus-like particles (VLPs). The self-assembled non-infectious VLPs present the overall structure of the viral capsid or virion. For example, in hepatitis E virus (HEV), *in vitro* expressed capsid assembly[54], followed a high resolution Cryo-EM reconstruction for its VLPs[55]. Notably, the *in vitro* produced HEV-VLPs are much smaller than the authentic infectious virions, and are never detected in infected individuals. As compared to the larger native icosahedral virions (T = 3, n = 180), the HEV-VLPs display T = 1, n = 60 symmetry where T = 1 projection however, appears as spikes decorated with spherical rings akin to native virions[56]. The observed T = 1 VLPs instead of T = 3 has been suggested because of its energetically unfavorable configuration in the absence of genomic RNA. Moreover, similar to plant T = 3 capsids, the HEV-VLPs display threefold protrusions formed by P1 and twofold spikes made of P2 adopting the jelly-roll motif. Also, based on the T = 1 VLP structure, a T = 3 capsid of HEV has been modeled by using the quasi-equivalent capsid of TBSV[56].

THERAPEUTIC APPLICATIONS

A detailed 3D image of a virus particle is essential for understanding the mechanisms of capsid assembly/disassembly, antigenicity, interaction with host cell-receptors, and for designing therapeutic strategies[57,58]. The self-assembled, non-infectious VLPs mimic the real virus and present its structural immunogenic proteins as vaccine candidates. The different stages of therapeutic VLP design and development includes selection of antigenic protein or component (epitope), its expression in prokaryotic or eukaryotic system, purification and immune assays. However, to further maximize the magnitude and duration of the immunity, most of the licensed VLP-based vaccines also utilize adjuvants like, liposomes, agonists of pathogen recognition receptors, polymeric particles, emulsions, cytokines and bacterial toxins[59]. For example, some licensed prophylactic vaccines against HEV, human papilloma virus, and porcine circovirus are VLP-based vaccines. VLP technology combined with synthetic biology allows for more precise and predictable control over the composition and assembly of the capsids towards generating multivalent or cross-protective vaccines[60]. Moreover, a broad range of molecular manipulations such as encapsulation, chemical conjugation and genetic engineering further present VLPs as promising delivery agents for targeted gene therapy[61].

In addition, several viral capsid and envelope glycoproteins are exploited as drug-

delivery vehicles *in vitro* and *in vivo*[62-64]. For example, brome mosaic virus (B MV) capsid assembles into different-sized therapeutic nanoparticles[64]. Recently, determination of a 1.4 Å resolution crystal structure of the novel SARS-CoV-2 nucleocapsid's N2b domain has been determined, revealing its compact, intertwined architecture and self-assembly properties very similar to that of SARSCoV-1 and MERS-CoV[65]. Further, cryo-EM structure of its "spike" glycoprotein ectodomain-trimer has been also elucidated towards designing of vaccines and inhibitor candidates of viral entry[66]. Likewise, therapeutic virosomes are hybrid drug-delivery system that can carry genetically-modified nucleic acids, peptides, proteins and small organic molecules. A number of such prophylactic and therapeutic virosomes, especially anti-cancer products with high safety profiles are currently commercially available[67].

Compared to other enveloped icosahedral viruses, the adenovirus capsid surface has remarkable long, thin fibers primarily responsible for tethering of the viral capsid to the cell-receptor. Adenoviral capsid vectors have therefore, achieved substantial use in broad ranging therapeutic applications (*e.g.* hemophilia, cancer, and cystic fibrosis) in preclinical animal models and human trials[68]. Adeno-associated virus has been developed as gene therapy vector[69]. In addition, reconstituted pseudovirions of fusion-competent Sendai virus and influenza virus have been used as therapeutic gene delivery vehicles or nanoparticles[70]. Moreover, phage T4 capsid nanoparticles carrying reporter genes, vaccine candidates, enzymes, and ligands have been efficiently delivered *in vitro* and *in vivo*[71].

CONCLUSION

Viruses have remarkable differences in their geometric shapes, sizes and biomolecular compositions. Advances in molecular biology, X-ray crystallography, Cryo-EM and MDS have elucidated atomic-level understanding the structures of virions, including mechanisms of capsid assembly/disassembly, antigenicity, cell-receptor interaction, and designing therapeutic interventions. Cryo-EM combined with image analysis has provided 3D structures of icosahedral capsids that fail to form large crystals. Also, the structural details of influenza virus hemagglutinin and neuraminidase spikes, and adenovirus hexon unit are now known. These structures have further enhanced the information on antigenic surface for neutralizing antibodies, the cell-receptor site and fusion, polyprotein processing during maturation and egress as well as the interfering molecules of capsid functionality. In addition, several viral envelope and capsid proteins are exploited as targeted drug/gene-delivery vehicles. Because viral surface/envelope protein glycosylation influences antigenicity, further incorporating models of their glycan moieties would be a key to enhance full-scale virion simulations. This may further provide crucial insights into capsid assembly/disassembly, nucleation of other components, viral genome packaging, antigenicity, interaction with cell-receptors, and therefore, exploiting for therapeutic strategies.

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REFERENCES

1 **World Health Organization**. Available from: https://www.who.int/ emergencies/diseases/novel-coronavirus-2019
2 **Parvez MK**, Parveen S. Evolution and Emergence of Pathogenic Viruses: Past, Present, and Future. *Intervirology* 2017; **60**: 1-7 [PMID: 28772262 DOI: 10.1159/000478729]
3 **ICTV**. Virus taxonomy: the classification and nomenclature of viruses. Available from: https://talk.ictvonline.org/ictv-reports/ ictv_ online_ report/
4 **Butler PJ**, Klug A. Assembly of tobacco mosaic virus *in vitro*: effect of state of polymerization of the protein component. *Proc Natl Acad Sci USA* 1972; **69**: 2950-2953 [PMID: 4342969 DOI: 10.1073/pnas.69.10.2950]
5 **Butler PJ**, Klug A. The assembly of a virus. *Sci Am* 1978; **239**: 62-69 [PMID: 734434 DOI: 10.1038/scientificamerican1178-62]
6 **Franklin RE**, Holmes KC. Tobacco mosaic virus: application of the method of isomorphous replacement to the determination of the helical parameters and radial density distribution. *Acta Crystallographica* 1958; **11**:

213-220
7 **Champness JN**, Bloomer AC, Bricogne G, Butler PG, Klug A. The structure of the protein disk of tobacco mosaic virus to 5A resolution. *Nature* 1976; **259**: 20-24 [PMID: 1250335 DOI: 10.1038/259020a0]
8 **Bloomer AC**, Champness JN, Bricogne G, Staden R, Butler PJ, Klug A. Assembly of tobacco mosaic virus *in vitro*: effect of state of polymerization of the protein component. *Proc Nat AcadSci* 1972; **69**: 2950-2953
9 **Wang IH**, Burckhardt CJ, Yakimovich A, Greber UF. Imaging, Tracking and Computational Analyses of Virus Entry and Egress with the Cytoskeleton. *Viruses* 2018; **10** [PMID: 29614729 DOI: 10.3390/v10040166]
10 **Lee KK**, Johnson JE. Complementary approaches to structure determination of icosahedral viruses. *Curr Opin Struct Biol* 2003; **13**: 558-569 [PMID: 14568610 DOI: 10.1016/j.sbi.2003.09.007]
11 **Andreev VM**, Grigor'ev VB, Klimenko SM. [Structure of bacteriophage T2 capsid]. *Dokl Akad Nauk SSSR* 1975; **224**: 701-703 [PMID: 1204451]
12 **Tama F**, Brooks CL. Symmetry, form, and shape: guiding principles for robustness in macromolecular machines. *Annu Rev Biophys Biomol Struct* 2006; **35**: 115-133 [PMID: 16689630 DOI: 10.1146/annurev.biophys.35]
13 **Arkhipov A**, Freddolino PL, Schulten K. Stability and dynamics of virus capsids described by coarse-grained modeling. *Structure* 2006; **14**: 1767-1777 [PMID: 17161367 DOI: 10.1016/j.str.2006.10.003]
14 **Zhao G**, Perilla JR, Yufenyuy EL, Meng X, Chen B, Ning J, Ahn J, Gronenborn AM, Schulten K, Aiken C, Zhang P. Mature HIV-1 capsid structure by cryo-electron microscopy and all-atom molecular dynamics. *Nature* 2013; **497**: 643-646 [PMID: 23719463 DOI: 10.1038/nature12162]
15 **Mateu MG**. Assembly, stability and dynamics of virus capsids. *Arch Biochem Biophys* 2013; **531**: 65-79 [PMID: 23142681 DOI: 10.1016/j.abb.2012.10.015]
16 **Caspar DL**. Structure of bushy stunt virus. *Nature* 1956; **177**: 475-476 [PMID: 13309340]
17 **Klug A**, Finch JT, Franklin RE. The structure of turnip yellow mosaic virus; x-ray diffraction studies. *Biochim Biophys Acta* 1957; **25**: 242-252 [PMID: 13471561 DOI: 10.1016/0006-3002(57)90465-1]
18 **Jones TA**, Liljas L. Structure of satellite tobacco necrosis virus after crystallographic refinement at 2.5 A resolution. *J Mol Biol* 1984; **177**: 735-767 [PMID: 6481804 DOI: 10.1016/0022-2836(84)90047-0]
19 **Hogle JM**, Chow M, Filman DJ. Three-dimensional structure of poliovirus at 2.9 A resolution. *Science* 1985; **229**: 1358-1365 [PMID: 2994218 DOI: 10.1126/science.2994218]
20 **Steven AC**, Serwer P, Bisher ME, Trus BL. Molecular architecture of bacteriophage T7 capsid. *Virology* 1983; **124**: 109-120 [PMID: 6823742 DOI: 10.1016/0042-6822(83)90294-5]
21 **Miyazaki N**, Uehara-Ichiki T, Xing L, Bergman L, Higashiura A, Nakagawa A, Omura T, Cheng RH. Structural evolution of reoviridae revealed by oryzavirus in acquiring the second capsid shell. *J Virol* 2008; **82**: 11344-11353 [PMID: 18787002 DOI: 10.1128/JVI.02375-07]
22 **Yuan S**, Wang J, Zhu D, Wang N, Gao Q, Chen W, Tang H, Wang J, Zhang X, Liu H, Rao Z, Wang X. Cryo-EM structure of a herpesvirus capsid at 3.1 Å. *Science* 2018; **360** [PMID: 29622627 DOI: 10.1126/science.aao7283]
23 **Liu H**, Jin L, Koh SB, Atanasov I, Schein S, Wu L, Zhou ZH. Atomic structure of human adenovirus by cryo-EM reveals interactions among protein networks. *Science* 2010; **329**: 1038-1043 [PMID: 20798312 DOI: 10.1126/science.1187433]
24 **Yan X**, Olson NH, Van Etten JL, Bergoin M, Rossmann MG, Baker TS. Structure and assembly of large lipid-containing dsDNA viruses. *Nat Struct Biol* 2000; **7**: 101-103 [PMID: 10655609 DOI: 10.1038/72360]
25 **Yan X**, Chipman PR, Castberg T, Bratbak G, Baker TS. The marine algal virus PpV01 has an icosahedral capsid with T=219 quasisymmetry. *J Virol* 2005; **79**: 9236-9243 [PMID: 15994818 DOI: 10.1128/JVI.79.14.9236-9243.2005]
26 **Tao Y**, Olson NH, Xu W, Anderson DL, Rossmann MG, Baker TS. Assembly of a tailed bacterial virus and its genome release studied in three dimensions. *Cell* 1998; **95**: 431-437 [PMID: 9814712 DOI: 10.1016/s0092-8674(00)81773-0]
27 **Chen T**, Glotzer SC. Simulation studies of a phenomenological model for elongated virus capsid formation. *Phys Rev E Stat Nonlin Soft Matter Phys* 2007; **75**: 051504 [PMID: 17677070 DOI: 10.1103/PhysRevE.75.051504]
28 **Linger BR**, Kunovska L, Kuhn RJ, Golden BL. Sindbis virus nucleocapsid assembly: RNA folding promotes capsid protein dimerization. *RNA* 2004; **10**: 128-138 [PMID: 14681591 DOI: 10.1261/rna.5127104]
29 **Stubbs G**, Kendall A. Helical viruses. *Adv Exp Med Biol* 2012; **726**: 631-658 [PMID: 22297534 DOI: 10.1007/978-1-4614-0980-9_28]
30 **Quemin ER**, Pietilä MK, Oksanen HM, Forterre P, Rijpstra WI, Schouten S, Bamford DH, Prangishvili D, Krupovic M. Sulfolobus Spindle-Shaped Virus 1 Contains Glycosylated Capsid Proteins, a Cellular Chromatin Protein, and Host-Derived Lipids. *J Virol* 2015; **89**: 11681-11691 [PMID: 26355093 DOI: 10.1128/JVI.02270-15]
31 **Steger G**, Riesner D. Viroid research and its significance for RNA technology and basic biochemistry. *Nucleic Acids Res* 2018; **46**: 10563-10576 [PMID: 30304486 DOI: 10.1093/nar/gky903]
32 **Diener TO**. Viroids and the nature of viroid diseases. *Arch Virol Suppl* 1999; **15**: 203-220 [PMID: 10470280 DOI: 10.1007/978-3-7091-6425-9_15]
33 **Flores R**, Owens RA, Taylor J. Pathogenesis by subviral agents: viroids and hepatitis delta virus. *Curr Opin Virol* 2016; **17**: 87-94 [PMID: 26897654 DOI: 10.1016/j.coviro.2016.01.022]
34 **Kronenberg S**, Kleinschmidt JA, Böttcher B. Electron cryo-microscopy and image reconstruction of adeno-associated virus type 2 empty capsids. *EMBO Rep* 2001; **2**: 997-1002 [PMID: 11713191 DOI: 10.1093/embo-reports/kve234]
35 **Baker ML**, Jiang W, Rixon FJ, Chiu W. Common ancestry of herpesviruses and tailed DNA bacteriophages. *J Virol* 2005; **79**: 14967-14970 [PMID: 16282496 DOI: 10.1128/JVI.79.23.14967-14970.2005]
36 **Crick FH**, Watson JD. Structure of small viruses. *Nature* 1956; **177**: 473-475 [PMID: 13309339 DOI: 10.1038/177473a0]
37 **Zandi R**, Reguera D, Bruinsma RF, Gelbart WM, Rudnick J. Origin of icosahedral symmetry in viruses. *Proc Natl Acad Sci USA* 2004; **101**: 15556-15560 [PMID: 15486087 DOI: 10.1073/pnas.0405844101]
38 **Nguyen HD**, Brooks CL 3rd. Generalized structural polymorphism in self-assembled viral particles. *Nano*

Lett 2008; **8**: 4574-4581 [PMID: 19367856 DOI: 10.1021/nl802828v]
39 **Rapaport DC**, Johnson JE, Skolnick J. Supramolecular self-assembly: molecular dynamics modeling of polyhedral shell formation. *Comput Phys Commun* 1999; **121**: 231-235
40 **Prevelige PE Jr**, Thomas D, King J. Nucleation and growth phases in the polymerization of coat and scaffolding subunits into icosahedral procapsid shells. *Biophys J* 1993; **64**: 824-835 [PMID: 8471727 DOI: 10.1016/S0006-3495(93)81443-7]
41 **Crowther RA**, Kiselev NA, Böttcher B, Berriman JA, Borisova GP, Ose V, Pumpens P. Three-dimensional structure of hepatitis B virus core particles determined by electron cryomicroscopy. *Cell* 1994; **77**: 943-950 [PMID: 8004680 DOI: 10.1016/0092-8674(94)90142-2]
42 **Böttcher B**, Wynne SA, Crowther RA. Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy. *Nature* 1997; **386**: 88-91 [PMID: 9052786 DOI: 10.1038/386088a0]
43 **Katen S**, Zlotnick A. The thermodynamics of virus capsid assembly. *Methods Enzymol* 2009; **455**: 395-417 [PMID: 19289214 DOI: 10.1016/S0076-6879(08)04214-6]
44 **Mukhopadhyay S**, Chipman PR, Hong EM, Kuhn RJ, Rossmann MG. *In vitro*-assembled alphavirus core-like particles maintain a structure similar to that of nucleocapsid cores in mature virus. *J Virol* 2002; **76**: 11128-11132 [PMID: 12368355 DOI: 10.1128/jvi.76.21.11128-11132.2002]
45 **Wang JC**, Nickens DG, Lentz TB, Loeb DD, Zlotnick A. Encapsidated hepatitis B virus reverse transcriptase is poised on an ordered RNA lattice. *Proc Natl Acad Sci USA* 2014; **111**: 11329-11334 [PMID: 25034253 DOI: 10.1073/pnas.1321424111]
46 **Zhang X**, Walker SB, Chipman PR, Nibert ML, Baker TS. Reovirus polymerase lambda 3 localized by cryo-electron microscopy of virions at a resolution of 7.6 A. *Nat Struct Biol* 2003; **10**: 1011-1018 [PMID: 14608373 DOI: 10.1038/nsb1009]
47 **Hafenstein S**, Palermo LM, Kostyuchenko VA, Xiao C, Morais MC, Nelson CD, Bowman VD, Battisti AJ, Chipman PR, Parrish CR, Rossmann MG. Asymmetric binding of transferrin receptor to parvovirus capsids. *Proc Natl Acad Sci USA* 2007; **104**: 6585-6589 [PMID: 17420467 DOI: 10.1073/pnas.0701574104]
48 **Dent KC**, Thompson R, Barker AM, Hiscox JA, Barr JN, Stockley PG, Ranson NA. The asymmetric structure of an icosahedral virus bound to its receptor suggests a mechanism for genome release. *Structure* 2013; **21**: 1225-1234 [PMID: 23810697 DOI: 10.1016/j.str.2013.05.012]
49 **Pierson EE**, Keifer DZ, Selzer L, Lee LS, Contino NC, Wang JC, Zlotnick A, Jarrold MF. Detection of late intermediates in virus capsid assembly by charge detection mass spectrometry. *J Am Chem Soc* 2014; **136**: 3536-3541 [PMID: 24548133 DOI: 10.1021/ja411460w]
50 **Wang JC**, Chen C, Rayaprolu V, Mukhopadhyay S, Zlotnick A. Self-Assembly of an Alphavirus Core-like Particle Is Distinguished by Strong Intersubunit Association Energy and Structural Defects. *ACS Nano* 2015; **9**: 8898-8906 [PMID: 26275088 DOI: 10.1021/]
51 **Hagan MF**, Chandler D. Dynamic pathways for viral capsid assembly. *Biophys J* 2006; **91**: 42-54 [PMID: 16565055 DOI: 10.1529/]
52 **McPherson A**. Micelle formation and crystallization as paradigms for virus assembly. *Bioessays* 2005; **27**: 447-458 [PMID: 15770675 DOI: 10.1002/bies.20196]
53 **Ceres P**, Zlotnick A. Weak protein-protein interactions are sufficient to drive assembly of hepatitis B virus capsids. *Biochemistry* 2002; **41**: 11525-11531 [PMID: 12269796 DOI: 10.1021/bi0261645]
54 **Li TC**, Yamakawa Y, Suzuki K, Tatsumi M, Razak MA, Uchida T, Takeda N, Miyamura T. Expression and self-assembly of empty virus-like particles of hepatitis E virus. *J Virol* 1997; **71**: 7207-7213 [PMID: 9311793]
55 **Xing L**, Kato K, Li T, Takeda N, Miyamura T, Hammar L, Cheng RH. Recombinant hepatitis E capsid protein self-assembles into a dual-domain T = 1 particle presenting native virus epitopes. *Virology* 1999; **265**: 35-45 [PMID: 10603315 DOI: 10.1006/viro.1999.0005]
56 **Guu TS**, Liu Z, Ye Q, Mata DA, Li K, Yin C, Zhang J, Tao YJ. Structure of the hepatitis E virus-like particle suggests mechanisms for virus assembly and receptor binding. *Proc Natl Acad Sci USA* 2009; **106**: 12992-12997 [PMID: 19622744 DOI: 10.1073/pnas.0904848106]
57 **Harrison SC**. Principles of virus structure. In: KnipeDM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Strauss SE. FieldsVirol.Vol.1. Philadelphia: Lippincott Williams Wilkins, 2007: 59-98
58 **Rossmann MG**. Structure of viruses: a short history. *Q Rev Biophys* 2013; **46**: 133-180 [PMID: 23889891 DOI: 10.1017/S0033583513000012]
59 **Cimica V**, Galarza JM. Adjuvant formulations for virus-like particle (VLP) based vaccines. *Clin Immunol* 2017; **183**: 99-108 [PMID: 28780375 DOI: 10.1016/j.clim.2017.08.004]
60 **Charlton Hume HK**, Vidigal J, Carrondo MJT, Middelberg APJ, Roldão A, Lua LHL. Synthetic biology for bioengineering virus-like particle vaccines. *Biotechnol Bioeng* 2019; **116**: 919-935 [PMID: 30597533 DOI: 10.1002/bit.26890]
61 **Chen C**, Kwak ES, Stein B, Kao CC, Dragnea B. Packaging of gold particles in viral capsids. *J Nanosci Nanotechnol* 2005; **5**: 2029-2033 [PMID: 16430136 DOI: 10.1166/jnn.2005.506]
62 **Dixit SK**, Goicochea NL, Daniel MC, Murali A, Bronstein L, De M, Stein B, Rotello VM, Kao CC, Dragnea B. Quantum dot encapsulation in viral capsids. *Nano Lett* 2006; **6**: 1993-1999 [PMID: 16968014 DOI: 1021/nl061165u]
63 **Dragnea B**, Chen C, Kwak ES, Stein B, Kao CC. Gold nanoparticles as spectroscopic enhancers for *in vitro* studies on single viruses. *J Am Chem Soc* 2003; **125**: 6374-6375 [PMID: 12785770 DOI: 10.1021/]
64 **Sun J**, DuFort C, Daniel MC, Murali A, Chen C, Gopinath K, Stein B, De M, Rotello VM, Holzenburg A, Kao CC, Dragnea B. Core-controlled polymorphism in virus-like particles. *Proc Natl Acad Sci USA* 2007; **104**: 1354-1359 [PMID: 17227841 DOI: 10.1073/pnas.0610542104]
65 **Ye QZ**, West AMV, Silletti S, Corbett KD. Architecture and self-assembly of the SARS-CoV-2 nucleocapsid protein. BioRxiv 2020 [PMID: 32511359 DOI: 10.1101/2020.05.17.100685]
66 **Walls AC**, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 2020; **181**: 281-292.e6 [PMID: 32155444 DOI: 10.1016/j.cell.2020.02.058]
67 **Liu H**, Tu Z, Feng F, Shi H, Chen K, Xu X. Virosome, a hybrid vehicle for efficient and safe drug delivery and its emerging application in cancer treatment. *Acta Pharm* 2015; **65**: 105-116 [PMID: 26011928 DOI:

10.1515/acph-2015-0019]
68 **Nicklin SA**, Wu E, Nemerow GR, Baker AH. The influence of adenovirus fiber structure and function on vector development for gene therapy. *Mol Ther* 2005; **12**: 384-393 [PMID: 15993650 DOI: 10.1016/j.ymthe.2005.05.008]
69 **Xie Q**, Bu W, Bhatia S, Hare J, Somasundaram T, Azzi A, Chapman MS. The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. *Proc Natl Acad Sci USA* 2002; **99**: 10405-10410 [PMID: 12136130 DOI: 10.1073/pnas.162250899]
70 **Kaneda Y**. Development of liposomes and pseudovirions with fusion activity for efficient gene delivery. *Curr Gene Ther* 2011; **11**: 434-441 [PMID: 22023472 DOI: 10.2174/156652311798192789]
71 **Tao P**, Mahalingam M, Marasa BS, Zhang Z, Chopra AK, Rao VB. In vitro and in vivo delivery of genes and proteins using the bacteriophage T4 DNA packaging machine. *Proc Natl Acad Sci USA* 2013; **110**: 5846-5851 [PMID: 23530211 DOI: 10.1073/pnas]

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ABOUT COVER

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FRONTIER

Hypothesis of design of biological cell robot as human immunodeficiency virus vaccine

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Abstract

High genetic variability of human immunodeficiency virus (HIV) has been a major intractable challenge to the practical design of vaccines. But a recent pioneer study published in PNAS Xenobots, is likely to revolutionize HIV prevention as it presented the world's first living robot made of cells. In the advent of this discovery, we herein discuss the possibility of using living biological cell robots to target HIV-infected T lymphocytes, and the prospects of this approach being a new HIV vaccine. We capture the current research status and trend of advances in biological cell robots' design as a new HIV vaccine. The key differences between this novel vaccine and other HIV vaccines are highlighted.

Key Words: Human immunodeficiency virus; New vaccine; Biologically inspired microrobots; Human immunodeficiency virus target cell surrogate; CD4

Core Tip: In February 2020, the birth of the world's first live-cell robot has brought hope for the artificial design of human live cells. Therefore, herein we propose a hypothesis: Can we artificially design a cell as an alternative target cell for human immunodeficiency virus (HIV) infection and use it as a new acquired immune deficiency syndrome vaccine to prevent HIV infection.

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INTRODUCTION

While preventive human immunodeficiency virus (HIV) vaccine development has been a constant goal since the discovery of HIV, interest in a therapeutic vaccine for HIV-infected people has fluctuated. Many people thought that therapeutic vaccines were impossible because until recently there were no examples of such vaccines being used for other diseases. With the emergence of more capable, simple, and relatively non-toxic combination drug therapies, there have been fewer calls for the development of a therapeutic acquired immune deficiency syndrome (AIDS) vaccine. However, the world's first living somatic cell robot has rekindled interest in a therapeutic vaccine. The vaccine can not only be used as a stand-in for HIV target cells, through alternative ways to protect the body's healthy immune cells, but can also enhance immune-mediated clearance of virus-producing cells and/or assist in the destruction of the reservoir of latently infected cells that drug therapy alone does not seem to be able to eliminate[1]. So, herein we propose a hypothesis that artificial design of the live-cell robot can be considered as a new AIDS vaccine.

CURRENT STATUS OF HIV VACCINES

Since the discovery of AIDS in 1981, massive resources have been directed at research aimed at developing preventive and curative agents for affected patients. Nearly 40 years later, AIDS has become a global public health threat claiming many lives. A few years ago, a meeting was held in Keystone Symposia (March 21 to 26, 2012) to focus on basic aspects of immunology and HIV-1 virology to highlight issues that challenge the field. The genetic diversity of circulating HIV-1 variants puts extreme demands on the quality of the response that a prophylactic vaccine will need to elicit. The recent trials of therapeutic HIV vaccines were introduced and the results of therapeutic vaccines in non-human primate models were discussed. It is clear that therapeutic vaccine development trials and studies follow a standard preventive vaccine development path. After conceptualizing the product, 10 to 15 years of animal model testing are performed prior to 4 to 6 years of GMP product development, allowing another 5 to 10 years of phase I, phase II, and then phase III clinical trials for licensing and distribution of specific vaccine candidate products before they occur. We can see that this path is frustratingly slow and may not be the best way to address several of the key issues to be discussed in the development of therapeutic vaccines. It is risky for researchers to try to design a vaccine that solves such a complex problem by reasoning through aspects of the final product before testing it. At the end of a long trial, however, it is likely to fail completely because it does not contain a necessary ingredient or contains unnecessary ingredients that undermine the overall efficacy. We have long believed that it is postulated that the HIV vaccine is the most effective approach to control the AIDS pandemic. Although much progress has been made to achieve this goal[1,2], no licensed HIV vaccine has been put on the market to prevent HIV infections. It was clear that therapeutic HIV vaccine development requires addressing several very different issues including: (1) How to correctly understand the mechanism of HIV infection? (2) How does the vaccine respond to HIV mutations? and (3) How to choose the optimal way to block HIV infection?

MECHANISM BY WHICH HIV INFECTS HOST CELLS

HIV selectively infects helper T lymphocytes, dendritic cells, and macrophages because these cells express CD4 molecules. The HIV infection process is complex, with several stages: Adsorption, entry, uncoating, reverse transcription, integration, replication, transcription, translation, assembly, and maturation (Figure 1). Current research provides evidence that HIV infection requires not only CD4 molecules and helper receptors (CXCR4 and CCR5), but also proteins encoded by HIV genes, such as gp120 and gp41. A detailed list of proteins encoded by genes associated with HIV infection in host cells is provided in Table 1[3]. When HIV enters the human bloodstream, it selectively invades host cells expressing CD4 molecules on their cell surface. HIV binds to the CD4 receptor on the surface of the host cell *via* its surface envelope protein gp120[4]. Upon binding, gp120 protein undergoes structural alterations exposing another envelope protein gp41. Meanwhile, the gp120-CD4 dimers formed to interact with the host's cell surface auxiliary receptor CXCR4/CCR5 to create three molecular complexes constituting gp120-CD4-CXCR4/CCR5. These

Table 1 Human immunodeficiency virus-related genes and their coding proteins, specific functions, and host-cell related proteins

Gene	Encoding protein	Protein function	Host cell-related proteins
Structural genes			
gag	MA	Matrix proteins	Karyopherins, HO3, Calmodulin, VAN/NAF1, TRIM5α, CyPA
	CA	Capsid protein	HP68/RNase L inhibitor, Actin
	NC	Nucleocapsid protein	ESCRT, Tsg-101, AIP-1, Nedd4, Ubiquitin
	p6	Nucleocapsid protein	-
pol	RT	A viral genome that can be transcribed and copied	-
	PR	Cut polymerized protein	-
	IN	Integrate viral DNA with cellular DNA	INI1/hSNF5, LEDGF/p75, BAF, HMGal, ATR, ATM, Karyopherins, XRCC5
env	gp120	Attach the virus to the surface of the cell	CD4, CCR5. CXCR4, DC-SIGN, DC-SIGNR, MR, CD207
	gp41	Fusion with host cells	-
Necessary regulatory genes			
tat	Tat	Trans-activated proteins that activate HIV gene transcription	NF-κB, cyclin T, CDK9, Med28
rev	Rev	A regulator of viral protein expression that regulates mRNA splicing and promotes mRNA transport to the cytoplasm	TNPO3, importin β, Crm1, Ran GTPase, Sam68, p32
Nonessential regulatory genes			
nef	Nef	Negative regulatory factors, which change cell signals, reduce the expression of CD4 and MHC-I molecules, and reduce the killing of HIV infected cells by CTL, representing essential factors in the development of infection into AIDS	PACS-l, ASK1, PAK, PI3-K, Lck, VAN/NAFl
vif	Vif	Viral infectious factors that promote viral assembly and maturation	APOBEC3G
vpr	Vpr	Viral protein regulatory, which transports viral DMA to the nucleus and inhibits cell growth	Karyopherins, Uracil-DNA glycosylase, Weel
vpu	Vpu	Viral protein U that promotes the release of the virus	CD317 (Tetherin, BST-2)

HO3: Heme oxygenase-3; TRIM5α: TRIpartite motif-5α; CyPA: Cyclophilin A; ESCRT: Endosomal sorting complex required for transport; Tsg-101: Tumor susceptibility gene-101; AIP-1: Apoptosis-linked gene-2-interacting protein 1; Nedd4: Neural precursor cell expressed, developmentally down-regulated 4; INI1: Integrase interactor 1; BAF: Barrier-to-autointegration factor; ATR: Ataxia telangiectasia and RAD3-associated kinase; ATM: Ataxia telangiectasia-mutated gene; SIGN: Specific intercellular adhesion molecule-3-grabbing nonintergrin; DC-SIGNR: Dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintergrin receptor; MR: Mannose receptor; HIV: Human immunodeficiency virus; NF-κB: Nuclear factor-κB; CDK9: Cyclin-dependent protein kinase 9; Med28: Mediator of RNA polymerase II transcription, subunit 28 homolog; TNPO3: Transportin-3; Crm1: Chromosome maintenance protein 1; Ran: Ras-related nuclear protein; Sam68: Src mitogen-associated protein 68 kDa; Nef: Negative regulate factor; CTL: Cytotoxic T lymphocytes; AIDS: Acquired immune deficiency syndrome; ASK1: Apoptotic signal-regulated kinase 1; PAK: p21 activated kinases; PI3-K: Phosphatidylinositol 3-kinases; Lck: Lymphocyte-specific protein-tyrosine kinase; VAN/NAF1: Virion associated nuclear shuttling protein/nef-associated factor 1; Vif: Viral infectivity factor; Vpr: Viral protein regulatory; Weel: Weel kinase is an important regulator to cell cycles; Vpu: Viral protein U.

complexes expose the host cell membrane and the envelope protein gp41, which is hydrophobic, enabling the HIV to be coated with the host cell membrane followed by HIV and host cell fusion. Finally, HIV nucleic acid is released into the host cell (Figure 2).

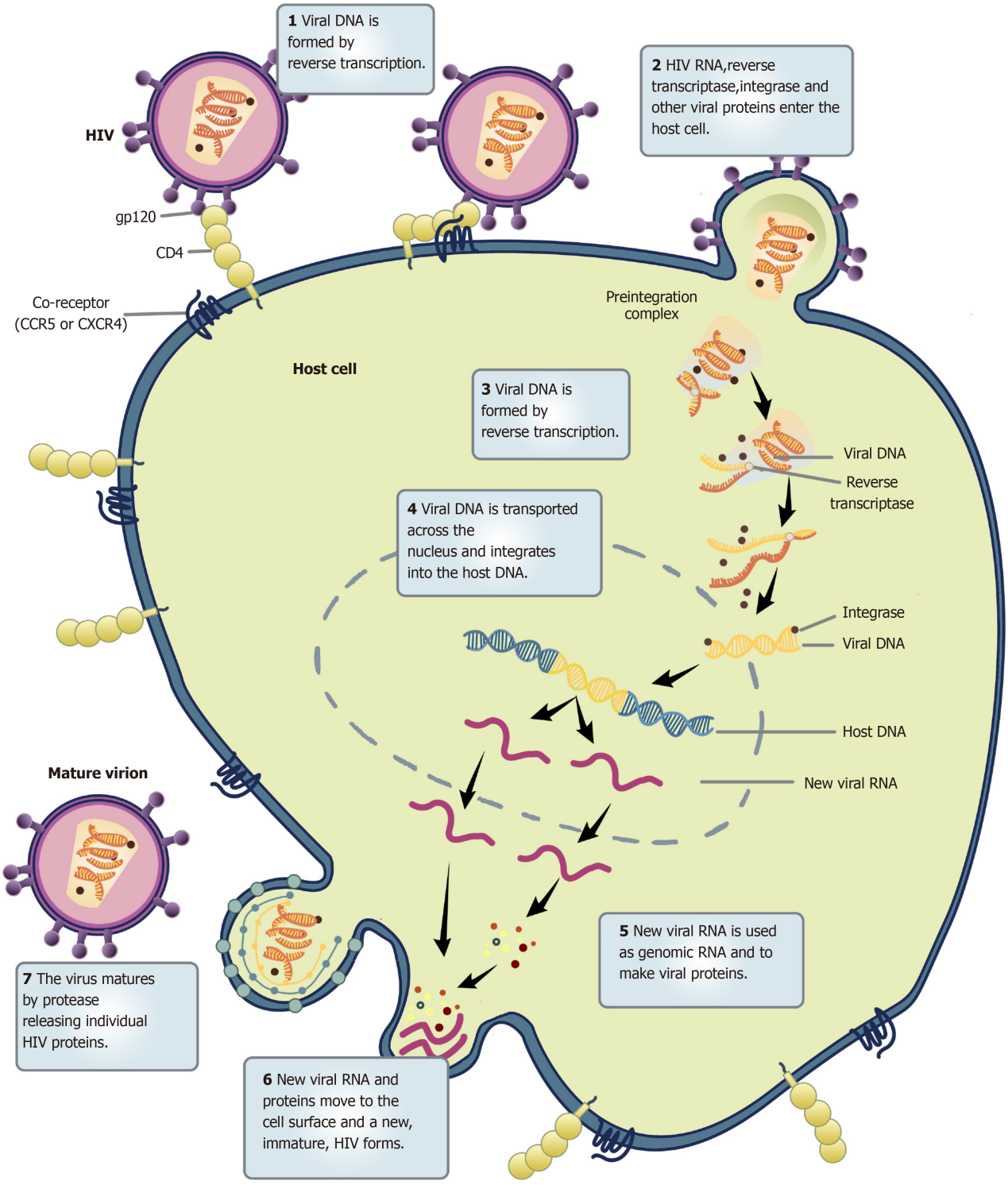

Figure 1 The mechanism by which human immunodeficiency virus attacks a target cell. HIV: Human immunodeficiency virus.

HIV IS HIGHLY VARIABLE

HIV is the most complex of all retroviruses. Its genetic material ranges between 9.2 and 9.8 kb of RNA. Structurally, it is not very stable and has a high mutation rate. Once HIV is coated and fused with the host cell membrane, it releases its capsid protein HIV p24, which is gradually degraded within the host cell to release HIV RNA and reverse transcriptase[5]. The reverse transcriptase uses the RNA as a template to synthesize viral DNA. The HIV DNA is integrated into the host cell nucleus chromosome by the integrase enzyme. Subsequently, the viral DNA uses existing host cell gene copies and protein replication machinery to synthesize its proteins.

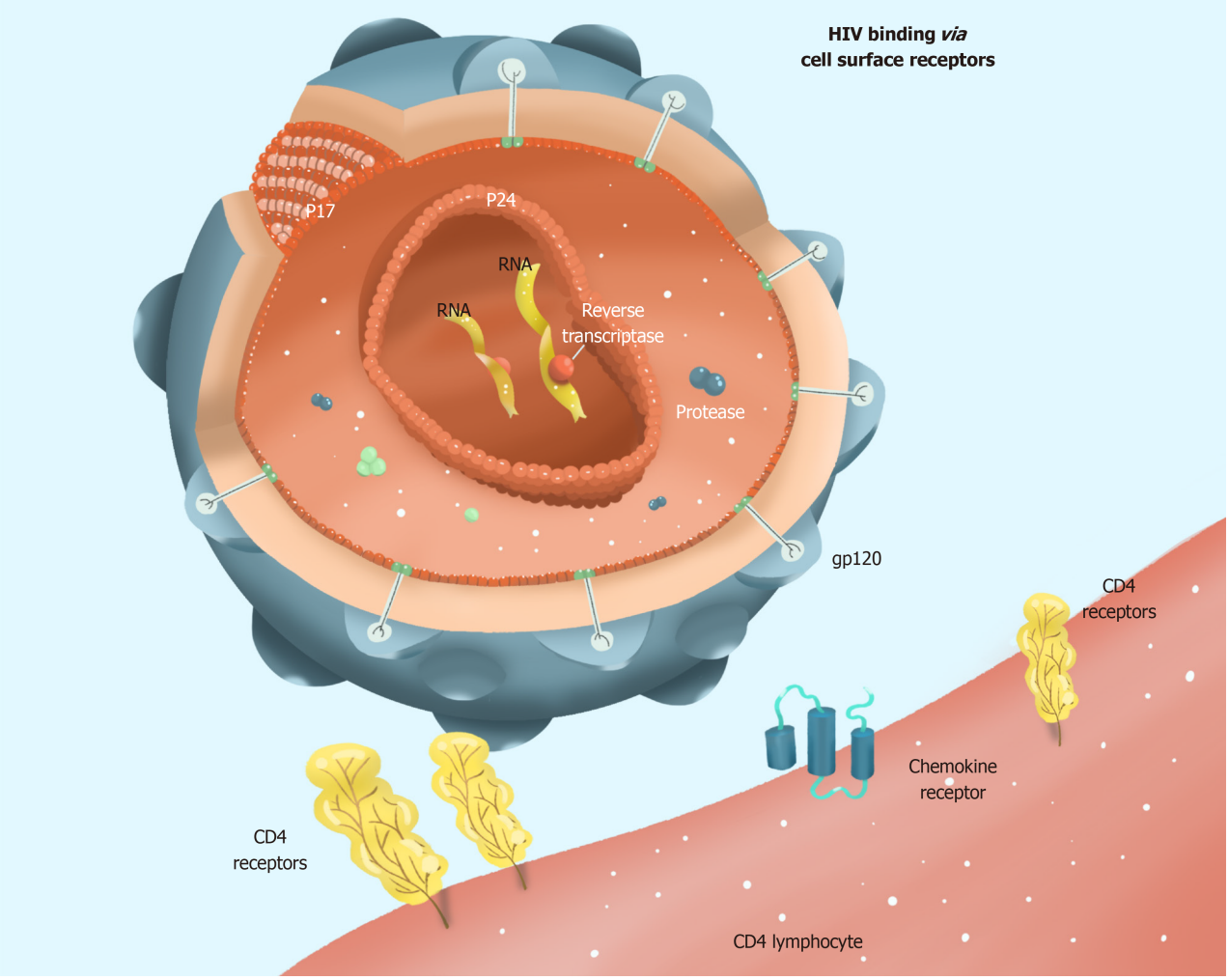

Figure 2 Human immunodeficiency virus recognizes and binds a CD4 lymphocyte. HIV: Human immunodeficiency virus.

Activation of HIV-infected cells triggers the transcription of pre-viral DNA into viral RNA, further translated into structural proteins of HIV. These proteins assemble in the cytoplasm, forming several new viral particles. Eventually, viral particles are exported to the cell surface in the form of buds, which recognize and attack other target cells. Currently, it is known that HIV exhibits high RNA variability. More importantly, the reverse transcriptase of HIV is prone to the problem of base mismatch in the reverse transcriptase synthesis of DNA using RNA as a template, because the reverse transcriptase lacks base proofreading. This leads to a failure to remove the mis-introduced nucleotides in time for replication. An error occurs about once in every replication cycle, causing the virus to replicate with random mutations that are high-frequency and non-directional. The high genetic variability translates to high variability in the encoded proteins. A comparison of antigens extracted from wild type strains of HIV with those from AIDS patients in which HIV has already undergone many replications reveals that the structure and amino acid sequence of proteins from these two groups are different. It has been observed that the injection of vaccines based on HIV antigen into AIDS patients fails to induce the formation of immune cells and antibodies to neutralize HIV. This is because HIV surface antigen molecules undergo rapid mutations that help HIV-infected cells to escape immune recognition due to decreased affinity of produced antibodies for the mutant HIV antigens.

ALTERNATIVE TARGET CELLS FOR HIV INFECTION

We analyzed the recently reported world's first living bio-robot, which we believe could be a new HIV vaccine. This vaccine differs significantly from conventional vaccines. We know that HIV has a high degree of antigenic variability. For this reason, HIV samples used for vaccine development contain mutated versions of HIV, hence

such vaccines are not sufficient for controlling AIDS. This has been the biggest challenge hindering HIV vaccine research and development. Notably, we have observed that despite its many variants, HIV always targets cells expressing CD4 molecules, and the expression of the vast majority of CD4 particles is conserved; variations are rare. This means that human CD4 units are the primary receptors for HIV infection. Based on the recent publication by Kriegman *et al*[6], we believe that a living biological cell robot can be designed to target CD4 molecules.

The surface molecules of this bio-bot are highly similar to the target cells, which not only express CD4 molecules but also other helper receptors needed for HIV infection (CXCR4/CCR5). We believe that this somatic cell robot can be an alternative target cell for HIV infection. This robot can be programmed using supercomputers and gene recombination technologies, to shorten its lifespan and make it undergo rapid apoptosis after HIV infection. Other cellular automata that are not infected with HIV will also degrade themselves within a short time, to avoid unnecessary immune responses. We can also enzymatically engineer the cell robot to synthesize HIV-RNase in the cell, degrade the HIV-RNA that enters the battery, or make the cell robot carry drugs that inhibit HIV replication (Figure 3). In this way, the reproduction of HIV in host cells can be immediately suppressed. To improve the binding rate of HIV to the cell robot, the target cells should be selectively and temporarily sealed with CD4 molecules in the original body using antibodies with radioactive isotopes. Blocking immune cells with CD4 molecules carrying the antibodies may reduce immunity in HIV-infected people, but only temporarily. A recent study showed that the use of live-cell robots as alternative target cells for HIV infection, combined with HIV fusion inhibitors, may reduce the use of CD4 blocking antibodies, which will indirectly enhance the new vaccine's effect without reducing the function of the patient's immune system. Besides, HIV fusion inhibitors can block the entry of HIV into human cells. This novel mechanism requires further research. Strategies that inhibit HIV fusion hold massive promise in solving the problem of HIV resistance. Such agents can be used as adjuvant treatments to the new vaccine, given after vaccination to reduce the adverse reactions of the new vaccine (Figure 4).

RESULTS AND DISCUSSION

The new live-cell robot vaccine is still in the developmental stage and may remain an idea that requires further research. However, we note that it is a better alternative to other HIV vaccines because it overcomes the high variability of HIV. Traditional HIV vaccines currently being studied are primarily based on subunit vaccines, live attenuated vaccines, and inactivated vaccines. But these approaches are proving difficult due to the high antigenic variability of HIV, which hinders the identification of crucial representative genotypes or specific protein antigens. Live attenuated vaccines are associated with safety concerns. In a rhesus monkey model, the simian immunodeficiency virus (SIV) mutant strain lacking the nef gene prevents attack by pathogenic SIV. It protects monkeys from developing AIDS, but it cannot safeguard vaccinated monkeys against the over-infection of wild-type virus. Moreover, SIV without the nef gene can still cause AIDS, especially when given orally to young monkeys. Of note, genetic mutations or deletions in HIV may attenuate viral reproduction, but at the cost of reducing the vaccine's effectiveness. For inactivated vaccines, physical or chemical methods are needed to kill the virus, which requires that the antigenic nature of HIV be changed. These inactivated HIV antigens cannot effectively activate the body's immune system to produce immune responses, and the produced antibody titers are also very low[7]. This calls for collaborative research between computer science and biological science. In natural sciences, cellular simulations based on the ability of HIV to recognize and bind to CD4 receptors in host cells should be developed. Such simulations should consider some conserved proteins such as gp120 and gp41 to design alternative target cells for HIV infection. In computer science, supercomputers with well-designed evolutionary algorithms, through trial and error approaches, should be employed to program cell robots[5]. The development of "surrogate target cells" for HIV infections will lead to "HIV suicide" because they cannot replicate and reproduce[8].

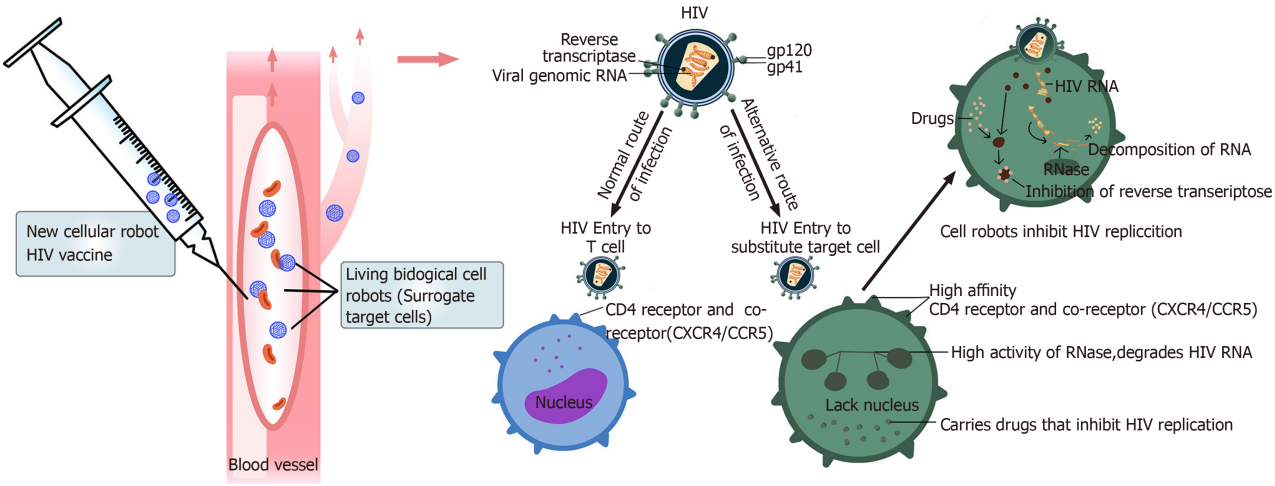

Figure 3 Functions of a living biological cell robot. After entering the body, the living cell robot mediates human immunodeficiency virus (HIV) infection by the high-affinity receptor expressed on the cell surface, and HIV is killed by drugs or RNase carried by the cell. HIV: Human immunodeficiency virus.

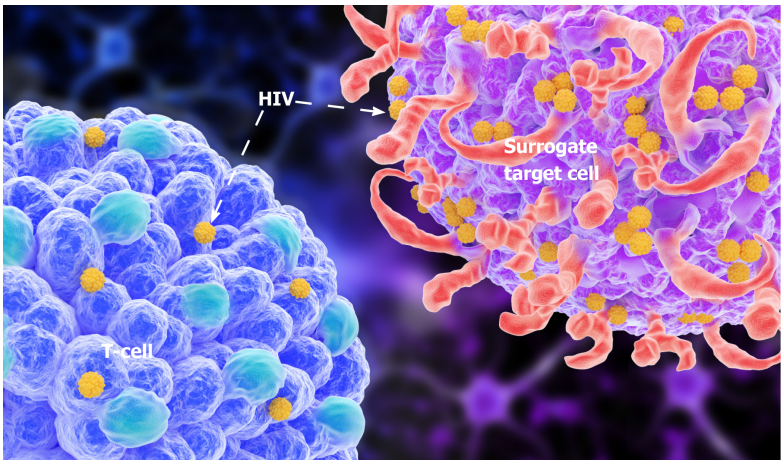

Figure 4 A simulation of human immunodeficiency virus infecting a surrogate target cell by the alternative infection. By blocking the receptor on the T cell surface with the blocking antibody, the probability of human immunodeficiency virus infection surrogate target cell will increase. HIV: Human immunodeficiency virus.

CONCLUSION

However, we note that the new cell robot vaccine is still in the early stages of development, and many fundamental questions need to be addressed before it can be put into practical use. If cell robots can be made from patients' cells, the technology could be used for drug delivery in humans. Otherwise, it may elicit problematic immune responses. The most successful HIV vaccine is still in early trial stages at the population level. Although the results are promising, further large-scale clinical trials are needed before it can be deemed suitable for clinical application. The HIV vaccine induces antibody response in the body, but it does not show that it can effectively fight HIV and prevent AIDS. HIV mainly attacks the body's immune system, and the production of antibodies in traditional vaccines is inseparable from the immune system, which often causes failure of vaccination. So far, there has not been a single HIV self-healing case, suggesting that our immune system alone cannot suppress or eliminate HIV. More importantly, the primary defense against HIV in humans relies on cytotoxic T lymphocytes (CTL), which secrete a variety of cytokines involved in immune function. In the fight against HIV, the central role of CTL is to kill cells that have been invaded by the virus, thereby halting the reproduction of HIV. However, none of the vaccines developed so far are effective at activating CTL. Another challenge in vaccine development is the long incubation period of HIV/AIDS, which can last for years or decades. As a result, highly active antiretroviral therapy remains by far the most popular treatment for HIV, and we are still a long way from a truly

widespread HIV vaccine.

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REFERENCES

1 **Shapiro SZ**. A proposal to use iterative, small clinical trials to optimize therapeutic HIV vaccine immunogens to launch therapeutic HIV vaccine development. *AIDS Res Hum Retroviruses* 2015; **31**: 49-55 [PMID: 25286142 DOI: 10.1089/aid.2014.0172]

2 **Ncube B**, Ansong J, Daniels K, Campbell-Stennett D, Jolly PE. Sexual risk behavior among HIV-positive persons in Jamaica. *Afr Health Sci* 2017; **17**: 32-38 [PMID: 29026375 DOI: 10.4314/ahs.v17i1.6]

3 **Chen M**, Jiang SB, Liu SW. Host cell proteins associated with HIV infection. *Xibao Yu Fenzi Mianyixue Zazhi* 2009; **25**: 662-664 [DOI: 10.3321/j.issn:1007-8738.2009.07.029]

4 **Redd AD**, Laeyendecker O, Kong X, Kiwanuka N, Lutalo T, Huang W, Gray RH, Wawer MJ, Serwadda D, Eshleman SH, Quinn TC; Rakai Health Sciences Program. Efficiency of CCR5 coreceptor utilization by the HIV quasispecies increases over time, but is not associated with disease progression. *AIDS Res Hum Retroviruses* 2012; **28**: 289-294 [PMID: 21663455 DOI: 10.1089/aid.2011.0006]

5 **Giraut A**, Song XP, Froeyen M, Marlière P, Herdewijn P. Iminodiacetic-phosphoramidates as metabolic prototypes for diversifying nucleic acid polymerization in vivo. *Nucleic Acids Res* 2010; **38**: 2541-2550 [PMID: 20097909 DOI: 10.1093/nar/gkp1246]

6 **Kriegman S**, Blackiston D, Levin M, Bongard J. A scalable pipeline for designing reconfigurable organisms. *Proc Natl Acad Sci USA* 2020; **117**: 1853-1859 [PMID: 31932426 DOI: 10.1073/pnas.1910837117]

7 **Sun Y**, Xu GL. Advances in HIV vaccine research. *Guoji Shengwuzhipinxue Zazhi* 2008; **4**: 167-171 [DOI: 10.3760/cma.j.issn.1673-4211.2008.04.007]

8 **Yamamoto T**, Kanuma T, Takahama S, Okamura T, Moriishi E, Ishii KJ, Terahara K, Yasutomi Y. STING agonists activate latently infected cells and enhance SIV-specific responses ex vivo in naturally SIV controlled cynomolgus macaques. *Sci Rep* 2019; **9**: 5917 [PMID: 30976083 DOI: 10.1038/s41598-019-42253-3]

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OPINION REVIEW

Current status of COVID-19 treatment: An opinion review

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Abstract

The pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has garnered the attention of scientists worldwide in the search for an effective treatment while also focusing on vaccine development. Several drugs have been used for the management of coronavirus disease 2019 (COVID-19), which has affected many hospitals and health centers worldwide. Statistically significant results are lacking on the effectiveness of the experimented drugs in reducing COVID-19 morbidity or mortality, as there are very few published randomized clinical trials. Despite this, the literature offers some material for study and reflection. This opinion review attempts to address three burning questions on COVID-19 treatment options. (1) What kind of studies are currently published or ongoing in the treatment of patients with COVID-19? (2) What drugs are currently described in the literature as options of treatment for patients affected by the infection? And (3) Are there specific clinical manifestations related to COVID-19 that can be treated with a customized and targeted therapy? By answering these questions, we wish to create a summary of current COVID-19 treatments and the anti-COVID-19 treatments proposed in the recent clinical trials developed in the last 3 mo, and to describe examples of clinical manifestations of the SARS-CoV-2 infection with a cause-related treatment.

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Core Tip: The pandemic spread of coronavirus disease 2019 (COVID-19) has led to the need to standardize a therapeutic approach in order to offer the same indications for all patients admitted to the hospital admissions for severe acute respiratory syndrome coronavirus 2 infection. However, no specific drug or drug regimen has been approved for treatment. This opinion review describes the recent literature on this topic and summarizes the treatment strategies currently in use for COVID-19 related complications.

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INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, defined as coronavirus disease 2019 (COVID-19), was originally discovered and identified as the cause of numerous viral pneumonia cases occurring in Wuhan (Hubei Province, China)[1]. It has now spread worldwide with recent epidemiological data reporting 18614177 infected and 702642 deaths[2]. In the context of a growing global health emergency, medical professionals demand a need for up-to-date guidelines for the management and treatment of this novel infection. Currently, there are multiple approved treatments (drugs, monoclonal antibodies, vaccines) for COVID-19. According to the WHO Interim Guidance on the 2019 coronavirus, early treatment is suggested if patients have confirmed diagnosis with mild symptoms associated with co-morbidities, increased risk of mortality, or moderate-severe clinical manifestations. Due to the swab time delay for confirmation of results, in the presence of a strong suggestive clinical presentation it is reasonable to start antiviral treatment as soon as possible[3]. Due to the severity of clinical symptoms and no statistically significant recommended treatment regimen, experimental use of a drug not yet approved may be necessary to improve patients' outcomes. To get more oriented among the numerous options of treatments proposed, this opinion review will summarize and clarify the role of each drug that has been used against COVID-19 in the clinical practice and those now under scientific examination.

WHAT KIND OF STUDIES ARE CURRENTLY PUBLISHED OR ONGOING IN THE TREATMENT OF PATIENTS WITH COVID-19?

The treatments proposed in the literature for COVID-19 are mainly based on the results of retrospective or observational studies, making it more difficult to hypothesize evidence-based therapies. Due to the need for more reliable data, the number of ongoing clinical trials are increasing. According to the International Clinical Trial Registry Platform database there are 1918 reported studies, with 1744 ongoing on COVID-19 patients[4]. Of these studies, 1661 are specific for the treatment of SARS-CoV-2 infection.

The most common classes of drugs used include antimalarial drugs, immunomodulators, convalescent plasma (CP), antiretrovirals, antibacterial drugs, lipid-lowering medications, anticoagulants and recently ivermectin. In this context, a large volume of data will soon be available and provide valuable novel recommendations regarding pathogenesis, treatment and prognosis[5].

WHAT DRUGS ARE CURRENTLY DESCRIBED IN THE LITERATURE AS OPTIONS OF TREATMENT FOR PATIENTS AFFECTED BY SARS-COV-2 INFECTION?

There is currently no therapy for COVID-19 infection whose efficacy has been proven. Nonetheless due to the current global crisis, it is crucial to be able to formulate an effective therapeutic strategy based on the evidence existing in the literature. From the analysis of all of the clinical trials designed on COVID-19 infection, we found a wide number of drugs employed in a multimodal treatment.

Hydroxychloroquine, an old anti-malaria drug, displayed the ability to inhibit coronavirus replication *in vitro*. Real-life data are currently discordant in recognizing its anti-SARS-CoV-2 claimed effect[6,7]. Since the virus was found to utilize the cell surface receptor angiotensin-converting enzyme 2 (ACE2) expressed in the lung, heart, kidney, and intestine[8], it has been hypothesized that hydroxychloroquine may also interfere with ACE2 receptor glycosylation, thus preventing SARS-CoV-2 binding to target cells[9]. In addition, hydroxychloroquine can inhibit the acidification of lysosomes and endosomes, interfering with the fusion process of the virus with the host[10]. The results of the Phase 3 clinical trial NCT: 04315948 may clarify the role of hydroxychloroquine in COVID-19 patients' prognosis.

Chloroquine alone or in combination with remdesivir and/or tocilizumab (under investigation by the clinical trial NCT: 04303507) may be effective against COVID-19 despite the more dangerous side effects than compared to hydroxychloroquine[11].

Among the drugs that seem to possess immunomodulatory benefits and reduce SARS-CoV-2 cell penetration, there are statins (the most prescribed ones are the atorvastatin 20 mg/d or an equivalent dose of rosuvastatin 40 mg/d). Statins act by reducing chemokine release, adhesion molecules, and modulating T-cell activity. Rosuvastatin, in particular, appears to have direct antiviral properties by binding and inhibiting the active site of the main protease enzyme (Mpro) of SARS-CoV-2. In a retrospective analysis Zhan *et al*[12] found that statin treatment among 13981 patients with COVID-19 was associated with a lower risk of all-cause mortality. Furthermore, the addition of ACE inhibitors or angiotensin II receptor blockers did not affect statin-associated outcomes in the studied cohort[12].

Remdesivir is a nucleoside analogue with a promising virus-inhibitory effect. It exhibits *in vitro* antiviral activity against coronaviruses[13] and *in vivo* has been shown to curb severe acute respiratory syndrome caused by coronavirus infection[14]. The drug can also inhibit viral replication interfering with the nascent viral-RNA chain resulting in its premature termination[15]. In a Phase 1 clinical trial the security and pharmacological effects of remdesivir were assessed[16]. Recently, in patients with severe COVID-19 receiving remdesivir clinical improvement was observed in 68% of cases (36 of 53 patients)[17]. However in a randomized, double-blind, placebo-controlled, multicenter trial, remdesivir was not associated with statistically significant clinical benefits[18]. Ongoing clinical trials (NCT: 04292899 and 04292730) should provide additional data on its effectiveness.

Azithromycin has shown *in vitro* antiviral activity against SARS-CoV-2, documented in literature at dosages similar to those used to treat bacterial pneumonia[19,20]. The mechanism of action is not well understood. It is believed to interfere with the acidification processes of lysosomes and endosomes[21] or amplification of the antiviral action of interferon in the host[22]. The use of azithromycin in combination with chloroquine/hydroxychloroquine has been described in the treatment of COVID-19 but the available clinical data is derived from retrospective, observational or uncontrolled studies[23,24]. The randomized telemedicine-based trial NCT: 04332107, now in Phase 3, may elicit further information.

Lopinavir and ritonavir are protease inhibitors used in HIV infections. Their use in combination allows the increase in half-life of lopinavir by enzymatic induction[25]. It has demonstrated *in vitro* antiviral activity for SARS-CoV[26] and MERS-CoV through inhibition of the 3-chymotrypsin-like protease[27]. Currently, there is no statistically significant evidence of its efficacy against SARS-CoV-2 *in vitro*. The studies available on the use of the lopinavir/ritonavir combination for the treatment of COVID-19 are mainly reports or retrospective studies, making it difficult to evaluate its effectiveness. In a randomized, controlled, open-label Chinese trial, no benefit was observed with lopinavir-ritonavir treatment[28]. There are several ongoing clinical trials. Among them NCT: 02735707 in its recruiting phase, is structured to compare the administration of lopinavir-ritonavir with no antiviral treatment.

Tocilizumab and sarilumab are monoclonal antibodies directed against the interleukin 6 (IL-6) receptor in which COVID-19 appears to target in the severe

inflammatory process and cytokine storm causing critical damage to the lungs and other organs[29,30]. Tocilizumab appears to be a viable treatment strategy in COVID-19 patients with risk of developing cytokine storm[30]. Studies supporting this thesis are mainly case-reports and retrospective analyses. There are a few randomized clinical trials (RCTs) in development (ChiCTR: 200002976, EuCTR: 2020-001110-38 NCT: 04320615) for the evaluation of the efficacy and safety of Tocilizumab, alone or in combination, in the treatment of severe pneumonia in COVID-19 hospitalized patients. Sarilumab is currently being studied in a multicenter Phase 2-3 study for the treatment of severe forms of COVID-19 (NCT: 04315298).

Anakinra is another monoclonal antibody used in the treatment of patients in critical condition. (NCT: 04330638). By blocking the IL-1 receptor, the drug could help reduce the cytokine storm triggered by the virus[31].

The monoclonal antibody eculizumab, which prevents the cleavage of the C5 fraction of the complement in the C5a and C5b, could also reduce this cytokine cascade. Currently, an encouraging case series has been published on the topic by Diurno *et al*[32] (2020) and we are looking forward to the results NCT: 04288713.

Among the immunomodulatory drugs with a possible action in reducing cytokine storm, colchicine has also been used. It is a non-selective inhibitor of NLRP3 inflammasome, and inhibitor of microtubule polymerization and leukocyte infiltration[33]. The COLCORONA trial is now ongoing in the recruiting phase (NCT: 04322682), while the GRECCO-19 study (NCT: 04326790) of 189 patients has recruited the necessary samples[34].

Several studies have analyzed the role of corticosteroids. Such drugs could theoretically act as immunomodulators. Wang *et al*[35] completed a randomized controlled trial, albeit with few patients, on the use of methylprednisolone highlighting that the short-term administration of the drug could be beneficial. In February 2020, Villar *et al*[36] published a randomized clinical study (NCT: 01731795) of 277 patients that defined the usefulness of the early administration of dexamethasone in reducing days of endotracheal intubation and overall mortality. Although these studies seem encouraging, further evidence of efficacy is needed[36].

In addition, CP has frequently been used as supplement therapy. It is a classic adaptive immunotherapy that was successfully and safely used in the treatment of infections caused by viruses similar to SARS-CoV-2[37], such as SARS, MERS, and in the 2009 H1N1 pandemic[38,39]. Data from the meta-analysis conducted By Mair-Jenkins *et al*[40] reported that this treatment can reduce the mortality of patients with COVID-19 especially if administered early to the onset of symptoms. The limitation of this treatment is the scarce availability of donor plasma considering that only recovered COVID-19 patients with neutralizing antibody titers above 1:640 are considered good plasma donors. Once the plasma is collected from donors, it is adequately treated and then infused into clinically symptomatic patients. A single 200 mL transfusion of CP is generally well tolerated and followed by improvement of the clinical symptoms. There is a subsequent increase of oxyhemoglobin saturation within 3 d and a rapid neutralization of the viremia[41]. The clinical trial NCT: 04321421 would clarify the usefulness of this treatment.

Among the integrative treatments, vitamin C infusion may produce an increase in the synthesis of norepinephrine and vasopressin[42], reduce cytokine levels[43], and prevent neutrophil activation and trap formation promoting vascular injury[44]. Its role is being investigated by the clinical trial NCT: 04264533.

Due to the increased incidence of thrombo-inflammation and hypercoagulability related to COVID-19, enoxaparin which inhibits factor Xa and thrombin is frequently present in almost all the clinical practice protocols of treatment[45]. The clinical trial identified as NCT: 04367831 is investigating the role of enoxaparin in COVID-19 and is currently ongoing.

There has also been an increase in tumor necrosis factor alpha (TNF-α) and IL-17 in peripheral blood samples of COVID-19 patients, but evidence in customized treatment is still lacking. Encouraging results arrived from real-life data in a large cohort of psoriatic patients on biologic agents. They demonstrated an increased risk of infection rates but without an increased risk of intensive care unit hospitalization or death[46]. Two trials were registered in the Chinese Clinical Trial Registry (ChiCTR2000030089, ChiCTR2000030703) that evaluate the potential use of adalimumab (anti-TNF-α) and ixekizumab (anti-IL-17) in the armamentarium to treat severe COVID-19 patients. Apremilast, a phosphodiesterase type 4 inhibitor, was a candidate treatment because it demonstrated inhibition of neutrophil, monocyte and lymphocyte migration during lung inflammation and decreased pro-inflammatory cytokine production[47].

A wide array of drugs used in current clinical practice but not yet approved and investigated by more than three ongoing clinical trials are presented in Table 1.

Table 1 Summary of the most investigated drugs for the treatment of coronavirus disease 2019

Therapeutic agent	Mechanism of action	Ongoing trials, *n*	Associations	Suggested dosage	Route of administration	Principal side effects	Ref.	NCT identifier
Hydroxychloroquine	Changes the pH of endosomes, prevents viral entry, transport and post-entry replication	224	Azithromycin, tocilizumab, lopinavir-ritonavir	200 mg BID or TID (10 d)	Oral-intravenous	Retinal toxicity, QT prolongation, nausea	[6-10]	NCT04315948
Chloroquine	Increases the endosomal pH interfering with the process of virus/cell fusion	225	Remdesivir, tocilizumab	2.5 g (3 d)	Oral-intravenous	Retinal toxicity, QT prolongation, nausea	[11]	NCT04303507
Convalescent plasma	Adaptive immunotherapy (neutralizing antibody tiers above 1:640)	129	Remdesivir, Interferon-alpha, oseltamivir, antibacterial and antifungal frugal drugs, methylprednisolone	200 ml single dose	Intravenous	Evanescent facial red spot	[37-41]	NCT04321421
Lopinavir/Ritonavir	Inhibition of the HIV protease/inhibition of CitP450-iso3A4 and augmented plasmatic concentration of lopinavir	67	Hydroxychloroquine, azithromycin, dexamethasone	200 mg/50 mg BID	Oral	Gastrointestinal upset, augmented plasmatic concentration of colchicine And HGAM-CoA reductase inhibitors	[25-28]	NCT02735707
Azithromycin	Prophylaxis of bacterial super-infection	59	Hydroxychloroquine, tocilizumab, atovaquone	500 mg	Oral-intravenous	QT prolongation	[19-24]	NCT04332107
Tocilizumab	Monoclonal antibody which targets the IL-6 receptor	50	lopinavir-ritonavir, remdesivir, chloroquine, hydroxychloroquine	Dosing according to weight range	Intravenous	Runny or stuffy nose, sinus pain, sore throat, headache, gastrointestinal upset, urinary tract infection	[30]	NCT04320615
Ivermectin	Suppression of SARS-CoV-2 viral replication in cell cultures (*in vitro*)	30	Hydroxychloroquine Dutasteride Azithromycin Proxalutamide	600 mcg/kg	Oral	Tiredness, loss of energy, stomach pain, loss of appetite, nausea, vomiting, diarrhea, dizziness	[48]	NCT04381884
Statin	Reduces chemokine release, adhesion molecules, and modulating T cell activity	23	Standard of care; colchicine + rosuvastatin	20 mg/d atorvastatin Rosuvastatin 40 mg/d or equivalent	Oral	rabdomiolisis	[12]	NCT04472611
Remdesivir	Nucleotide analogue that is incorporated into the nascent viral RNA chain resulting in its premature termination	20	Hydroxychloroquine, chloroquine, tocilizumab, convalescent plasma	200 mg 1st day – 100 mg (10 d)	Intravenous	Phlebitis, constipation, headache, ecchymosis, nausea, pain in extremities	[13-18]	NCT04292899
Methylprednisolone	Immunosuppression against cytokine storm	17	Siltuximab, tacrolimus	40 mg BID (5 d) - f 1-2 mg/kg/d (5-7 d)	Oral-intravenous	Headache, nausea, weight gain, excitement, infections	[35]	NCT04323592
Sarilumab	Monoclonal antibody which targets the IL-6 receptor	17	Not available	400 mg or 200mg single dose	Intravenous	Neutropenia, increased ALT, injection site redness, upper respiratory infections, nasal congestion, sore throat, urinary tract infections,	[29]	NCT04315298

						thrombocytopenia		
Colchicine	Non-selective inhibitor of NLRP3 inflammasome, inhibitor of microtubule polymerization and leukocyte infiltration	17	Not available	0.5 mg per os (BID) for 3 d - then once daily for the last 27 d	Oral-intravenous	Gastrointestinal upset, low blood cells count and rhabdomyolysis	[33-34]	NCT04322682
Heparin	Inhibition of Xa factor and thrombin	15	Methylprednisolone	Dosed to target activated partial thromboplastin time (aPTT) between 1.5-2.0 times the normal value	Subcutaneous injection	Reduced creatinine clearance	[45]	NCT04485429
Anakinra	Monoclonal antibody which targets the IL-1 receptor	11	Siltuximab or tocilizumab (single i.v. injection)	1 injection a day (max 28 d)	Subcutaneous injection	Gastrointestinal upset, headache, joint pain, flu symptoms, redness-bruising-pain in the injection site	[30,31]	NCT04330638
Dexamethasone	Immunosuppression against cytokine storm	10	Not available	20 mg/d (5 d) then 10 mg/d (5 d)	Intravenous	Headache, weight gain, excitement, infections	[36]	NCT04325061
Enoxaparin	Inhibition of Xa factor and thrombin	5	Not available	4000 UI/d or 100 UI/kg	Subcutaneous injection	Skin irritation in injection site, bleeding, heparin-induced thrombocytopenia, fatigue, fever	[45]	NCT04367831
Eculizumab	Monoclonal antibody which targets C5 inhibiting its cleavage in C5a and C5b	3	Hydroxychloroquine, lopinavir-ritonavir, ceftriaxone, vitamin C	3600 mg/wk (8-22 wk)	Intravenous	Fever, headache, nausea and vomiting, body aches, confusion, increased sensitivity to light, stiffness	[32]	NCT04288713
Vitamin C	Antioxidant, increases the synthesis of norepinephrine and vasopressin, attenuate increases in cytokine levels	3	All mentioned drugs	12 g/12 h (7 d) or 50 mg/kg/6 h (4 d)	Oral-intravenous	Gastrointestinal upset	[42-44]	NCT04264533

In this table are the most utilized and investigated drugs for the treatment of coronavirus disease 2019. It provides the name of the therapeutic agent, mechanism of action, number of ongoing trials, drug association if available, suggested dosage and maximum period of time the drug should be administered, route of administration, principal side effects, a reference on the drug, and an example of randomized clinical trial (RCT) identified by the national clinical trial number. Drugs investigated in less than three RCTs have been excluded. BID: Bis in die; IL-1: Interleukin 1; NCT: National clinical trial; RCT: Randomized clinical trial; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; TID: Tris in die.

Among these drugs is Ivermectin, an FDA-approved anti-parasitic. This drug showed to have broad-spectrum anti-viral activity only *in vitro*[48], and results of a Phase 1 study are absolutely needed before using ivermectin. There are not enough data to support a recommendation for its use in a higher-than-approved dosage.

ARE THERE SPECIFIC CLINICAL MANIFESTATIONS RELATED TO COVID-19 THAT CAN BE TREATED WITH A CUSTOMIZED AND TARGETED THERAPY?

The pathogenesis of the damage induced by the SARS-CoV-2 virus is presently being analyzed. There are two clinical manifestations that are most frequently found in infected patients, namely respiratory failure and systemic coagulopathy. Currently it appears, according to a report by Magro *et al*[49], that the multiple district damage induced by the infection is caused by a hyper-activation of the complement system and exacerbation of the cytokine cascade. On histological samples of patients' lung and skin tissue who died from COVID-19, there was a discernable deposit of C5b9, C4d, and the mannose-binding lectin-associated serine protease 2. These proteins are the residual products of complement activation[49]. Once the host's barriers are overcome, the virus stimulates the innate immunity and enters the cell by binding to the ACE2 receptor. It goes on to destroy the endothelial cells of all organs whose cells express the ACE2 receptor widely. The same cell destruction increases tissue permeability and facilitates the systemic release of the virus. This results in hyperproduction of interleukins (cytokine storm and intracellular activation of the inflammasome) and hypercoagulability with diffuse thrombosis in the microcirculation.

Due to the unproven efficacy of antiviral drugs alone, there is strong reason to believe it useful to administer other drug treatments to facilitate meaningful recovery. Drugs such as eculizumab, which act by blocking the cleavage/activation of complement factor C5; tocilizumab, sarilumab, and anakinra which block the interleukin receptors by limiting the cytokine cascade; colchicine which acts by interfering with the inflammasome NLRP3; vitamin C which may reduce the activation of neutrophils and stimulate the endogenous production of vasopressors; and enoxaparin which assists in the prevention and treatment of hypercoagulation thrombosis. The mechanisms of drug action according to cell damage, complement activation and cytokine storm are described in Figure 1.

CONCLUSION

Although a short period of time has passed since the novel coronavirus was initially described, several treatment options have been introduced. To simplify the current therapeutic armamentarium, Table 1 summarizes the most investigated options for the treatment of COVID-19 in decreasing order by number of ongoing RCTs. Nonetheless there is still no proven evidence based therapeutic plan that can offer the best survival chance to patients infected. The inconsistent results presented in the literature on the treatment of SARS-CoV-2, is likely due to the lack of well-controlled studies with an adequate sample size. A meticulous understanding of the pathophysiology and immunological response of the host is also still necessary. Additional data are required to provide a proper risk stratification for patients and an adequate place in therapy of current investigational options.

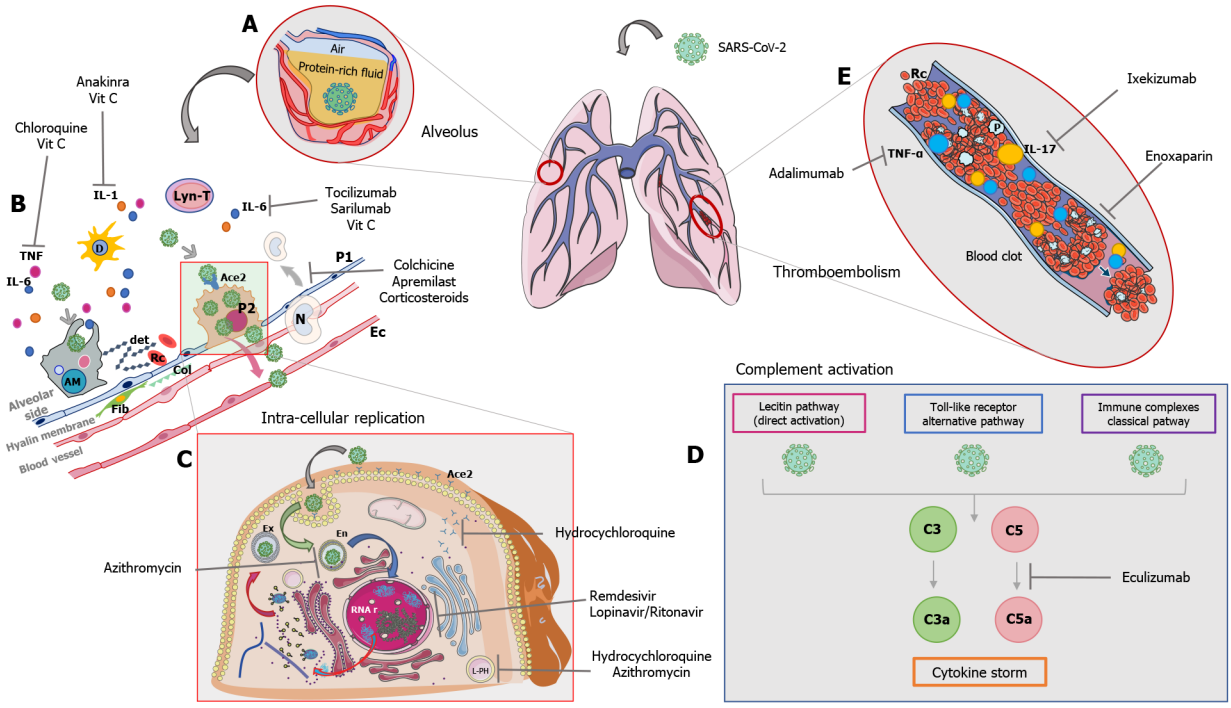

Figure 1 Severe acute respiratory syndrome coronavirus 2 infection, consequences and rationale of treatment. A: The virus in the alveolus induces the production of a protein rich fluid interfering with ventilation gas exchanges; B: Description of what happens in the alveolus after the infection and drugs involved in this phase. The virus binds the angiotensin-converting enzyme 2 (ACE2) receptor and replicates in the Type 2 pneumocyte (P2), leading to apoptosis (accumulation of cellular dendrites), activation of alveolar macrophages, dendritic cells, lymphocytes T, and neutrophils. There is an augmentation of cytokines levels, especially interleukin 6 (IL-6), IL-1, and tumor necrosis factor (TNF). The inflammatory process leads to damage of the alveolar barrier and to the formation of the hyaline membrane between the alveolar side and the blood vessels. In this phase, chloroquine reduces the levels of TNF; vitamin C reduces the levels of TNF, IL-1, and IL-6; anakinra inhibits IL-1 binding to its receptor; tocilizumab and sarilumab inhibit IL-6 binding to its receptor; and colchicine, apremilast, and corticosteroids reduce the migration of lymphoid cells into the alveolus; C: Synthesis of the intra-cellular replication of the virus. Hydroxychloroquine reduces the exposition of ACE2 receptors on the surface of P2, and as azithromycin reduces the pH in lysosomes and endosomes. Remdesivir and lopinavir/ritonavir interfere with viral replication; D: The virus is capable of activating the complement system that can be inhibited by eculizumab. E: Enoxaparin may be effective against the typical thromboembolism induced by the virus. Furthermore, in the plasmatic torrent, the high levels of TNF-alpha and IL-17 can be reduced by selective binders such as adalimumab and ixekizumab, respectively. Original picture by Di Franco S and Alfieri A (2020).

REFERENCES

1 **Zhou P**, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**: 270-273 [PMID: 32015507 DOI: 10.1038/s41586-020-2012-7]

2 **World Health Organization**. Coronavirus disease 2019 (COVID-19) Situation Report – 199 - 6 Aug 2020: World Health Organization; 2020. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200806-covid-19-sitrep-199.pdf?sfvrsn=6b9d262d_2

3 **World Health Organization**. Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected: interim guidance, 28 January 2020. Contract No: WHO/nCoV/Clinical/2020.3 Available from: https://apps.who.int/iris/handle/10665/330893

4 **World Health Organization**. International Clinical Trials Registry Platform (ICTRP). Consulted on May 2020. Available from: https://apps.who.int/trialsearch/

5 **Bragazzi NL**, Dai H, Damiani G, Behzadifar M, Martini M, Wu J. How Big Data and Artificial Intelligence Can Help Better Manage the COVID-19 Pandemic. *Int J Environ Res Public Health* 2020; 17 [PMID: 32370204 DOI: 10.3390/ijerph17093176]

6 **Gendelman O**, Amital H, Bragazzi NL, Watad A, Chodick G. Continuous hydroxychloroquine or colchicine therapy does not prevent infection with SARS-CoV-2: Insights from a large healthcare database analysis. *Autoimmun Rev* 2020; **19**: 102566 [PMID: 32380315 DOI: 10.1016/j.autrev.2020.102566]

7 **McKee DL**, Sternberg A, Stange U, Laufer S, Naujokat C. Candidate drugs against SARS-CoV-2 and COVID-19. *Pharmacol Res* 2020; **157**: 104859 [PMID: 32360480 DOI: 10.1016/j.phrs.2020.104859]

8 **Wang PH**, Cheng Y. Increasing Host Cellular Receptor—Angiotensin-Converting Enzyme 2 (ACE2) Expression by Coronavirus may Facilitate 2019-nCoV Infection. 2020 Preprint. Available from: bioRxiv [DOI: 10.1101/2020.02.24.963348]

9 **Devaux CA**, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? *Int J Antimicrob Agents* 2020; **55**: 105938 [PMID: 32171740 DOI: 10.1016/j.ijantimicag.2020.105938]

10 **Zhou D**, Dai SM, Tong Q. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. *J Antimicrob Chemother* 2020; **75**: 1667-1670 [PMID: 32196083 DOI:

10.1093/jac/dkaa114]
11 **Wang M**, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020; **30**: 269-271 [PMID: 32020029 DOI: 10.1038/s41422-020-0282-0]
12 **Zhang XJ**, Qin JJ, Cheng X, Shen L, Zhao YC, Yuan Y, Lei F, Chen MM, Yang H, Bai L, Song X, Lin L, Xia M, Zhou F, Zhou J, She ZG, Zhu L, Ma X, Xu Q, Ye P, Chen G, Liu L, Mao W, Yan Y, Xiao B, Lu Z, Peng G, Liu M, Yang J, Yang L, Zhang C, Lu H, Xia X, Wang D, Liao X, Wei X, Zhang BH, Zhang X, Yang J, Zhao GN, Zhang P, Liu PP, Loomba R, Ji YX, Xia J, Wang Y, Cai J, Guo J, Li H. In-Hospital Use of Statins Is Associated with a Reduced Risk of Mortality among Individuals with COVID-19. *Cell Metab* 2020; **32**: 176-187.e4 [PMID: 32592657 DOI: 10.1016/j.cmet.2020.06.015]
13 **Al-Tawfiq JA**, Al-Homoud AH, Memish ZA. Remdesivir as a possible therapeutic option for the COVID-19. *Travel Med Infect Dis* 2020; **34**: 101615 [PMID: 32145386 DOI: 10.1016/j.tmaid.2020.101615]
14 **Sheahan TP**, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, Mackman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med* 2017; **9** [PMID: 28659436 DOI: 10.1126/scitranslmed.aal3653]
15 **Agostini ML**, Andres EL, Sims AC, Graham RL, Sheahan TP, Lu X, Smith EC, Case JB, Feng JY, Jordan R, Ray AS, Cihlar T, Siegel D, Mackman RL, Clarke MO, Baric RS, Denison MR. Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. *mBio* 2018; **9** [PMID: 29511076 DOI: 10.1128/mBio.00221-18]
16 **World Health Organization**. WHO R&D Blueprint – Ad-hoc Expert Consultation on clinical trials for Ebola Therapeutics. 11 October 2018. Available from: https://www.who.int/ebola/drc-2018/treatments-approved-for-compassionate-use-update/en/
17 **Grein J**, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, Feldt T, Green G, Green ML, Lescure FX, Nicastri E, Oda R, Yo K, Quiros-Roldan E, Studemeister A, Redinski J, Ahmed S, Bernett J, Chelliah D, Chen D, Chihara S, Cohen SH, Cunningham J, D'Arminio Monforte A, Ismail S, Kato H, Lapadula G, L'Her E, Maeno T, Majumder S, Massari M, Mora-Rillo M, Mutoh Y, Nguyen D, Verweij E, Zoufaly A, Osinusi AO, DeZure A, Zhao Y, Zhong L, Chokkalingam A, Elboudwarej E, Telep L, Timbs L, Henne I, Sellers S, Cao H, Tan SK, Winterbourne L, Desai P, Mera R, Gaggar A, Myers RP, Brainard DM, Childs R, Flanigan T. Compassionate Use of Remdesivir for Patients with Severe Covid-19. *N Engl J Med* 2020; **382**: 2327-2336 [PMID: 32275812 DOI: 10.1056/NEJMoa2007016]
18 **Wang Y**, Zhang D, Du G, Du R, Zhao J, Jin Y, Fu S, Gao L, Cheng Z, Lu Q, Hu Y, Luo G, Wang K, Lu Y, Li H, Wang S, Ruan S, Yang C, Mei C, Wang Y, Ding D, Wu F, Tang X, Ye X, Ye Y, Liu B, Yang J, Yin W, Wang A, Fan G, Zhou F, Liu Z, Gu X, Xu J, Shang L, Zhang Y, Cao L, Guo T, Wan Y, Qin H, Jiang Y, Jaki T, Hayden FG, Horby PW, Cao B, Wang C. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020; **395**: 1569-1578 [PMID: 32423584 DOI: 10.1016/S0140-6736(20)31022-9]
19 **Touret F**, Gilles M, Barral K, Nougairède A, Decroly E, de Lamballerie X, Coutard B. In vitro screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. 2020 Preprint. Available from: bioRxiv [DOI: 10.1101/2020.04.03.023846]
20 **Andreani J**, Le Bideau M, Duflot I, Jardot P, Rolland C, Boxberger M, Wurtz N, Rolain JM, Colson P, La Scola B, Raoult D. In vitro testing of combined hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect. *Microb Pathog* 2020; **145**: 104228 [PMID: 32344177 DOI: 10.1016/j.micpath.2020.104228]
21 **Tyteca D**, Van Der Smissen P, Mettlen M, Van Bambeke F, Tulkens PM, Mingeot-Leclercq MP, Courtoy PJ. Azithromycin, a lysosomotropic antibiotic, has distinct effects on fluid-phase and receptor-mediated endocytosis, but does not impair phagocytosis in J774 macrophages. *Exp Cell Res* 2002; **281**: 86-100 [PMID: 12441132 DOI: 10.1006/excr.2002.5613]
22 **Li C**, Zu S, Deng YQ, Li D, Parvatiyar K, Quanquin N, Shang J, Sun N, Su J, Liu Z, Wang M, Aliyari SR, Li XF, Wu A, Ma F, Shi Y, Nielsevn-Saines K, Jung JU, Qin FX, Qin CF, Cheng G. Azithromycin Protects against Zika virus Infection by Upregulating virus-induced Type I and III Interferon Responses. *Antimicrob Agents Chemother* 2019; **63**: e00394-19 [PMID: 31527024 DOI: 10.1128/AAC.00394-19]
23 **Gautret P**, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Tissot Dupont H, Honoré S, Colson P, Chabrière E, La Scola B, Rolain JM, Brouqui P, Raoult D. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020; **56**: 105949 [PMID: 32205204 DOI: 10.1016/j.ijantimicag.2020.105949]
24 **Gautret P**, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, Mailhe M, Doudier B, Aubry C, Amrane S, Seng P, Hocquart M, Eldin C, Finance J, Vieira VE, Tissot-Dupont HT, Honoré S, Stein A, Million M, Colson P, La Scola B, Veit V, Jacquier A, Deharo JC, Drancourt M, Fournier PE, Rolain JM, Brouqui P, Raoult D. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study. *Travel Med Infect Dis* 2020; **34**: 101663 [PMID: 32289548 DOI: 10.1016/j.tmaid.2020.101663]
25 **Sheahan TP**, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, Montgomery SA, Hogg A, Babusis D, Clarke MO, Spahn JE, Bauer L, Sellers S, Porter D, Feng JY, Cihlar T, Jordan R, Denison MR, Baric RS. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nat Commun* 2020; **11**: 222 [PMID: 31924756 DOI: 10.1038/s41467-019-13940-6]
26 **Chu CM**, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, Kao RY, Poon LL, Wong CL, Guan Y, Peiris JS, Yuen KY; HKU/UCH SARS Study Group. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax* 2004; **59**: 252-256 [PMID: 14985565 DOI: 10.1136/thorax.2003.012658]
27 **de Wilde AH**, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, van den Hoogen BG, Neyts J, Snijder EJ. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob*

Agents Chemother 2014; **58**: 4875-4884 [PMID: 24841269 DOI: 10.1128/AAC.03011-14]
28 **Cao B**, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Wang J, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med* 2020; **382**: 1787-1799 [PMID: 32187464 DOI: 10.1056/NEJMoa2001282]
29 **Lu CC**, Chen MY, Lee WS, Chang YL. Potential therapeutic agents against COVID-19: What we know so far. *J Chin Med Assoc* 2020; **83**: 534-536 [PMID: 32243270 DOI: 10.1097/JCMA.0000000000000318]
30 **Zhang C**, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. *Int J Antimicrob Agents* 2020; **55**: 105954 [PMID: 32234467 DOI: 10.1016/j.ijantimicag.2020.105954]
31 **Mehta P**, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; **395**: 1033-1034 [PMID: 32192578 DOI: 10.1016/S0140-6736(20)30628-0]
32 **Diurno F**, Numis FG, Porta G, Cirillo F, Maddaluno S, Ragozzino A, De Negri P, Di Gennaro C, Pagano A, Allegorico E, Bressy L, Bosso G, Ferrara A, Serra C, Montisci A, D'Amico M, Schiano Lo Morello S, Di Costanzo G, Tucci AG, Marchetti P, Di Vincenzo U, Sorrentino I, Casciotta A, Fusco M, Buonerba C, Berretta M, Ceccarelli M, Nunnari G, Diessa Y, Cicala S, Facchini G. Eculizumab treatment in patients with COVID-19: preliminary results from real life ASL Napoli 2 Nord experience. *Eur Rev Med Pharmacol Sci* 2020; **24**: 4040-4047 [PMID: 32329881 DOI: 10.26355/eurrev_202004_20875]
33 **Swanson KV**, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol* 2019; **19**: 477-489 [PMID: 31036962 DOI: 10.1038/s41577-019-0165-0]
34 **Deftereos SG**, Siasos G, Giannopoulos G, Vrachatis DA, Angelidis C, Giotaki SG, Gargalianos P, Giamarellou H, Gogos C, Daikos G, Lazanas M, Lagiou P, Saroglou G, Sipsas N, Tsiodras S, Chatzigeorgiou D, Moussas N, Kotanidou A, Koulouris N, Oikonomou E, Kaoukis A, Kossyvakis C, Raisakis K, Fountoulaki K, Comis M, Tsiachris D, Sarri E, Theodorakis A, Martinez-Dolz L, Sanz-Sánchez J, Reimers B, Stefanini GG, Cleman M, Filippou D, Olympios CD, Pyrgakis VN, Goudevenos J, Hahalis G, Kolettis TM, Iliodromitis E, Tousoulis D, Stefanadis C. The Greek study in the effects of colchicine in COvid-19 complications prevention (GRECCO-19 study): Rationale and study design. *Hellenic J Cardiol* 2020; **61**: 42-45 [PMID: 32251729 DOI: 10.1016/j.hjc.2020.03.002]
35 **Wang Y**, Jiang W, He Q, Wang C, Wang B, Zhou P, Dong N, Tong Q. Early, low-dose and short-term application of corticosteroid treatment in patients with severe COVID-19 pneumonia: single-center experience from Wuhan, China. 2020 Preprint. Available from: medRxiv [DOI: 10.1101/2020.03.06.20032342]
36 **Villar J**, Ferrando C, Martínez D, Ambrós A, Muñoz T, Soler JA, Aguilar G, Alba F, González-Higueras E, Conesa LA, Martín-Rodríguez C, Díaz-Domínguez FJ, Serna-Grande P, Rivas R, Ferreres J, Belda J, Capilla L, Tallet A, Añón JM, Fernández RL, González-Martín JM; dexamethasone in ARDS network. Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial. *Lancet Respir Med* 2020; **8**: 267-276 [PMID: 32043986 DOI: 10.1016/S2213-2600(19)30417-5]
37 **Chen L**, Xiong J, Bao L, Shi Y. Convalescent plasma as a potential therapy for COVID-19. *Lancet Infect Dis* 2020; **20**: 398-400 [PMID: 32113510 DOI: 10.1016/S1473-3099(20)30141-9]
38 **Cheng Y**, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, Chan P, Wong KC, Leung CB, Cheng G. Use of convalescent plasma therapy in SARS patients in Hong Kong. *Eur J Clin Microbiol Infect Dis* 2005; **24**: 44-46 [PMID: 15616839 DOI: 10.1007/s10096-004-1271-9]
39 **Ko JH**, Seok H, Cho SY, Ha YE, Baek JY, Kim SH, Kim YJ, Park JK, Chung CR, Kang ES, Cho D, Müller MA, Drosten C, Kang CI, Chung DR, Song JH, Peck KR. Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: a single centre experience. *Antivir Ther* 2018; **23**: 617-622 [PMID: 29923831 DOI: 10.3851/IMP3243]
40 **Mair-Jenkins J**, Saavedra-Campos M, Baillie JK, Cleary P, Khaw FM, Lim WS, Makki S, Rooney KD, Nguyen-Van-Tam JS, Beck CR; Convalescent Plasma Study Group. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. *J Infect Dis* 2015; **211**: 80-90 [PMID: 25030060 DOI: 10.1093/infdis/jiu396]
41 **Duan K**, Liu B, Li C, Zhang H, Yu T, Qu J, Zhou M, Chen L, Meng S, Hu Y, Peng C, Yuan M, Huang J, Wang Z, Yu J, Gao X, Wang D, Yu X, Li L, Zhang J, Wu X, Li B, Xu Y, Chen W, Peng Y, Hu Y, Lin L, Liu X, Huang S, Zhou Z, Zhang L, Wang Y, Zhang Z, Deng K, Xia Z, Gong Q, Zhang W, Zheng X, Liu Y, Yang H, Zhou D, Yu D, Hou J, Shi Z, Chen S, Chen Z, Zhang X, Yang X. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci USA* 2020; **117**: 9490-9496 [PMID: 32253318 DOI: 10.1073/pnas.2004168117]
42 **Carr AC**, Shaw GM, Fowler AA, Natarajan R. Ascorbate-dependent vasopressor synthesis: a rationale for vitamin C administration in severe sepsis and septic shock? *Crit Care* 2015; **19**: 418 [PMID: 26612352 DOI: 10.1186/s13054-015-1131-2]
43 **Matthay MA**, Aldrich JM, Gotts JE. Treatment for severe acute respiratory distress syndrome from COVID-19. *Lancet Respir Med* 2020; **8**: 433-434 [PMID: 32203709 DOI: 10.1016/S2213-2600(20)30127-2]
44 **Fowler AA 3rd**, Truwit JD, Hite RD. Morris PE, DeWilde C, Priday A, Fisher B, Thacker LR 2nd, Natarajan R, Brophy DF, Sculthorpe R, Nanchal R, Syed A, Sturgill J, Martin GS, Sevransky J, Kashiouris M, Hamman S, Egan KF, Hastings A, Spencer W, Tench S, Mehkri O, Bindas J, Duggal A, Graf J, Zellner S, Yanny L, McPolin C, Hollrith T, Kramer D, Ojielo C, Damm T, Cassity E, Wieliczko A, Halquist M. Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients With Sepsis and Severe Acute Respiratory Failure: The CITRIS-ALI Randomized Clinical Trial. *JAMA* 2019; **322**: 1261-1270 [PMID: 31573637 DOI: 10.1001/jama.2019.11825]
45 **Connors JM**, Levy JH. Thromboinflammation and the hypercoagulability of COVID-19. *J Thromb Haemost*

2020; **18**: 1559-1561 [PMID: 32302453 DOI: 10.1111/jth.14849]
46 **Damiani G**, Pacifico A, Bragazzi NL, Malagoli P. Biologics increase the risk of SARS-CoV-2 infection and hospitalization, but not ICU admission and death: Real-life data from a large cohort during red-zone declaration. *Dermatol Ther* 2020; e13475 [PMID: 32356577 DOI: 10.1111/dth.13475]
47 **Bridgewood C**, Damiani G, Sharif K, Quartuccio L, McGonagle D. Rationale for use of PDE4 inhibition for severe inflammation in COVID-19 Pneumonia. 2020 Preprint. [DOI: 10.13140/RG.2.2.12421.50407]
48 **Caly L**, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res* 2020; **178**: 104787 [PMID: 32251768 DOI: 10.1016/j.antiviral.2020.104787]
49 **Magro C**, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, Baxter-Stoltzfus A, Laurence J. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. *Transl Res* 2020; **220**: 1-13 [PMID: 32299776 DOI: 10.1016/j.trsl.2020.04.007]

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ORIGINAL ARTICLE

Observational Study

Chinese medical students' interest in COVID-19 pandemic

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Abstract

BACKGROUND

The outbreak of coronavirus disease 2019 (COVID-19) happened in early December and it has affected China in more ways than one. The societal response to the pandemic restricted medical students to their homes. Although students cannot learn about COVID-19 through clinical practice, they can still pay attention to news of COVID-19 through various channels. Although, as suggested by previous studies, some medical students have already volunteered to serve during the COVID-19 pandemic, the overall willingness of Chinese medical students to volunteer for such has not been systematically examined.

AIM

To study Chinese medical students' interest in the relevant knowledge on COVID-19 and what roles they want to play in the pandemic.

METHODS

approval for this study (IRB No. S-K1173).

Informed consent statement: Our study has not involved the patients' consent because it is not a clinical study.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

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Medical students at Peking Union Medical College were surveyed *via* a web-based questionnaire to obtain data on the extent of interest in the relevant knowledge on COVID-19, attitude towards volunteerism in the pandemic, and career preference. Logistic regression modeling was used to investigate possible factors that could encourage volunteerism among this group in a pandemic.

RESULTS

A total of 552 medical students responded. Most medical students showed a huge interest in COVID-19. The extent of students' interest in COVID-19 varied among different student-classes ($P < 0.05$). Senior students had higher scores than the other two classes. The number of people who were 'glad to volunteer' in COVID-19 represented 85.6% of the respondents. What these students expressed willingness to undertake involved direct, indirect, and administrative job activities. Logistic regression analysis identified two factors that negatively influenced volunteering in the pandemic: Student-class and hazards of the voluntary job. Factors that positively influenced volunteering were time to watch COVID-19 news, predictable impact on China, and moral responsibility.

CONCLUSION

More innovative methods can be explored to increase Chinese medical students' interest in reading about the relevant knowledge on COVID-19 and doing voluntary jobs during the pandemic.

Key Words: COVID-19; Chinese medical students; Volunteer; Medical education; Public health emergency of international concern

Core Tip: Our survey of Chinese medical students showed an overall strong initiative for volunteerism in the coronavirus disease 2019 (known as COVID-19) pandemic. These students were willing to play direct, indirect, or administrative roles. Student-class and hazards of the voluntary job were the negative influencing factors of volunteering in the pandemic; thus, reducing students' fear of being infected, such as by providing strong personal protection, can improve their willingness to volunteer. As for their future career preference, nearly half of the students expressed reluctance to engage in pandemic-related specialties, which could imply measures needed to attract potential practitioners in the future.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), broke out in Hubei Province, China in December 2019[1]. The World Health Organization later declared this outbreak a pandemic, due to its rapid spread across the world[2]. Wuhan, the capital of Hubei Province, was locked down on January 23, 2020. As of March 8, 2020 — according to data published by the National Health Commission of China — about 42000 medical staff had been dispatched to different regions of Hubei during the lock-down[3]. Among these was a multidisciplinary team of 186 doctors and nurses from Peking Union Medical College (PUMC) hospital, who managed an intensive care unit (referred to as ICU) from February 4 to April 12 in the Sino-French New City Branch of Tongji Hospital (Wuhan), a designated hospital for COVID-19. No medical student was included in this medical team.

Because the outbreak of COVID-19 coincided with the Chinese Spring Festival, most of the medical students in China were scattered across the country and consequently

self-quarantined in their hometowns. The global outbreak has affected medical students worldwide, in many different ways. In the Chinese medical education system, medical students learn basic sciences and clinical medical courses in their junior and middle class-years, respectively; each class has very limited access to clinical practice during this time. Senior class students, on the other hand, enter hospitals for clerkship, internship, or clinical rotation as residents. With the help of various Internet-based learning technologies, the coursework of junior and middle class-year medical students was hardly affected by the pandemic lock-down. However, the clinical practice of senior students had to be suspended.

This disruption in medical school training was not exclusive to China. Medical students at Oxford University Hospitals faced a similar situation[4]. While their medical training was nearly completely suspended, medical students embarked on laboratory jobs and administrative tasks to alleviate the general understaffing burden brought on by the pandemic. Some scholars have advocated such involvement of medical students in the pandemic[5,6]. Yet, there is little data to show medical students' willingness, particularly for those in China. Thus, we designed and carried out a survey to assess Chinese medical students' willingness to know more about COVID-19 and participate in the pandemic, and investigate whether COVID-19 had increased their interest in specialties related to the prevention and treatment of severe infectious diseases.

MATERIALS AND METHODS

Questionnaire design

An 18-item questionnaire was designed to evaluate Chinese medical students' involvement in reading the relevant knowledge of COVID-19, their willingness to volunteer in the pandemic, and whether the outbreak of COVID-19 had any impact on their career choice. The design was adapted from a questionnaire verified by Mortelmans *et al*[7], inspired by surveys of the influenza pandemic[8] and Middle East respiratory syndrome[9,10], and based on a psychological survey conducted in the early stage of the COVID-19 outbreak[11]. The majority of items — including willingness to learn about COVID-19, interest level in the relevant knowledge on COVID-19, perceived personal and nationwide impact of COVID-19, and preference of professional choices - were evaluated using a Likert 5-point scale, with 1 being strongly disagree/unwilling and 5 being strongly agree/willing. For other items, the 5-point qualitative scale was as follows: 1-2: "a little"; 3: "moderate"; and 4-5: "very much". Further, interviewees answered "Yes" or "No" to the question "Are you willing to be a volunteer in the COVID-19 pandemic?", and selected their access to pandemic information and the type of pandemic-relevant department that they were willing to join. Before distribution, the questionnaire was assessed by an internal consistency test, and the Cronbach-α coefficient was determined to be 0.802. All information was anonymous and informed consent was obtained from respondents. The study was approved by the Medical Ethics Committee of PUMC Hospital.

Participants

A total of 916 medical students at PUMC, distributed among eight student classes, were invited to fill out the web-based questionnaire. Respondents could submit only a single time and had to answer each question under the platform system settings. From April 10 to April 18, the invitation to fill out the questionnaire was delivered three times, to make sure that every student received the message and with the ultimate goal of maximizing the response rate. By that time, the pandemic crisis-level had been downgraded in China and the lock-down of Wuhan had been lifted (on April 8); the new semester had not yet started at PUMC and the students had been restricted to their homes for more than 2 mo.

Statistical analysis

All analyses were carried out with the SPSS statistical software package (v23; IBM Corp., Armonk, NY, United States). Quantitative data are expressed as the mean ± SD. Qualitative data are described as constituent ratios. Kruskal-Wallis test was applied for difference analysis between student-class and interest in the relevant knowledge on COVID-19. Logistic regression modeling was used for influencing-factor analysis of willingness to be a volunteer in the COVID-19 pandemic. A *P* value less than 0.05 was considered as the threshold for statistical significance.

RESULTS

A total of 552 questionnaires (response rate of 60.3%) were returned from 33 provincial administrative regions of China during the 8-d survey period (Figure 1). Among all the responders, 57.8% were female and 42.2% were male. The total respondent pool was divided into three groups according to their curriculum setups, as follows: Junior students, whose coursework involved basic sciences and little medical knowledge; middle-grade students, who received medical education but had no access to clinical practice; and senior students who were currently in clinical rotations. The respondent distribution and the response rate of each group are provided in Table 1.

Involvement in reading about COVID-19

Seventy-one percent of the respondents showed willingness to follow the progress of the COVID-19 pandemic and sixty-eight percent of them reported that they spent 15-60 min per day on it. The most popular way to access the relevant information was social media (90.9%), followed by news app (67.4%) and television (52.0%). The question "Which aspect of the relevant knowledge on COVID-19 do you know best?" is designated to determine students' involvement in reading about COVID-19 upon they were self-quarantined at home and to assess which aspect of COVID-19 they will be most interested in when they followed news. Table 2 shows that medical students were most interested in preventive measures in daily life (4.38 ± 0.65) but less interested in diagnostic criteria and treatment procedures (3.12 ± 0.95). The medical students' preference varied among the different classes. The senior students showed a greater interest in clinical knowledge, such as in-hospital prevention and diagnosis and treatment procedures of COVID-19, followed by middle-grade students ($P < 0.05$), while the junior students appeared to have the least interest to these aspects. There was no statistically significant difference between the different classes for interest in pathogenesis. As for prevention in daily life, there was a tendency for the seniors to be more into protecting themselves in daily routine than the junior students ($P = 0.05$) (Table 2).

Attitude towards volunteering in pandemic

The number of people who were willing to offer spontaneous support and help in COVID-19 accounted for 85.6% of the respondents. The questionnaire was set up with some items about the role that medical students would prefer to play as a volunteer in the pandemic. Students could arbitrarily choose the task that they wanted to undertake, without being restricted to choose only one item. One-half (50.2%) expressed willingness to provide direct medical services, mainly involving management of patients under the guidance of superior physicians; importantly, this service has a possibility of direct clinical exposure. Indirect medical activities were more popular (69.4%), including working on the clinical front-line but not directly treating the patient. The majority of students (80.4%) expressed willingness to assist in administrative work, such as managing paper files and designing community pamphlets; this work carries the lowest risk of infection.

The incentives cited by the respondent Chinese medical students to volunteer in a pandemic are summarized in Table 3. Binary logistic regression modeling was used to investigate possible factors that could affect medical students' willingness to volunteer. Female medical students were found to be more likely to volunteer than their male counterparts. Notably, willingness to volunteer decreased with seniority. Next, it was remarkable that students were more willing to be a volunteer with increasingly more time spent on watching news and stronger will to learn about COVID-19. Not surprisingly, students who held the opinion that COVID-19 exerted a huge impact on China and those who thought that doctors volunteer because of moral obligation were more inclined to volunteer. Sixty-three percent of the respondents agreed or strongly agreed that health care professionals have a moral obligation to voluntarily provide medical services in a pandemic such as COVID-19. These medical students were significantly more willing to volunteer as well.

COVID-19 pandemic and career preference

How the pandemic was affecting career preference of medical students is illustrated in Figure 2. When asked to rate their inclination to join pandemic-related specialties (more than one option was available), nearly half of the students expressed reluctance. Public health specialties were the most popular among all related specialties, followed by ICU. Among the students who were interested in COVID-19-related specialties, only 18% chose infectious disease, making it the least popular option.

Table 1 Chinese medical student respondents

Characteristic	Junior	Middle	Senior	Total
Study sample, *n*	268	173	475	916
Returned questionnaire, *n*	157	134	261	552
Response rate	58.6%	77.5%	54.9%	60.3%

Table 2 Interest level of Chinese medical student respondents in relevant knowledge on coronavirus disease 2019

Item	Junior		Middle		Senior		Total		*P* value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Pathogenesis	3.45	0.87	3.53	0.91	3.62	0.85	3.55	0.87	0.098
Prevention in life	4.29	0.68	4.37	0.61	4.44	0.65	4.38	0.65	0.05
In-hospital prevention	3.23	1.08	3.43	0.92	3.84	0.87	3.57	0.98	< 0.001
Diagnosis and treatment	2.85	0.97	2.99	0.95	3.34	0.87	3.12	0.95	< 0.001

SD: Standard deviation.

DISCUSSION

It has been debated whether medical students should serve in a pandemic such as COVID-19, owing to the possibility of their getting infected during the clinical practice activities and their lack in knowledge about severe infectious disease[6,11]. So far, the different countries affected by COVID-19 have reacted differently. Portugal declared the closure of medical schools after 31 cases were confirmed[12]. On March 17, 2020, the American College of Medicine Association (United States) recommended suspension of all direct patient contact responsibilities for medical students[13] but policies differed among districts. New York University offered voluntary opportunities for senior students who met all graduation requirements to graduate in advance of the pandemic, and planned to have them in internal medicine and emergency departments[14].

At the same time, some medical students were also enthusiastic to offer their help during the pandemic. Compared with clinical jobs, non-clinical jobs seemed to be more acceptable. Medical students at Columbia University (New York, NY, United States) initiated a virtual volunteer group to perform the necessary chores for hospital staff and to participate in a COVID-19 laboratory program[15]. More than 500 medical students at Harvard Medical School (Boston, MA, United States) spontaneously formed volunteer teams to fulfill their potential through community mobilization[16]. To our relief, the study conducted at PUMC showed that Chinese medical students were also likely to offer support and help in a pandemic. This trend was more obvious when students sensed the threat of COVID-19 to China.

The self-assessment of results from PUMC, presented herein, indicate that students showed preference to know COVID-19 daily life prevention than hospital settings. Not surprisingly, the senior medical students had higher scores than their junior class counterparts for interest in disease prevention. This suggests that younger volunteers, who still lacked sufficient working experience in hospital, have not been aware of the significance of disease prevention yet, thus indicating that more consciousness and knowledge about self-protection can be instilled into these younger students.

According to our survey, several factors influenced the Chinese medical student's willingness to serve in a pandemic. It seems that senior students are more reluctant to volunteer. This may be because most of the senior students have their specialty of choice already. Moreover, the possibility of getting infected may have deterred them. Other influencing factors have been seen in previous studies, in which several lines of evidence have been obtained to suggest that inefficiency, prior training, financial security, and access to protective equipment can affect medical students' enthusiasm to be volunteers[17]. Ultimately, focusing on these collective factors will not only improve medical students' volunteerism but their protection as well.

This study also indicated that medical students preferred to get information from social media and news apps. According to data published by the China Internet

Table 3 Odds ratios for willingness to volunteer in the coronavirus disease 2019 pandemic

Item		*n* (%)	OR (95%CI)
Sex	Male	233 (42.2)	1
	Female	319 (57.8)	1.52 (0.86-2.72)
Student class	Junior	157 (28.4)	1
	Middle	134 (24.3)	0.79 (0.36-1.73)
	Senior	261 (47.3)	0.59 (0.29-1.21)
Time to watch news	< 15 min	125 (22.6)	1
	15-30 min	265 (48.0)	1.4 (0.7-2.81)
	30-60 min	111 (20.1)	2.13 (0.86-5.26)
	> 60 min	51 (9.2)	2.13 (0.65-6.95)
Willingness to know	Little	160 (29.0)	1
	Moderate	242 (43.8)	1.65 (0.86-3.18)
	Very much	150 (27.2)	0.85 (0.38-1.88)
Impact on personal life	Little	85 (15.4)	1
	Moderate	155 (28.1)	0.49 (0.16-1.57)
	Very much	312 (56.5)	0.62 (0.18-2.08)
Impact on China	Little	97 (17.6)	1
	Moderate	163 (29.5)	1.55 (0.6-4.05)
	Very much	292 (52.9)	2.16 (0.81-5.76)
Doctors' obligation	Little	202 (36.6)	1
	Moderate	216 (39.1)	4.29 (2.25-8.16)
	Very much	134 (24.3)	28.22 (6.03-131.98)
Hazards of voluntary job	Little	236 (42.8)	1
	Moderate	256 (46.4)	0.43 (0.22-0.85)
	Very much	60 (10.9)	0.24 (0.1-0.58)

Odds ratio and 95% confidence interval in binary logistic regression modeling were used for assessing a "Yes" answer to the question "Are you willing to be a volunteer in the COVID-19 pandemic?" OR: Odds ratio; CI: Confidence interval.

Network Information Center at the end of March of this year, 265 million students turned to online education. The number of online education users in China reached 423 million, equating to an increase of 110.2% from the end of 2018[18]. After the pandemic, schools can still consider a combination of in-class learning and some online learning modalities[19]. Our findings also confirmed the popularity and feasibility of this way. It can be a good chance for schools to penetrate into students' social circles and raise their intention of being volunteers by means of, for instance, posting high-quality videos about COVID-19 on social media[20].

The outbreak of COVID-19 also exposes the potential understaffing. It is imperative to take measures to appeal more practitioners. Although preventive medical courses are provided to students, there is no curriculum about public principles in response to emerging infectious diseases, and gradually students are reluctant to become doctors related to epidemic control[21]. It is reassuring that even not in clinical practice, medical students take a vivid public health course through COVID-19, which may increase students' emphasis on epidemic-related specialties. Although it remains unclear to know what extent their plans to specialize in a specialty relevant to the pandemic was altered by the pandemic, nearly 80% of students believed that this outbreak improved their interest and understanding of public health, which can be a good sign. Thus far, previous studies have revealed a correlation between lack of incentive mechanisms, little perception of public health, and students' choice of community medicine[22]. This suggests that students should be made clear of the significance of epidemic-relevant

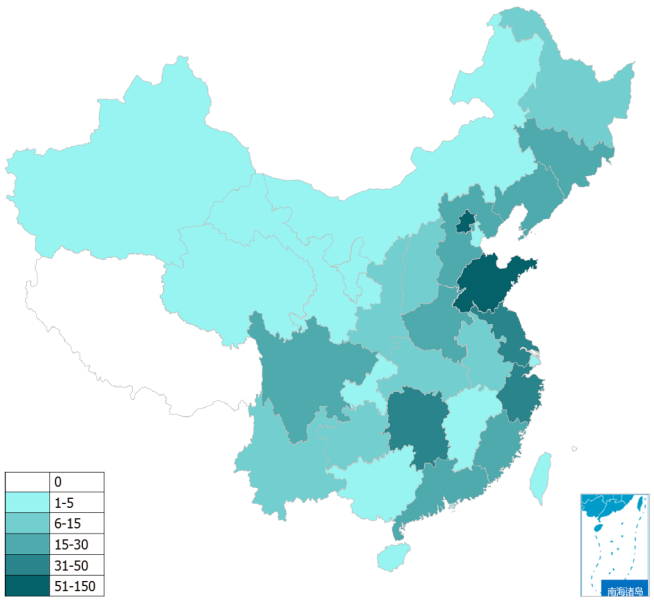

Figure 1 Distribution of medical students participating in this survey across China (*n* = 552).

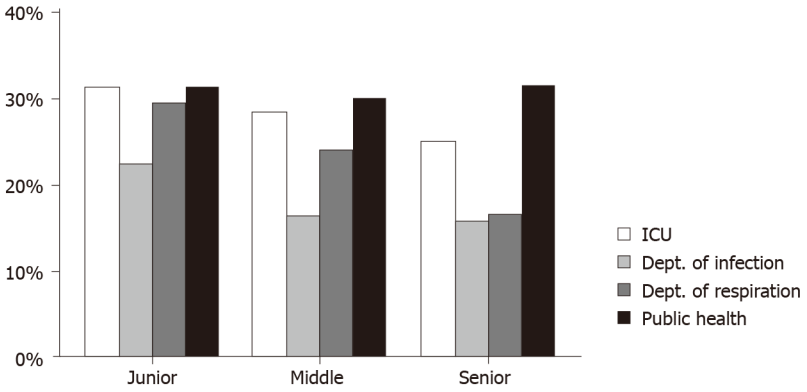

Figure 2 Career choice of medical students about pandemic-related specialties. ICU: Intensive care unit.

specialties, and encouraged by role models who have worked in epidemic areas.

This study has several limitations that must be considered when interpreting our findings. First, for the purpose of a higher response rate, only students at PUMC were surveyed; thus, the data collected might not be representative of the entire student population in China. However, despite studying at the same college (PUMC), the students involved in our study originated from across the entire country. Undoubtedly, our findings should be further confirmed by a multi-center study. A web-based questionnaire also has particular benefits for our study population, as it complements the geographic restriction caused by the pandemic. Second, the questionnaire was delivered in early April, when the pandemic in China had been basically controlled, and students were inherently more familiar with COVID-19. Hence, the results might be less optimistic if it had been conducted at an earlier stage of the pandemic.

CONCLUSION

In this study, a web-based questionnaire was used to reveal Chinese medical students' interest in the international public health event, COVID-19. We found that this emerging pandemic triggered students' curiosity and prompted their interest in reading about and responding to related events. Overall, students tended to read more about daily life prevention of COVID-19, and they expressed their passion to participate in volunteer activities in different ways.

ARTICLE HIGHLIGHTS

Research background

Coronavirus disease 2019 (COVID-19) has raged across the world. The dramatically increasing numbers of infected cases consequently caused a heavy burden on medical staff worldwide. With the intent of helping ease the burden of medical systems, some medical students have been willing to volunteer in the pandemic but there is little systematic evidence to show that among Chinese medical students.

Research motivation

As medical students will emerge as the practitioners during future outbreaks and pandemics, it is essential to determine the profile of incentivizing factors for such volunteer work today. This knowledge will also help to construct strategies that will improve their enthusiasm for volunteerism.

Research objectives

A total of 552 medical students at Peking Union Medical College responded to the study questionnaire.

Research methods

This study was online-based and conducted through a questionnaire that explored students' interest in the relevant knowledge on COVID-19, attitude towards volunteerism in the pandemic, and career preference. Logistic regression modeling was used to investigate possible factors that could encourage medical students to volunteer in a pandemic.

Research results

Chinese medical students expressed a strong initiative to aid in COVID-19 by means of taking on direct, indirect, or administrative responsibilities. There were two negative influencing factors, namely, student-class and hazards associated with the voluntary job, which suggested that reducing students' fear of being infected and offering sufficient personal protection could help improve volunteerism in a pandemic. In terms of future career preference, nearly half of the students expressed reluctance to engage in pandemic-related specialties, which could imply more measures to attract potential practitioners in the future.

Research conclusions

Most Chinese medical students take initiatives to learn about COVID-19 and are glad to volunteer in a pandemic. However, hazards associated with the voluntary job can likely damp down students' enthusiasm for volunteerism, which means more innovative methods, such as Internet platforms, sufficient personal protection, specialized knowledge, and full training in advance, can be explored.

Research perspectives

Multi-center studies are needed, taking racial, geographic distribution, educational background, parental background, income and academic performance, *etc*. into consideration. In addition, more standard assessment questionnaires should be made and enacted to evaluate students' comprehensive understanding of COVID-19, in order to reduce the bias of different surveys conducted in different regions.

REFERENCES

1 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]

2 **Grasselli G**, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, Cereda D, Coluccello A, Foti G, Fumagalli R, Iotti G, Latronico N, Lorini L, Merler S, Natalini G, Piatti A, Ranieri MV, Scandroglio AM, Storti E, Cecconi M, Pesenti A; COVID-19 Lombardy ICU Network. Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. *JAMA* 2020; **323**: 1574-1581 [PMID: 32250385 DOI: 10.1001/jama.2020.5394]

3 Press conference of the joint prevention and control mechanism of the state council [Press release]. Beijing: Propaganda department, April 7, 2020

4 **Armstrong A**, Jeevaratnam J, Murphy G, Pasha M, Tough A, Conway-Jones R, Mifsud RW, Tucker S. A

plastic surgery service response to COVID-19 in one of the largest teaching hospitals in Europe. *J Plast Reconstr Aesthet Surg* 2020; **73**: 1174-1205 [PMID: 32359857 DOI: 10.1016/j.bjps.2020.03.027]
5 **Thomson E**, Lovegrove S. 'Let us Help'-Why senior medical students are the next step in battling the COVID-19 Pandemic. *Int J Clin Pract* 2020; e13516 [PMID: 32301206 DOI: 10.1111/ijcp.13516]
6 **Miller DG**, Pierson L, Doernberg S. The Role of Medical Students During the COVID-19 Pandemic. *Ann Intern Med* 2020; **173**: 145-146 [PMID: 32259194 DOI: 10.7326/M20-1281]
7 **Mortelmans LJ**, De Cauwer HG, Van Dyck E, Monballyu P, Van Giel R, Van Turnhout E. Are Belgian senior medical students ready to deliver basic medical care in case of a H5N1 pandemic? *Prehosp Disaster Med* 2009; **24**: 438-442 [PMID: 20066648 DOI: 10.1017/s1049023x00007287]
8 **Rosychuk RJ**, Bailey T, Haines C, Lake R, Herman B, Yonge O, Marrie TJ. Willingness to volunteer during an influenza pandemic: perspectives from students and staff at a large Canadian university. *Influenza Other Respir Viruses* 2008; **2**: 71-79 [PMID: 19453473 DOI: 10.1111/j.1750-2659.2008.00042.x]
9 **Al-Mohrej A**, Agha S. Are Saudi medical students aware of middle east respiratory syndrome coronavirus during an outbreak? *J Infect Public Health* 2017; **10**: 388-395 [PMID: 27502524 DOI: 10.1016/j.jiph.2016.06.013]
10 **Liu M**, Jiang C, Donovan C, Wen Y, Sun W. Middle East Respiratory Syndrome and Medical Students: Letter from China. *Int J Environ Res Public Health* 2015; **12**: 13289-13294 [PMID: 26512679 DOI: 10.3390/ijerph121013289]
11 **Wang C**, Pan R, Wan X, Tan Y, Xu L, Ho CS, Ho RC. Immediate Psychological Responses and Associated Factors during the Initial Stage of the 2019 Coronavirus Disease (COVID-19) Epidemic among the General Population in China. *Int J Environ Res Public Health* 2020; **17** [PMID: 32155789 DOI: 10.3390/ijerph17051729]
12 **Mahase E**. Covid-19: Portugal closes all medical schools after 31 cases confirmed in the country. *BMJ* 2020; **368**: m986 [PMID: 32156675 DOI: 10.1136/bmj.m986]
13 **Rose S**. Medical Student Education in the Time of COVID-19. *JAMA* 2020 [PMID: 32232420 DOI: 10.1001/jama.2020.5227]
14 **DeWitt DE**. Fighting COVID-19: Enabling Graduating Students to Start Internship Early at Their Own Medical School. *Ann Intern Med* 2020; **173**: 143-144 [PMID: 32259191 DOI: 10.7326/M20-1262]
15 **Iserson KV**. Augmenting the Disaster Healthcare Workforce. *West J Emerg Med* 2020; **21**: 490-496 [PMID: 32302286 DOI: 10.5811/westjem.2020.4.47553]
16 **Soled D**, Goel S, Barry D, Erfani P, Joseph N, Kochis M, Uppal N, Velasquez D, Vora K, Scott KW. Medical Student Mobilization During A Crisis: Lessons From A COVID-19 Medical Student Response Team. *Acad Med* 2020; **95**: 1384-1387 [PMID: 32282373 DOI: 10.1097/ACM.0000000000003401]
17 **Gouda P**, Kirk A, Sweeney AM, O'Donovan D. Attitudes of Medical Students Toward Volunteering in Emergency Situations. *Disaster Med Public Health Prep* 2019; 1-4 [PMID: 31475653 DOI: 10.1017/dmp.2019.81]
18 **CINI Center**. The 45th China statistical report on internet development. 2020
19 **Eltayar AN**, Eldesoky NI, Khalifa H, Rashed S. Online faculty development using cognitive apprenticeship in response to COVID-19. *Med Educ* 2020; **54**: 665-666 [PMID: 32324934 DOI: 10.1111/medu.14190]
20 **Bauchner H**, Sharfstein J. A Bold Response to the COVID-19 Pandemic: Medical Students, National Service, and Public Health. *JAMA* 2020; Online ahead of print [PMID: 32267488 DOI: 10.1001/jama.2020.6166]
21 **Schröder-Bäck P**, Duncan P, Sherlaw W, Brall C, Czabanowska K. Teaching seven principles for public health ethics: towards a curriculum for a short course on ethics in public health programmes. *BMC Med Ethics* 2014; **15**: 73 [PMID: 25288039 DOI: 10.1186/1472-6939-15-73]
22 **Zhang L**, Bossert T, Mahal A, Hu G, Guo Q, Liu Y. Attitudes towards primary care career in community health centers among medical students in China. *BMC Fam Pract* 2016; **17**: 75 [PMID: 27423474 DOI: 10.1186/s12875-016-0472-5]

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OPINION REVIEW

Management strategies in a thoracic surgery ward during COVID-19 pandemic: Experience from West China Hospital

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Abstract

The coronavirus disease 2019 was first reported in Wuhan in December 2019 and then spread rapidly throughout the world. On March 11, 2020, the World Health Organization declared coronavirus disease 2019 a pandemic. In response to the pandemic, the management division of West China Hospital oversaw the implementation of hospital-wide emergency measures. In accordance with these measures, the hospital's thoracic surgery ward implemented a new management system by reformulating staff training plans, patient admission procedures, and other systems for managing the ward and protecting perioperative patients. Overall, the ward was successful in restoring normal working order, protecting all staff from occupational exposures, and ensuring the safety of inpatients and their families.

Key Words: COVID-19; Thoracic surgery; Thoracic surgery ward management; SARS-CoV-2; Epidemic prevention and control; Nosocomial infection

Core Tip: This study describes a specific approach to preventing a coronavirus disease 2019 outbreak in the thoracic surgery ward at a hospital in West China. We believe that our study will make a significant contribution to the literature. It documents lessons learned in developing and deploying a system to protect staff and vulnerable inpatients

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during the unprecedented and highly challenging situation that has been the coronavirus disease 2019 pandemic.

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INTRODUCTION

Shortly after the emergence of the novel coronavirus in Wuhan, Hubei Province, China in December 2019, other regions in China detected cases of the disease. Subsequently, the coronavirus disease-19 (COVID-19) epidemic spread throughout the world. On February 11, 2020, the International Committee on Taxonomy of Viruses officially named the virus the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1], and the World Health Organization named the disease caused by the virus the COVID-19. Then, on March 11, 2020, the World Health Organization declared the epidemic a global pandemic. The total number of globally confirmed cases reached 2.2 million by April 19, 2020. According to published research[2,3], the virus is a β-coronavirus, sharing up to 85% of its homology-based genes with a bat SARS-like coronavirus. The major sources of infection are confirmed COVID-19 patients, though asymptomatic patients can also be infection sources. The disease is highly contagious, and its main transmission routes include droplets and close person-to-person contact; there is also the possibility for aerosol transmission in closed environments. A retrospective study published by Zhongnan Hospital of Wuhan University reported that 41.3% (57/138) of patients diagnosed with COVID-19 had nosocomial infections from the early stages of the epidemic[4].

The majority of thoracic surgery ward inpatients have malignant tumor diagnoses. These patients are usually immunocompromised and may be more vulnerable to viral infections, as postoperative respiratory rehabilitation training may increase the risk of viral transmission[5]. SARS-CoV-2 infections may increase these patients' postoperative complications as well as their perioperative mortality. Therefore, it is extremely important to prevent outbreaks and the subsequent spread of COVID-19 in thoracic surgery wards. After the outbreak of COVID-19 in China, the West China Hospital (the authors' hospital) made it a top priority to prevent and control the disease, and quickly established a system to manage the COVID-19 outbreak. In accordance with this system[6], the thoracic surgery department (the authors' department) constructed a detailed protocol for ward management, which included patient admission procedures, overseeing accompanying family members, and ward disinfection and isolation systems during the outbreak. As a result, the authors were able to restore working order in the ward, and they continued to treat inpatients in a timely fashion. Furthermore, no hospital-acquired infections occurred in the authors' ward. This paper describes the specific thoracic surgery ward management system in response to COVID-19.

STAFF TRAINING IN EPIDEMIC PREVENTION AND CONTROL

The authors' hospital implemented measures to train staff on the prevention, diagnosis, and treatment of COVID-19 through online courses, weekly video-conferences, and WeChat postings[6]. Training covered basic knowledge about COVID-19 including its epidemiology, symptoms, diagnosis, and treatment. Staff were instructed on how to report COVID-19 cases and collect specimens as well as on disinfection, isolation, and self-protection. The department encouraged all staff to participate in the online discussions and complete the training and assessments. By acquiring the relevant knowledge and skills, the staff developed the capability to tackle the outbreak. According to Fu *et al*[7], inconsistent protection standards and improper donning and removal of protective equipment are the key causes of medical staff occupational exposures. Therefore, the authors' department informed all staff on

these and other issues through online courses and periodic face-to-face training. Staff were trained to manage patient admissions, screen inpatients and their families for COVID-19, properly use protective equipment and so on. In addition, emergency preplans were developed and carried out based on the capabilities of the department, particularly for activities such as donning, removing, and disposing of protective equipment; reporting suspected/confirmed cases; and transporting infected patients. The director of the department was responsible for training doctors; the head nurse oversaw the nurse trainings; and the hospital's infection control nurse trained cleaning staff and others. These measures ensured that everyone could continue to perform their normal duties during the outbreak as well as effectively contribute to the prevention and control of COVID-19.

DYNAMIC MANAGEMENT OF INPATIENT ADMISSION ACCORDING TO SURGICAL INDICATIONS

Taking into consideration the thoracic surgery specialty and patients who have been admitted to the department in the past, inpatients in the authors' department generally fall into one of three categories: Those requiring emergency operations, those needing limited operations, and those undergoing selective operations. Indications for emergency operations include spontaneous hemopneumothorax, esophageal rupture, open chest trauma, giant tracheal tumors, and other emergencies requiring immediate surgical treatment. Indications for limited operations include esophageal cancer, invasive lung cancer, thymic carcinoma, thoracic malignancies with rapid progression, and other conditions requiring surgical treatment in the near future. Indications for selective operations include benign esophageal or pulmonary diseases, pulmonary ground-glass opacities, mediastinal cysts and benign tumors, palmar hyperhidrosis, and other situations that allow for a more flexible scheduling of the surgical procedure.

In the early stages of the COVID-19 outbreak, the epidemiology, diagnosis, and treatment of the disease were unclear. Therefore, as part of a hospital-wide deployment, the authors' department stopped seeing outpatients and postponed limited and selective operations for one week. This gave us time to develop protocols and procedures to manage the epidemic as well as reserve the necessary protective equipment. After one week, those requiring limited operations could once again be admitted. Patients with more severe disease were prioritized, but those who lived outside Sichuan Province had their surgeries further postponed to reduce their risk of contracting COVID-19 *en route* to the hospital. At that point, the domestic epidemic was under control. In the authors' department, the admission of patients for elective operations returned to normal, and those who were waiting the longest times for their surgeries were the first to be admitted. However, all those seeking admission to the hospital were required to pass a three-step screening procedure[8]. Prospective patients had to provide complete histories, first, when sending their admission notice; second, at the hospital admissions department; and third, in the surgical ward reception area. The histories documented any recent symptoms of cough, fatigue, diarrhea and so on in the prospective patient and his or her family members. A prospective patient also had to describe any recent travel and have his or her body temperature taken. Finally, after a chest computed tomography (CT) examination to exclude COVID-19 infection, the patient could be admitted into the hospital. These were the measures taken to prevent COVID-19 outbreaks in our medical institution.

Patients requiring emergency operations would undergo emergent treatment and history-taking at the same time based on the "screening while treating" principle. Then, after a chest CT examination and throat-swabbing nucleic acid test to exclude COVID-19 infection, patients in stable condition could be transferred to the inpatient ward. Patients in severe condition who could not undergo the screening test before their operations were reported to both the thoracic surgery department and hospital administration. The operating room and the department of anesthesiology were then contacted, and the patients were treated as confirmed COVID-19 cases during their emergency operations with every precaution taken. After the operation, the screening tests for COVID-19 were performed, and those without COVID-19 infection would be admitted to the authors' department. Otherwise, the patients would be admitted into the isolation ward of the infectious disease department.

STANDARDIZED WARD MANAGEMENT TO ENSURE THE SAFETY OF STAFF AND PATIENTS

Management of ward environment

Ward access was restricted under a 24-h closed management system. Access to the ward was possible only at two points, based on our ward's configuration. Staff at both access points were equipped with non-contact thermometers as well as the "Log Book for Recording Personnel Entrance and Exit". Staff took the temperatures and histories (*i.e.*, symptoms and epidemiology) of patients and families entering the ward for the first time. Upon leaving the ward, inpatients and family members were required to have their temperatures taken again and provide the reason for leaving and the destination. Temperatures would be taken again upon their return. Patients were allowed access to the ward by showing their wrist strap, while family members were required to present a companionship certificate. Other visitors and personnel were strictly prohibited from entering. Furthermore, only card-carrying employees were permitted to pass through the staff passageway; and body temperatures were taken there, as well. Our ward reserved an appropriate number of empty rooms for isolation of suspected patients in case of emergency.

Maintenance of the ward environment and disinfection of equipment were all in accordance with hospital requirements[9]. For ventilation, the windows of the inpatient ward, medical staff office, and duty room would be opened at least twice a day for a minimum of one hour. The equipment surfaces in the nursing station, therapy room, dressing room, and ward were disinfected twice a day by wiping with an effective chlorine concentration of 1000 mg/L. Also twice per day, the ward floor and corridor passages were sprayed and cleaned using a wet disinfectant with an effective chlorine concentration of 1000 mg/L, and air disinfection machines were used to disinfect the therapy room and dressing room.

Management of staff

All staff had to complete the "Basic Information Backup Record for Returning to Work", and those with suspicious symptoms or travel histories were required to suspend clinical work. Staff who returned to work would undergo daily body temperature monitoring by specially assigned personnel, and those who had not yet returned to work would monitor their body temperatures at home. All staff had to complete daily health reports so that administrators could stay informed about the health status of the hospital's front-line workers. Staff were required to wear surgical masks and hats at work and to maintain strict hand hygiene practices. When performing tracheotomies, sputum aspirations, and throat swabs, staff had to wear medical masks, goggles or face screens, rubber gloves, and disposable medical gowns. Staff were required to wear protective clothing when caring for patients with suspected COVID-19. Special personnel for the hospital and in each department supervised and enforced the personal protection practices, providing on-site feedback and corrections in case of insufficient or excessive protection. During work breaks, staff continued to observe the preventive regulations. Protective equipment used in the ward was not allowed in the duty room, nor were eating and group conversations. Staff were required to observe strict coughing and hand hygiene in all areas and to keep the duty room neat and ventilated.

Family member and companion management

Preoperative patients could not have a companion present unless they were minors or seniors or others incapable of self-care. These patients were allowed to have one companion. Postoperative patients could keep one fixed companion as determined by the attending doctor and nurses, and individuals with a recent history of fever or cough, or a positive epidemiological history could not serve as companions. Companion family members had to complete the same symptom and epidemiological screenings as patients. During a severe outbreak, nucleic acid tests or chest CT scans would also be needed. Each remaining family member had to use their own identity document card to apply for a companionship certificate. This was to facilitate the verification of the information and to signify acceptance of the department's unified management system. Companions were required to have their body temperatures taken and symptoms recorded three times per day. They were asked to wash their hands frequently and to wear masks throughout the hospital. It was forbidden for family members to gather for conversation, meals, or other purposes. If a patient's family member was found to have a fever or other positive signs, a monitor would take the family member to the fever clinic, where he or she would be overseen by the

clinic's supervising personnel.

Perioperative management of patients

During the epidemic, the following steps were taken as part of routine patient care: (1) After admission, patients were not allowed to enter or leave the department at will. Only after the primary nurse or doctor was informed could a patient leave the ward for an examination, accompanied by a central transport employee; (2) Patients needed to wear surgical masks throughout their hospitalizations[10]. In the authors' department, some postoperative patients complained of dyspnea and feelings of suffocation while wearing masks; these symptoms were relieved after the patients were gently instructed to relax. It was reported that some postoperative patients on oxygen experienced a rise in oxygen saturation while wearing masks. This finding indicates a need for further studies on the pros and cons of wearing a mask after surgery and a mask's influence on breathing; (3) One-to-one bedside guidance was adopted to replace the original group health related education for patients and their companions in our ward. In addition to the conventional content, the guidance also included information on epidemic prevention. To reduce ineffective postoperative dry coughs, postoperative respiratory rehabilitation training techniques were emphasized, especially effective sputum expectoration[11]. Patients were instructed to observe airway hygiene by covering their mouths and noses with tissues when coughing or sneezing, to implement hand hygiene after touching respiratory secretions, and to maintain a distance of one meter or more from others. Nursing staff were required to locate themselves behind the patient or with their own heads to other side when assisting the patient to finish nebulizer inhalation or produce sputum. Any expectorated sputum was wrapped in tissues and immediately discarded into a garbage bag, which was then tightly fastened. Moreover, patients and family members were required to keep an appropriate distance from one another; (4) Patient body temperatures were taken four times per day. In the case of fever, the doctor in charge was to be informed immediately. In this case, while attending to the patient's symptoms, a rapid screening would be performed. If the fever was not due to the primary lesion, a consultation would be scheduled with the respiratory department. The suspected patient would then be isolated in a single room and undergo a blood test, nucleic acid test, and chest CT to further screen for COVID-19; (5) Studies have suggested that panic is one of the most detrimental aspects caused by the acute stress response during epidemic outbreak[12,13]. Along with concerns about surgical wounds and the illness itself, patients often suffer from severe generalized anxiety and depression, which can influence recovery. Therefore, patients should receive timely psychological evaluation and care upon admission. Doctors should communicate thoughtfully with patients if surgery or treatment needs to be delayed because of the pandemic. Patients with insomnia can take hypnotics in accordance with medical advice, and patients without companions can be instructed to connect with their families through cellphone or video chat to alleviate negative affect; and (6) After hospital discharge, patients would be instructed to protect themselves from COVID-19 through a healthy diet, exercise, and avoiding large social gatherings. Postoperative dressing changes and follow-up examinations could be completed at the nearest community hospital. Discharge follow-up could be done on the phone or *via* an internet platform.

EFFECTS OF PREVENTION AND CONTROL

During the epidemic period from January 24, 2020 to April 19, 2020, 17 emergency patients and 569 regular patients were admitted to the authors' department. All patients completed epidemiological histories and chest CT examinations before entering the ward. We conducted 14 emergency operations and 469 elective operations, among which nine suspected cases of COVID-19 were identified (8 were admitted patients and 1 was a family companion of a patient). All nine suspected cases underwent complete COVID-19 screening to rule out infection.

CONCLUSION

The COVID-19 epidemic has introduced many challenges and problems to management and clinical practice in the thoracic surgery ward. The following are

some examples: (1) Owing to the pandemic, patients whose operations are postponed may miss opportunities to receive the best treatment. Therefore, the doctor in charge must determine the treatment order of patients based on the "disease first" principle, and the waiting times of patients seeking ordinary elective surgeries can be extended; (2) The symptoms and imaging features of many thoracic diseases are similar to those of mild COVID-19, making identification of COVID-19 more difficult in these patients. Therefore, it is imperative that these patients and their families be screened for epidemiological history before admission. Travel and contact histories should carefully consider regions with high numbers of confirmed cases or where cases are increasing rapidly. Those taking histories should also enquire about confirmed cases in patients' communities and workplaces; (3) Postoperative symptoms such as fever, cough, chest pain, and dyspnea could indicate the possibility of infection. The nurse in charge should actively communicate with the doctor, closely monitor examination results and disease changes in patients, and actively identify causes. When necessary, treatment can progress to multi-disciplinary collaborations and joint diagnoses; (4) Once a patient is suspected as having COVID-19, he or she should immediately be transferred to a single room for isolation and be attended to by special personnel. The department should then be closed; admissions and discharges should stop right away. All personnel should be isolated on the spot and prevented from resuming normal work until disease is excluded; and (5) To prevent an inpatient surge after the pandemic, which would likely increase risks, admissions to the department should be restored gradually, according to the risk level of the pandemic. All management plans and processes should be dynamically adjusted according to the current situation.

As key institutions in society for treatment and recovery, hospitals tend to house large numbers of patients, who are transported from one location to another in relatively closed environments[14]. Hence, given the inpatient population's general susceptibility and the fact that COVID-19 is highly contagious through person-to-person transmission, hospital staff must pay attention to every level of epidemic prevention. In our experience, hospitals should initiate a "top-down" linked prevention and control mechanism. In this model, hospital administration plans the overall deployment, and then each department's managers construct specific protocols based on the unique features of their department. All on-duty hospital personnel must recognize the importance of epidemic prevention and be equipped with the proper screening skills and tools. Staff should be able to rapidly detect abnormalities and take prompt measures. By strictly applying the management strategies described above, the authors' department achieved the goal of "zero infection" for staff, patients, and family members while continuing to treat patients in a timely manner.

REFERENCES

1 **Coronaviridae Study Group of the International Committee on Taxonomy of Viruses**. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020; **5**: 536-544 [PMID: 32123347 DOI: 10.1038/s41564-020-0695-z]

2 **National health commission of China**. Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition); Available from: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml

3 **World Health Organization**. Clinical management of severe acute respiratory infection when novel coronavirus infection is suspected: Interim Guidance [S/OL]; Available from: https://www.who.int/csr/disease/coronavirus_infections/InterimGuidance_ClinicalManagement_NovelCoronavirus_11Feb13.pdf?ua=1

4 **Wang D**, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*2020 [PMID: 32031570 DOI: 10.1001/jama.2020.1585]

5 **Wang Q**, Kuang W, Ping W, Gao Y, Hao Z, Cai Y, Li Y, Zhang N, Fu X. Prevention and treatment of cross infection of novel coronavirus pneumonia in thoracic surgery ward. *Zhongguo Xiongxixueguan Waike Linchuang Zazhi* 2020; **27**: 371-374 [DOI: 10.7507//1007-4848.202002132]

6 **Wen J**, Zeng R, Xu CG, Zhao X, Zong ZY, Rao L, Li DJ, Wang M, Wu XD, Huang J, Zeng Y, Cheng NS, Huang Y, Zhang W, Li WM. Ten measures to fight against the 2019-nCoV pneumonia in West China Hospital. *Zhongguo Xunzheng Yixue Zazhi* 2020; **20**: 365-368 [DOI: 10.7507/1672-2531.202002014]

7 **Fu Q**, Zhang XY, Li SW. Strategies for risk management of medical staffs occupational exposure to COVID-19. *Zhonghua Yiyuan Ganranxue Zazhi* 2020; **30**: 801-805 [DOI: 10.11816/cn.ni.2020-200216]

8 **Lin L**, Zheng E, Mei JD, Che GW, Chen LQ, Ning N, Yang M. Treatment and management strategies of patients undergoing selective thoracic surgery in the epidemic situation of COVID-19. *Zhongguo Xiongxixueguan Waike Linchuang Zazhi* 2020; **27**: 1-4

9 **Li SY**, Huang WZ, Liao XL, Li DD, Du LY, Song JJ, Zhou YW, Zhao SZ, Wang Y, Cao XY, Wang J, Liu JL, Wang YN, Zhu SC, Li L, Hao QK, Zong ZY, Sun X, Li WM. Disease control of 2019 novel coronavirus infection in hospital: West China urgent recommendation. *Zhongguo Xunzheng Yixue Zazhi* 2020; **20**: 125-133 [DOI: 10.7507/1672-2531.202001121]

10 **Wang LY**, He L, Deng LJ, An Q, Zhang JM, Zhang FM, Chen LJ, Luo YL, Feng M, Luo BR, Tang ML. Urgent recommendation and practice of prevention and control of novel coronavirus disease 2019 (COVID-19) in intensive care units in West China Hospital of Sichuan University during the epidemics. *Zhongguo Xiongxixueguan Waike Linchuang Zazhi* 2020; **27**: 388-394 [DOI: 10.7507/1007-4848.202002066]

11 **National health commission of China**. Criteria of prevention and control of airborne nosocomial infections. (2016-12-27) [EB/OL]; Available from: http://www.nhc.gov.cn/wjw/s9496/201701/7e0e8fc6725843aabba8f841f2f585d2.shtml

12 **Mosolov SN**. [Problems of mental health in the situation of COVID-19 pandemic]. *Zh Nevrol Psikhiatr Im S S Korsakova* 2020; **120**: 7-15 [PMID: 32621462 DOI: 10.17116/jnevro202012005 17]

13 **Talevi D**, Socci V, Carai M, Carnaghi G, Faleri S, Trebbi E, di Bernardo A, Capelli F, Pacitti F. Mental health outcomes of the CoViD-19 pandemic. *Riv Psichiatr* 2020; **55**: 137-144 [PMID: 32489190 DOI: 10.1708/3382.33569]

14 **Muller MP**, Siddiqui N, Ivancic R, Wong D. Adenovirus-related epidemic keratoconjunctivitis outbreak at a hospital-affiliated ophthalmology clinic. *Am J Infect Control* 2018; **46**: 581-583 [PMID: 29305276 DOI: 10.1016/j.ajic.2017.11.011]

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ABOUT COVER

Editorial board member of *World Journal of Virology*, Dr. Simone Giannecchini is a Professor at the University of Florence, in Florence, Italy. He received his Bachelor's degree in Biology in 1993 and his PhD in Immunobiology of Viruses in 1998, both from the University of Pisa, Italy. He undertook the position of Researcher in Microbiology and Clinical Microbiology at University of Florence in 2004, where he advanced to Associate Professor in 2018. His ongoing research interests involve cellular and molecular biology applied to the study of pathogenesis of viral infections and their prevention. His most recent investigations focus on the role of association of viruses to extracellular vesicles in viral persistence. (L-Editor: Filipodia)

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MINIREVIEWS

Chronic hepatitis B-associated liver disease in the context of human immunodeficiency virus co-infection and underlying metabolic syndrome

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Abstract

Globally, a shift in the epidemiology of chronic liver disease has been observed. This has been mainly driven by a marked decline in the prevalence of chronic hepatitis B virus infection (CHB), with the greatest burden restricted to the Western Pacific and sub-Saharan African regions. Amidst this is a growing burden of metabolic syndrome (MetS) worldwide. A disproportionate co-burden of human immunodeficiency virus (HIV) infection is also reported in sub-Saharan Africa, which poses a further risk of liver-related morbidity and mortality in the region. We reviewed the existing evidence base to improve current understanding of the effect of underlying MetS on the development and progression of chronic liver disease during CHB and HIV co-infection. While the mechanistic association between CHB and MetS remains poorly resolved, the evidence suggests that MetS may have an additive effect on the liver damage caused by CHB. Among HIV infected individuals, MetS-associated liver disease is emerging as an important cause of non-AIDS related morbidity and mortality despite antiretroviral therapy (ART). It is plausible that underlying MetS may lead to adverse outcomes among those with concomitant CHB and HIV co-infection. However, this remains to be explored through rigorous longitudinal studies, especially in sub-Saharan Africa. Ultimately, there is a need for a comprehensive package of care that integrates ART programs with routine screening for MetS and promotion of lifestyle modification to ensure an improved quality of life among CHB and HIV co-infected individuals.

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Core Tip: Independently, chronic hepatitis B virus (HBV) infection, human immunodeficiency virus (HIV) infection and metabolic syndrome (MetS) are known risk factors of chronic liver disease. The presence of MetS components, including type 2 diabetes mellitus, central obesity and lipid abnormalities, are associated with adverse outcomes and altered treatment response among HBV and HIV infected individuals. While underlying MetS may have an additive effect on the development and progression of chronic liver disease among HBV-HIV co-infected individuals, the evidence from endemic regions like sub-Saharan Africa is limited and deserves further attention in the research agenda.

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INTRODUCTION

Chronic liver disease is a frequent clinical condition accounting for an estimated 2 million deaths each year worldwide[1]. It is characterized by a progressive deterioration of liver function, involving a continuous process of inflammation, destruction and regeneration of the cells of the liver. This often leads to complications such as liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC). A broad spectrum of etiologies is associated with chronic liver disease and typically includes alcohol use disorder, chronic exposure to toxins, viral hepatitis including chronic hepatitis B virus (HBV) infection and immune and metabolic disorders[1]. Globally, an epidemiological shift in the burden of chronic liver disease has been observed, mainly driven by diabolical factors. On the one hand are the global efforts that have led to the increased elimination of aflatoxins from food, improved safety of transfusions and transplantations, the establishment of viral hepatitis treatment programs and universal childhood hepatitis B vaccination programs; and on the other hand is the increasing burden of metabolic disorders worldwide and the persisting endemicity of chronic HBV infection (CHB) in regions such as the Western Pacific and sub-Saharan Africa[1-3].

It is well established that CHB is a leading cause of liver disease and death worldwide. The World Health Organization estimates that currently 257 million persons or 3.5% of the world's population are chronic carriers of HBV, 68.0% of whom live in the Western Pacific (115 million) and sub-Saharan African (60 million) regions alone[4]. The greater proportion of chronic carriers are persons who were born prior to the establishment of universal childhood hepatitis B vaccination programs. What further compounds the situation in a region like sub-Saharan Africa is the fact that it is also home to 71.0% of the global population (35 million) of people living with human immunodeficiency virus (PLHIV)[5,6]. Due to similar routes of transmission, co-infection with HBV and HIV are not uncommon. Of the 2.7 million HBV-HIV co-infected persons worldwide, 69.0% or 1.9 million live in sub-Saharan Africa[7]. With rapid expansion of antiretroviral treatment (ART) programs, there has been a dramatic decline in AIDS-related deaths and consequently an increase in the life expectancy of PLHIV, including those co-infected with HBV[5,6]. However, as PLHIV are living longer, an increased risk of chronic liver disease has been observed and is emerging as an important cause of non-AIDS-related mortality within this population[8,9]. Among PLHIV, liver-related mortality has been found to be up to 10 times of that occurring within the general population[10]. Development of chronic liver disease among PLHIV has been associated with underlying viral hepatitis (including CHB) and non-viral hepatitis risk factors such as lifelong exposure to components of ART regimens with hepatotoxic effects and the development of metabolic syndrome (MetS)[8].

MetS is a common yet complex condition characterized by a clustering of various metabolic disorders (Table 1) that are known to increase the risk of developing chronic liver disease or to worsen the prognosis among individuals with other underlying risk factors of chronic liver disease[11-14]. Chronic liver disease among individuals with MetS is often preceded by the accumulation of fats or triglycerides in the cells of the liver due to MetS components like insulin resistance, abnormal lipid metabolism and dysregulation of cytokines and adipokines, leading to a spectrum of fatty liver disorders known as non-alcoholic fatty liver disease (NAFLD). With significant liver inflammation and injury over time, a severe form of NAFLD develops, referred to as non-alcoholic steatohepatitis (NASH). NASH is associated with liver damage and progression to advanced liver cirrhosis and fibrosis[15]. The evidence on the association between MetS and other common risk factors of chronic liver disease, such as CHB, is oftentimes conflicting. In addition, the role of MetS in the development and prognosis of chronic liver disease among HBV-HIV co-infected individuals is unclear. With the concomitant high burden of CHB and HIV infection, and the growing prevalence of MetS and its associated complications, sub-Saharan Africa presents a unique case for continuously examining key risk factors of chronic liver disease in order to inform ongoing public health interventions[16].

We review evidence emerging over the last decade (2010-2020) to improve current understanding of the pathogenesis of chronic liver disease among CHB and HIV co-infected individuals with underlying MetS. We identify gaps in the evidence base and propose recommendations for future research, as well as current policy and practice. This review takes a special focus on sub-Saharan Africa where the burden of CHB and HIV co-infection is high, the prevalence of MetS is growing and the need for intervention is often the greatest.

CONFLICTING EVIDENCE ON THE ASSOCIATION BETWEEN METS AND CHB

With 6.1% of the population living with CHB, the burden of liver cirrhosis, fibrosis and HCC in sub-Saharan Africa is significant[4]. The association between MetS and the increased risk of chronic liver disease presents an added burden and calls for greater attention within this population. Despite this, our review of primary studies published within the last decade reveal a profound lack of data on CHB and MetS from sub-Saharan Africa.

Drawing on data from elsewhere, the combined prevalence of MetS among those with CHB varies from 5.0% to 30.1%[17,18]. In Europe, studies conducted in Slovakia report MetS prevalence rates of 27.8% among Roma[19] and 24.6% among both Caucasian and Roma[20] populations with CHB. This is comparable to findings from a study conducted in Spain that found that 24.0% of individuals with CHB had underlying MetS[21]. In both Slovakian studies, however, no significant association between MetS and HBV infection was found, as the prevalence of the condition was comparable between those with or without CHB (27.8% in CHB patients *vs* 29.6% in controls, $P = 0.785$[19]; and 24.6% in CHB patients *vs* 24.7% in controls, $P = 0.561$[20]), irrespective of age and sex. Instead, the studies did show that CHB patients with MetS presented with significantly higher HBV-DNA viral load and elevated liver enzymes, including alanine aminotransferase (ALT) and gamma-glutamyl transferase, compared to those without MetS, suggesting an additive effect of MetS on the liver damage caused by CHB[19,20]. Contrary to these findings, a large population-based study conducted in the United States (the NHANES III study) described a significantly lower prevalence of MetS in CHB patients compared to controls (10.4% *vs* 25.6%, $P = 0.019$). Stratified by sex, this inverse correlation between MetS and CHB was found to persist in males but not in females[22]. Unlike the Slovakian observations, CHB patients with high levels of ALT in the NHANES III study had a significantly lower rate of MetS compared with controls (2.1% *vs* 49.8%, $P < 0.001$). Given these findings, the authors hypothesized that chronic liver inflammation, instead of HBV itself, may be responsible for metabolic derangements in CHB patients[22]. It is worth noting that participants in the NHANES III study were relatively older than those in the Slovakian studies, and this may have influenced the conflicting findings. Evidence emerging from Asia on the association between CHB and MetS is no less conflicting than that discussed previously. For example, a case-series conducted in Taiwan found no correlation between CHB and MetS[23], while two other cross-sectional studies from Taiwan reported an inverse correlation between CHB and MetS[24,25]. Contrary to this, a positive association between latent HBV infection and MetS [hazard ratio (HR) = 2.27,

Table 1 Metabolic syndrome—definition, diagnostic criteria and association with chronic liver disease

Definition of MetS

A clustering of metabolic disorders that include hypertension, central obesity, impaired glucose metabolism including insulin resistance and abnormal cholesterol or triglyceride levels. MetS increases the risk of morbidity and mortality from cardiovascular disease, stroke, type 2 diabetes, chronic kidney disease and chronic liver disease

Diagnostic criteria[1]

NCEP/ATP III[27]	AHA/NHLBI[28]	IDF[29]	JIS[30]	WHO[31]
Presence of ≥ 3 of the following:	Presence of ≥ 3 of the following:	Central obesity; ethnicity-specific waist circumference values[2] or BMI > 30 kg/m^2 plus any 2 of the following:	Presence of ≥ 3 of the following:	Glucose intolerance, impaired glucose tolerance or diabetes mellitus and/or insulin resistance and any 2 of the following:
Abdominal obesity; > 102 cm in males and > 88 cm in females	Elevated waist circumference; ≥ 102 cm in males and ≥ 88 cm in females	Raised triglycerides; ≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality	Elevated waist circumference; population- and country-specific definitions[2]	Raised arterial pressure; ≥ 160/90 mmHg
Elevated triglycerides; ≥ 150 mg/dL or treatment for elevated triglycerides	Elevated triglycerides; ≥ 150 mg/dL (1.7 mmol/L) or treatment for elevated triglycerides	Reduced HDL cholesterol; < 40 mg/dL (1.03 mmol/L) in males and < 50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality	Elevated triglycerides; ≥ 150 mg/dL (1.7 mmol/L) or treatment for elevated triglycerides	Raised plasma triglyceride; ≥ 150 mg/dL, and/or low HDL cholesterol; < 35 mg/dL in males and < 39 mg/dL in females
Reduced HDL cholesterol; < 40 mg/dL in males and < 50 mg/dL in females	Reduced HDL cholesterol; < 40 mg/dL (1.03 mmol/L) in males and < 50 mg/dL (1.3 mmol/L) in females or treatment for reduced HDL cholesterol	Raised blood pressure; ≥ 130/≥ 85 mmHg, or treatment of previously diagnosed hypertension	Reduced HDL cholesterol; < 40 mg/dL (1.0 mmol/L) in males and < 50 mg/dL (1.3 mmol/L) in females, or treatment for reduced HDL cholesterol	Central obesity; waist/hip ratio > 0.90 in males and > 0.85 in females and/or BMI > 30 kg/m^2
Elevated blood pressure; ≥ 130/≥ 85 mmHg or treatment for elevated blood pressure	Elevated blood pressure; ≥ 130/≥ 85 mmHg or antihypertensive treatment	Raised fasting plasma glucose; ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes	Elevated blood pressure; ≥ 130/≥ 85 mmHg or anti-hypertensive treatment	Microalbuminuria; urinary albumin excretion rate ≥ 20 μg/min or albumin/creatinine ratio ≥ 20 μg/mg
Elevated fasting glucose; ≥ 110 mg/dL or treatment for elevated glucose	Elevated fasting glucose; ≥ 100 mg/dL or treatment for elevated glucose		Elevated fasting glucose; ≥ 100 mg/dL, or treatment of elevated glucose	

MetS and chronic liver disease

The association between MetS and chronic liver disease involves a complexity of risk factors which are yet to be fully understood. NAFLD which covers a spectrum of fatty liver disorders including NASH, is the most common cause of abnormal liver function among individuals with MetS. MetS components like insulin resistance may increase fatty acids in the liver, leading to fat or triglyceride accumulation in hepatocytes. NASH, which is an advanced form of NAFLD, is associated with liver inflammation and liver damage, leading to the development of liver cirrhosis and progression to advanced liver fibrosis. In addition, type 2 diabetes and obesity may increase the risk of HCC. The presence of MetS may have worse outcomes in individuals with other causes of chronic liver disease, such as viral hepatitis.

[1]NCEP/ATP III: National Cholesterol Education Program/Adult Treatment Panel III; AHA/NHLBI: American Heart Association/National Heart, Lung and Blood Institute; IDF: International Diabetes Federation; JIS: Joint Interim Statement; WHO: World Health Organization.

[2]Currently, ethnicity-specific waist circumference values have not been defined for populations from sub-Saharan Africa. BMI: Body mass index; HCC: Hepatocellular carcinoma; HDL: High-density lipoprotein; MetS: Metabolic syndrome; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

95%CI: 1.52-3.38] was demonstrated in a retrospective cohort study conducted in China, suggesting that latent HBV infection may be a risk factor for the development of MetS[26].

Evidently, the mechanistic association between CHB and MetS remains poorly

resolved. Reasons for the conflicting findings may include variations in the MetS diagnostic criteria used in the various studies (Table 1)[27-31]. The geographic heterogeneity of the published data also means that a comparison between studies may not always be feasible. Also, worth noting is the fact that a majority of the published studies are cross-sectional in nature, which often draws the weakest evidence for the establishment of causal associations between CHB and MetS, as opposed to robust longitudinal studies. Despite the disparities in the prevalence of MetS reported among those with CHB, the evidence base strongly suggests that older age[24] and female sex[18,25,32,33] may be predictors of MetS within this population.

Several studies have shown that underlying MetS increases the risk and progression of liver fibrosis, cirrhosis and HCC in patients with CHB[17,34-36]. A longitudinal population-based study involving 2979 participants aged 40-65 years, of whom 1690 had CHB, revealed that the presence of three or more metabolic risk factors, compared with no factors, significantly increased the risk of HCC by two- to three-fold among CHB patients[36]. This relationship persisted after controlling for viral factors such as high HBV-DNA viremia (≥ 10000 copies/mL) and other known risk factors of HCC[36]. These findings are consistent with observations made elsewhere[34]. Among these metabolic risk factors, insulin resistance and central obesity are independently associated with the development of liver damage and HCC. In a longitudinal cohort study conducted by Huang *et al*[37], a significantly higher cumulative incidence of cirrhosis [log-rank test, $P < 0.001$, with a relative risk (RR) of 3.43, 95% confidence interval (CI): 2.62-4.49] and decompensated cirrhosis (log-rank test, $P < 0.001$, with an RR of 4.11, 95%CI: 2.95-5.70) was noted among CHB patients with newly diagnosed diabetes as compared to those without diabetes. Adjusting for age, sex, CHB treatment, HCC and comorbidity index, type 2 diabetes mellitus (T2DM) remained an independent predictor for cirrhosis (HR = 2.015; 95%CI: 1.393-2.915; $P < 0.001$) and decompensated cirrhosis (HR = 1.792; 95%CI: 1.192-2.695; $P = 0.005$)[37]. Another study showed that pre-existing T2DM for > 5 years before cirrhosis diagnosis, insulin and/or sulphonylurea use and poor diabetic control (defined as glycated hemoglobin A1c ≥ 7.0%) were predictors of cirrhosis complications and HCC development[38]. These findings were confirmed by a longitudinal study that reported a significantly higher incidence of HCC (13.3% *vs* 10.0%; $P < 0.001$) and HCC-related mortality (7.5% *vs* 4.7%; $P < 0.001$) among 2966 CHB patients with T2DM compared to 2966 CHB patients without T2DM, after a median follow-up of 11.4 years[39]. Elevated serum adiponectin levels may also play a role in the increased risk of liver fibrosis, cirrhosis and HCC[40,41].

When investigating dyslipidemia, including hypercholesterolemia or hypertriglyceridemia, among CHB patients *vs* controls, several studies have reported significantly lower levels of total cholesterol and triglycerides among those with CHB[19,20,42,43]. Among CHB patients, significant disparities have been observed in the levels of triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) detected among males *vs* females and those aged ≤ 45 years *vs* > 45 years[18,23,32]. While the heterogeneity in the lipid profiles detected among CHB patients is not fully understood, some virus-specific risk factors have been linked with the lipid abnormalities observed within this population. For example, HBV infection is known to cause liver injury, which may lead to impaired liver function, thereby altering total cholesterol and triglyceride levels[42]. Moreover, it has been shown that the viral HBx protein inhibits the secretion of apolipoprotein B, which is an essential component for the formation of very-LDL and LDL, thereby lowering serum triglyceride levels and causing the accumulation of hepatic triglycerides[43,44]. This is particularly concerning as excessive accumulation of triglycerides in the liver leads to NAFLD.

Both CHB and NAFLD are recognized as significant causes of liver cirrhosis and fibrosis. Thus, it is only reasonable to examine the relationship between these conditions. Several studies have reported a positive association between hepatic steatosis and MetS components such as high body mass index or central obesity, elevated serum triglyceride and total cholesterol levels and insulin resistance, among those with CHB[45-47], although an inverse relationship with HBV replication and hepatitis B surface antigen positivity has also been reported[46,48-50]. These observations suggest host factors, and not HBV itself, as predictors of hepatic steatosis in those with CHB. Interestingly, Joo *et al*[51] found that HBV infection was significantly associated with a lower risk of incident NAFLD. By investigating the lipid profiles in these patients, the authors reported a significant decrease in total cholesterol levels over time among CHB patients compared with controls, suggesting that HBV infection could protect against the development of NAFLD, possibly through its effect on lipid metabolism.

SUBSTANTIAL BURDEN OF METS AND NAFLD AMONG PLHIV

Sub-Saharan Africa has a disproportionate burden of HIV infection, and there is evidence to suggest that a significant proportion of PLHIV within this region are at increased risk of developing MetS and its associated complications, including chronic liver disease[13,52]. It is worth noting however, that the true burden of MetS among PLHIV in sub-Saharan Africa is often difficult to ascertain, given the heterogeneity of the condition itself, the lack of ethnicity-specific diagnostic criteria for sub-Saharan African populations and the disparities in the associated risk factors (HIV-related *vs* host and environmental-related risk factors). Table 2 shows the variable prevalence rates of MetS among PLHIV from selected studies conducted in sub-Saharan African countries within the last decade[53-67]. These variations in the prevalence of MetS among PLHIV are not exclusive to sub-Saharan African countries but have been reported in similar population groups in Latin America[68] and Europe[69-72]. The commonly reported independent risk factors associated with MetS among PLHIV in sub-Saharan Africa include female sex, age > 40 years and central obesity[56,59,62]. The potential influence of host genetics in the development of MetS within this population has also been suggested previously[66].

A persistent matter of debate in the evidence-base has been the impact of lifelong exposure to ART on the burden of MetS among PLHIV. In a recent cross-sectional study conducted among PLHIV in Ghana, Obirikorang *et al*[65] found a higher prevalence of MetS among participants on ART compared to their ART-naïve counterparts, irrespective of the diagnostic criteria used. Consistent with this finding, a study conducted in Cameroon reported prevalence rates of 36.0% and 23.4% among those on ART compared to ART-naive individuals, respectively[57]. Mbunkah *et al*[60] further reported statistically significant (P = 0.02) variations in MetS prevalence among those on first-line ART (24.2%) and second-line or protease inhibitor-based ART (10.0%), compared to ART-naive (11.5%) Cameroonian PLHIV. Contrary to these findings, Ngatchou *et al*[63] found that ART-naïve individuals rather experience a two-fold increase in the prevalence of MetS, suggesting a possible influence of uncontrolled HIV replication, while Tesfaye *et al*[67] noted that the prevalence of MetS among PLHIV in Ethiopia was not influenced by whether or not they had initiated ART. To date, the findings from sub-Saharan Africa have been limited by the cross-sectional design adopted by most studies. Findings from a previous longitudinal study conducted in Italy show that after 3 years of follow-up, there was no significant difference in the incidence of MetS among those on ART and ART-naïve individuals. Instead, the authors posit that there may be different metabolic pathways underlying the development of MetS in ART-naïve individuals compared to those on ART[72]. While the findings on MetS among PLHIV in sub-Saharan Africa remain inconclusive, they do suggest a possible multi-factorial mechanism—involving viral, host and environmental factors—underlying the pathogenesis of MetS among PLHIV, which underscores the importance of the condition within this population.

Historically, the development of chronic liver disease among PLHIV has been associated with concomitant viral hepatitis, ART-associated hepatotoxicity and alcoholic liver disease[8]. Emerging evidence now shows that NAFLD is increasingly becoming an important cause of significant liver morbidity among PLHIV[73-75]. Unfortunately, evidence emerging from sub-Saharan Africa on the burden of NAFLD among PLHIV is limited, and this has been raised previously as a regional public health concern[76]. Our search for relevant sub-Saharan African studies published within the last decade on this topic returned only one output from South Africa that reported a hepatic steatosis prevalence rate of 28.0% among PLHIV[77]. This is considerably lower than prevalence rates reported for Asian populations (31.0%)[78] as well as from studies conducted in Canada (54.0%)[79] and Greece (55.0%)[80]. These studies also provide strong evidence suggesting that PLHIV are at high risk for developing NASH, fibrosis and HCC, spurred by a high burden of traditional MetS components such as insulin resistance, central obesity and dyslipidemia[78-81]. Several reports from sub-Saharan Africa do indicate that these traditional MetS components (insulin resistance, T2DM, central obesity and dyslipidemia) are in fact prevalent among PLHIV, which could suggest a significant risk for the development of NASH and other chronic liver complications, although this association is less well researched within the region[82-85]. The scarcity of evidence from sub-Saharan Africa means that the true burden and natural history of NAFLD among PLHIV may be underappreciated. This could have negative implications for the development of evidence-based public health interventions tailored to the sub-Saharan African context.

Table 2 Prevalence of metabolic syndrome among people living with human immunodeficiency virus in sub-Saharan Africa from selected studies

Ref.	Country	Study design	Sample size, *n*	MetS diagnostic criteria	Prevalence of MetS	Independent risk factors[1]
Adébayo *et al*[53]	Benin	Cross-sectional	244	IDF	18.4%	-
Ayodele *et al*[54]	Nigeria	Cross-sectional	291	NCEP/ATP III; IDF; JIS	12.7%; 17.2%; 21.0%	-
Berhane *et al*[55]	Ethiopia	Cross-sectional	313	NCEP/ATP III	21.1%	HAART > 12 mo, female sex
Bosho *et al*[56]	Ethiopia	Cross-sectional	286	NCEP/ATP III; IDF; JIS	23.5%; 20.5%; 27.6%	BMI ≥ 25 kg/m^2, formal education
Dimodi *et al*[57]	Cameroon	Cross-sectional	463	IDF; NCEP/ATP III	32.8%; 30.7%	-
Guira *et al*[58]	Burkina Faso	Cross-sectional	300	IDF	18.0%	-
Hirigo *et al*[59]	Ethiopia	Cross-sectional	185	IDF; NCEP/ATP III	24.3%; 17.8%	BMI ≥ 25 kg/m^2, female sex, age > 40 yr
Mbunkah *et al*[60]	Cameroon	Cross-sectional	173	NCEP/ATP III	15.6%	-
Muhammad *et al*[61]	Nigeria	Cross-sectional	200	NCEP/ATP III	15.0%	-
Muyanja *et al*[62]	Uganda	Cross-sectional	250	AHA/NHLBI	58.0%	Female sex, age > 40 yr
Ngatchou *et al*[63]	Cameroon	Cross-sectional	108	AHA/NHLBI	47.0%	-
Nguyen *et al*[64]	South Africa	Cross-sectional	748	JIS; IDF; NCEP/ATP III	28.2%; 26.5%; 24.1%	-
Obirikorang *et al*[65]	Ghana	Cross-sectional	433	NCEP/ATP III; WHO; IDF	48.3%; 24.5%; 42.3%	-
Sobieszczyk *et al*[66]	South Africa	Longitudinal	160	NCEP/ATP III	19.2%	Older age, time post HIV infection, family history of diabetes, human leukocyte antigen B 81:01 allele
Tesfaye *et al*[67]	Ethiopia	Cross-sectional	374	IDF; NCEP/ATP III	25.0%; 16.8%	Female sex, older age, BMI ≥ 25 kg/m^2, total cholesterol ≥ 200 mg/dL

[1]Based on multivariate analysis in the individual studies. AHA/NHLBI: American Heart Association/National Heart, Lung and Blood Institute; BMI: Body mass index; HAART: Highly active antiretroviral therapy; IDF: International Diabetes Federation; JIS: Joint Interim Statement; MetS: Metabolic syndrome; NCEP/ATP III: National Cholesterol Education Program/Adult Treatment Panel III; WHO: World Health Organization.

LIMITED EVIDENCE ON PLAUSIBLE SYNERGISTIC EFFECT BETWEEN METS AND HBV-HIV CO-INFECTION

Given the substantial risk of MetS and NAFLD among those with CHB and PLHIV, it is important to understand if there is a synergistic effect between MetS and HBV-HIV co-infection in the pathogenesis of chronic liver disease. It is well established that HBV-HIV co-infected individuals are at increased risk of chronic liver disease[86,87]. In addition to the widely recognized mechanisms underlying chronic liver disease in HBV-HIV co-infected individuals, it has now been shown that interactions between HIV gp120 and tat proteins with epithelial cells may induce epithelial-mesenchymal transition, leading to the development of fibrosis[88]. Thus, among those with HBV-HIV co-infection, HIV interactions with liver cells may synergize the development of fibrosis and cirrhosis. In comparison, very little is known of the effect of underlying MetS on the progression of chronic liver disease among HBV-HIV co-infected individuals. While a synergistic effect may be plausible, there is insufficient evidence to confirm this as very few studies report on the burden of MetS among HBV-HIV co-infected individuals. In fact, only three studies met the criteria for this review, one of which involved a sub-Saharan African population[89-91]. In this study involving 41891 ART-naïve HIV-infected individuals from Tanzania, Nagu *et al*[89] sought to identify independent risk factors of elevated ALT titers (> 40 IU/L) as a less sensitive but non-invasive predictor of liver injury and increased risk of mortality from liver disease. Multivariate analysis showed that MetS components including hypertriglyceridemia, hyperglycemia and central obesity, as well as immunosuppression due to uncontrolled

HIV infection and HBV co-infection, were significantly associated with higher risk of elevated ALT[88]. However, the cumulative effect of these risk factors on liver function was not investigated as part of this study.

In a study using the more sensitive transient elastography to assess liver fibrosis and determine associated risk factors among German PLHIV on ART, T2DM and central obesity were found to be associated with the presence of significant fibrosis with (n = 23, 18%) or without (n = 343, 10%) HBV co-infection[90]. Finally, when investigating the etiology of liver-related hospital admissions among PLHIV and CHB patients in the United States, Rajbhandari *et al*[91] found a high prevalence of NASH among HIV mono-infected patients (43.6%) and HBV-HIV co-infected patients (26.9%). In addition to this, a three-fold surge in in-hospital mortality was reported among PLHIV with concomitant HBV co-infection and cirrhosis or portal hypertension compared to those without these comorbidities (odds ratio: 3.00, 95%CI: 1.80-5.02)[91]. Taken together, these findings suggest high risk of adverse outcomes among HBV-HIV co-infected individuals with liver disease and some form of metabolic disorder. It will be important to explore these findings in sub-Saharan Africa where the burden of HBV-HIV co-infection is significantly higher.

While there is an obvious need for further research to improve our understanding of the association between HBV-HIV co-infection and MetS, it is still possible to draw some implications for the clinical management of this population. Comprehensive programs targeted at HBV-HIV co-infected individuals that integrate ART programs with routine screening for MetS components and promotion of lifestyle modifications could be low-hanging fruits for effectively reducing the risk of adverse outcomes including chronic liver disease. Where underlying MetS is left undetected and uncontrolled there may be negative implications for ART outcomes. For example, the presence of central obesity and T2DM has been associated with lower rates of fibrosis regression among patients with CHB undergoing long-term treatment with nucleotide/ nucleoside analogues[92]. The effect of nucleotide/nucleoside analogues on MetS components such as lipid abnormalities has also been investigated. A retrospective cohort study that compared tenofovir disoproxil fumarate (or TDF, which forms part of some ART regimens) and entecavir (ETV) therapy among CHB patients found that serum lipoprotein lipid levels significantly differed pre- and post-treatment for median total cholesterol (3.92 *vs* 4.42 mmol/L, $P < 0.01$), LDL-C (2.25 *vs* 2.51 mmol/L, $P < 0.01$) and HDL-C (1.14 *vs* 1.34 mmol/L, $P < 0.01$) in the TDF arm whereas no significant differences were observed in the ETV group[93]. In fact, TDF was shown to be an independent predictor of changes in lipid profiles, with TDF-treated patients being 14.0%, 13.0% and 20.0% more likely to attain a reduction in levels of total cholesterol, LDL-C and HDL-C, respectively, compared to those on ETV. However, triglycerides levels did not change over the follow-up period (median of 56 mo) in the TDF group[93]. Similarly, while a recent phase IV randomized control trial demonstrated the superiority (by 8.0%-10.0%) of pitavastatin over pravastatin in reducing LDL-C among PLHIV with dyslipidemia, there was no difference between either cholesterol-lowering drug in altering triglyceride levels[94].

CONCLUSION

As HBV-HIV co-infected individuals are living longer due to the benefits of ART, there is a need to ensure optimal quality of life, and this can be achieved by reducing the risk of comorbidities like MetS and chronic liver disease. There is a need to expand the research agenda in sub-Saharan Africa in order to improve our understanding of the role of MetS in the progression of chronic liver disease among the substantial population of CHB and HBV-HIV co-infected individuals within the region. Future research should include rigorous longitudinal studies to allow for the determination of the temporal sequence of the development and progression of chronic liver disease among CHB and HBV-HIV co-infected individuals with underlying MetS. In addition, a consensus on ethnicity-specific diagnostic criteria for sub-Saharan African populations is required in order to improve the assessment of MetS within the region.

REFERENCES

1 **Moon AM**, Singal AG, Tapper EB. Contemporary Epidemiology of Chronic Liver Disease and Cirrhosis. *Clin Gastroenterol Hepatol* 2019; Online ahead of print [PMID: 31401364 DOI: 10.1016/j.cgh.2019.07.060]

2 **Magnussen A**, Parsi MA. Aflatoxins, hepatocellular carcinoma and public health. *World J Gastroenterol* 2013; **19**: 1508-1512 [PMID: 23539499 DOI: 10.3748/wjg.v19.i10.1508]

3 **GBD 2017 Cirrhosis Collaborators**. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020; **5**: 245-266 [PMID: 31981519 DOI: 10.1016/S2468-1253(19)30349-8]

4 **World Health Organization**. Global hepatitis report, 2017. Geneva: World Health Organization, April, 2017. [cited 31 May 2020]. Available from: http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1

5 **Joint United Nations Programme on HIV/AIDS (UNAIDS)**. The Gap Report. 2014. [cited 31 May 2020]. Available from: https://www.unaids.org/en/resources/documents/2014/20140716_UNAIDS_gap_report

6 **Kharsany AB**, Karim QA. HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. *Open AIDS J* 2016; **10**: 34-48 [PMID: 27347270 DOI: 10.2174/1874613601610010034]

7 **Platt L**, French CE, McGowan CR, Sabin K, Gower E, Trickey A, McDonald B, Ong J, Stone J, Easterbrook P, Vickerman P. Prevalence and burden of HBV co-infection among people living with HIV: A global systematic review and meta-analysis. *J Viral Hepat* 2020; **27**: 294-315 [PMID: 31603999 DOI: 10.1111/jvh.13217]

8 **Price JC**, Thio CL. Liver disease in the HIV-infected individual. *Clin Gastroenterol Hepatol* 2010; **8**: 1002-1012 [PMID: 20851211 DOI: 10.1016/j.cgh.2010.08.024]

9 **Farahani M**, Mulinder H, Farahani A, Marlink R. Prevalence and distribution of non-AIDS causes of death among HIV-infected individuals receiving antiretroviral therapy: a systematic review and meta-analysis. *Int J STD AIDS* 2017; **28**: 636-650 [PMID: 26868158 DOI: 10.1177/0956462416632428]

10 **Hernando V**, Perez-Cachafeiro S, Lewden C, Gonzalez J, Segura F, Oteo JA, Rubio R, Dalmau D, Moreno S, Amo JD; CoRIS. All-cause and liver-related mortality in HIV positive subjects compared to the general population: differences by HCV co-infection. *J Hepatol* 2012; **57**: 743-751 [PMID: 22709620 DOI: 10.1016/j.jhep.2012.06.010]

11 **Wang CC**, Tseng TC, Kao JH. Hepatitis B virus infection and metabolic syndrome: fact or fiction? *J Gastroenterol Hepatol* 2015; **30**: 14-20 [PMID: 25092429 DOI: 10.1111/jgh.12700]

12 **Ren H**, Wang J, Gao Y, Yang F, Huang W. Metabolic syndrome and liver-related events: a systematic review and meta-analysis. *BMC Endocr Disord* 2019; **19**: 40 [PMID: 31023282 DOI: 10.1186/s12902-019-0366-3]

13 **Todowede OO**, Mianda SZ, Sartorius B. Prevalence of metabolic syndrome among HIV-positive and HIV-negative populations in sub-Saharan Africa-a systematic review and meta-analysis. *Syst Rev* 2019; **8**: 4 [PMID: 30606249 DOI: 10.1186/s13643-018-0927-y]

14 **Wang CC**, Cheng PN, Kao JH. Systematic review: chronic viral hepatitis and metabolic derangement. *Aliment Pharmacol Ther* 2020; **51**: 216-230 [PMID: 31746482 DOI: 10.1111/apt.15575]

15 **Rosselli M**, Lotersztajn S, Vizzutti F, Arena U, Pinzani M, Marra F. The metabolic syndrome and chronic liver disease. *Curr Pharm Des* 2014; **20**: 5010-5024 [PMID: 24320032 DOI: 10.2174/1381612819666131206111352]

16 **Jaspers Faijer-Westerink H**, Kengne AP, Meeks KAC, Agyemang C. Prevalence of metabolic syndrome in sub-Saharan Africa: A systematic review and meta-analysis. *Nutr Metab Cardiovasc Dis* 2020; **30**: 547-565 [PMID: 32143896 DOI: 10.1016/j.numecd.2019.12.012]

17 **Yoon H**, Lee JG, Yoo JH, Son MS, Kim DY, Hwang SG, Rim KS. Effects of metabolic syndrome on fibrosis in chronic viral hepatitis. *Gut Liver* 2013; **7**: 469-474 [PMID: 23898389 DOI: 10.5009/gnl.2013.7.4.469]

18 **Katoonizadeh A**, Ghoroghi S, Sharafkhah M, Khoshnia M, Mirzaei S, Shayanrad A, Poustchi H, Malekzadeh R. Chronic hepatitis B infection is not associated with increased risk of vascular mortality while having an association with metabolic syndrome. *J Med Virol* 2016; **88**: 1230-1237 [PMID: 26742819 DOI: 10.1002/jmv.24466]

19 **Janicko M**, Senajová G, Drazilová S, Veselíny E, Fedacko J, Siegfried L, Kristian P, Virág L, Pella D, Mareková M, Gecková AM, Kalanin P, Jarcuska P, Halánová M; HepaMeta Team. Association between metabolic syndrome and hepatitis B virus infection in the Roma population in eastern Slovakia: a population-based study. *Cent Eur J Public Health* 2014; **22** Suppl: S37-S42 [PMID: 24847613 DOI: 10.21101/cejph.a3900]

20 **Jarčuška P**, Janičko M, Kružliak P, Novák M, Veselíny E, Fedačko J, Senajová G, Dražilová S, Madarasová-Gecková A, Mareková M, Pella D, Siegfried L, Kristián P, Kolesárová E; HepaMeta Study Group. Hepatitis B virus infection in patients with metabolic syndrome: a complicated relationship. Results of a population based study. *Eur J Intern Med* 2014; **25**: 286-291 [PMID: 24445023 DOI: 10.1016/j.ejim.2014.01.006]

21 **Mena Á**, Pedreira JD, Castro Á, López S, Vázquez P, Poveda E. Metabolic syndrome association with fibrosis development in chronic hepatitis B virus inactive carriers. *J Gastroenterol Hepatol* 2014; **29**: 173-178 [PMID: 24219115 DOI: 10.1111/jgh.12432]

22 **Jinjuvadia R**, Liangpunsakul S. Association between metabolic syndrome and its individual components with viral hepatitis B. *Am J Med Sci* 2014; **347**: 23-27 [PMID: 23514672 DOI: 10.1097/MAJ.0b013e31828b25a5]

23 **Li WC**, Lee YY, Chen IC, Sun C, Chiu FH, Chuang CH. Association between the hepatitis B and C

viruses and metabolic diseases in patients stratified by age. *Liver Int* 2013; **33**: 1194-1202 [PMID: 23782533 DOI: 10.1111/liv.12224]

24 **Kuo YH**, Tsai MC, Kee KM, Chang KC, Wang JH, Lin CY, Lin SC, Lu SN. Associated Factors for Metabolic Syndrome in the Older Adults with Chronic Virus Hepatitis in the Community. *PLoS One* 2016; **11**: e0155544 [PMID: 27177024 DOI: 10.1371/journal.pone.0155544]

25 **Kuo YH**, Kee KM, Wang JH, Hsu NT, Hsiao CC, Chen Y, Lu SN. Association between chronic viral hepatitis and metabolic syndrome in southern Taiwan: a large population-based study. *Aliment Pharmacol Ther* 2018; **48**: 993-1002 [PMID: 30277595 DOI: 10.1111/apt.14960]

26 **Zhou Y**, Cui Y, Deng H, Yu J. Association between hepatitis B virus infection and metabolic syndrome: a retrospective cohort study in Shanghai, China. *BMC Public Health* 2014; **14**: 516 [PMID: 24885963 DOI: 10.1186/1471-2458-14-516]

27 **Expert Panel on Detection**, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486-2497 [PMID: 11368702 DOI: 10.1001/jama.285.19.2486]

28 **Grundy SM**, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F; American Heart Association; National Heart; Lung; and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; **112**: 2735-2752 [PMID: 16157765 DOI: 10.1161/CIRCULATIONAHA.105.169404]

29 **International Diabetes Federation**. The IDF consensus worldwide definition of metabolic syndrome. 2006. [cited 05 June 2020]. Available from: https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome

30 **Alberti KG**, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr; International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart; Lung; and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640-1645 [PMID: 19805654 DOI: 10.1161/CIRCULATIONAHA.109.192644]

31 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553 [PMID: 9686693 DOI: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S]

32 **Chung TH**, Kim MC, Kim CS. Association between Hepatitis B Surface Antigen Seropositivity and Metabolic Syndrome. *Korean J Fam Med* 2014; **35**: 81-89 [PMID: 24724003 DOI: 10.4082/kjfm.2014.35.2.81]

33 **Choi JS**, Han KJ, Lee S, Chun SW, Kim DJ, Kim HC, Kim HM. Serum HBV surface antigen positivity is associated with low prevalence of metabolic syndrome in Korean adult men. *J Epidemiol* 2015; **25**: 74-79 [PMID: 25283312 DOI: 10.2188/jea.JE20140053]

34 **Welzel TM**, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. *Hepatology* 2011; **54**: 463-471 [PMID: 21538440 DOI: 10.1002/hep.24397]

35 **Wong GL**, Chan HL, Yu Z, Chan AW, Choi PC, Chim AM, Chan HY, Tse CH, Wong VW. Coincidental metabolic syndrome increases the risk of liver fibrosis progression in patients with chronic hepatitis B--a prospective cohort study with paired transient elastography examinations. *Aliment Pharmacol Ther* 2014; **39**: 883-893 [PMID: 24612251 DOI: 10.1111/apt.12658]

36 **Yu MW**, Lin CL, Liu CJ, Yang SH, Tseng YL, Wu CF. Influence of Metabolic Risk Factors on Risk of Hepatocellular Carcinoma and Liver-Related Death in Men With Chronic Hepatitis B: A Large Cohort Study. *Gastroenterology* 2017; **153**: 1006-1017. e5 [PMID: 28711626 DOI: 10.1053/j.gastro.2017.07.001]

37 **Huang YW**, Wang TC, Lin SC, Chang HY, Chen DS, Hu JT, Yang SS, Kao JH. Increased risk of cirrhosis and its decompensation in chronic hepatitis B patients with newly diagnosed diabetes: a nationwide cohort study. *Clin Infect Dis* 2013; **57**: 1695-1702 [PMID: 24051864 DOI: 10.1093/cid/cit603]

38 **Hsiang JC**, Gane EJ, Bai WW, Gerred SJ. Type 2 diabetes: a risk factor for liver mortality and complications in hepatitis B cirrhosis patients. *J Gastroenterol Hepatol* 2015; **30**: 591-599 [PMID: 25250942 DOI: 10.1111/jgh.12790]

39 **Shyu YC**, Huang TS, Chien CH, Yeh CT, Lin CL, Chien RN. Diabetes poses a higher risk of hepatocellular carcinoma and mortality in patients with chronic hepatitis B: A population-based cohort study. *J Viral Hepat* 2019; **26**: 718-726 [PMID: 30739359 DOI: 10.1111/jvh.13077]

40 **Chen CL**, Yang WS, Yang HI, Chen CF, You SL, Wang LY, Lu SN, Liu CJ, Kao JH, Chen PJ, Chen DS, Chen CJ. Plasma adipokines and risk of hepatocellular carcinoma in chronic hepatitis B virus-infected carriers: A prospective study in Taiwan. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 1659-1671 [PMID: 24895413 DOI: 10.1158/1055-9965.EPI-14-0161]

41 **Hsu CS**, Liu WL, Chao YC, Lin HH, Tseng TC, Wang CC, Chen DS, Kao JH. Adipocytokines and

liver fibrosis stages in patients with chronic hepatitis B virus infection. *Hepatol Int* 2015; **9**: 231-242 [PMID: 25788201 DOI: 10.1007/s12072-015-9616-2]

42 **Chen JY**, Wang JH, Lin CY, Chen PF, Tseng PL, Chen CH, Chang KC, Tsai LS, Chen SC, Lu SN. Lower prevalence of hypercholesterolemia and hyperglyceridemia found in subjects with seropositivity for both hepatitis B and C strains independently. *J Gastroenterol Hepatol* 2010; **25**: 1763-1768 [PMID: 21039839 DOI: 10.1111/j.1440-1746.2010.06300.x]

43 **Liu PT**, Hwang AC, Chen JD. Combined effects of hepatitis B virus infection and elevated alanine aminotransferase levels on dyslipidemia. *Metabolism* 2013; **62**: 220-225 [PMID: 22938729 DOI: 10.1016/j.metabol.2012.07.022]

44 **Chiang CH**, Yang HI, Jen CL, Lu SN, Wang LY, You SL, Su J, Iloeje UH, Chen CJ; REVEAL-HBV Study Group. Association between obesity, hypertriglyceridemia and low hepatitis B viral load. *Int J Obes (Lond)* 2013; **37**: 410-415 [PMID: 22531094 DOI: 10.1038/ijo.2012.63]

45 **Zheng RD**, Xu CR, Jiang L, Dou AX, Zhou K, Lu LG. Predictors of hepatic steatosis in HBeAg-negative chronic hepatitis B patients and their diagnostic values in hepatic fibrosis. *Int J Med Sci* 2010; **7**: 272-277 [PMID: 20714438 DOI: 10.7150/ijms.7.272]

46 **Rastogi A**, Sakhuja P, Kumar A, Hissar S, Jain A, Gondal R, Sarin SK. Steatosis in chronic hepatitis B: prevalence and correlation with biochemical, histologic, viral, and metabolic parameters. *Indian J Pathol Microbiol* 2011; **54**: 454-459 [PMID: 21934202 DOI: 10.4103/0377-4929.85074]

47 **Cai S**, Ou Z, Liu D, Liu L, Liu Y, Wu X, Yu T, Peng J. Risk factors associated with liver steatosis and fibrosis in chronic hepatitis B patient with component of metabolic syndrome. *United European Gastroenterol J* 2018; **6**: 558-566 [PMID: 29881611 DOI: 10.1177/2050640617751252]

48 **Wong VW**, Wong GL, Chu WC, Chim AM, Ong A, Yeung DK, Yiu KK, Chu SH, Chan HY, Woo J, Chan FK, Chan HL. Hepatitis B virus infection and fatty liver in the general population. *J Hepatol* 2012; **56**: 533-540 [PMID: 22027575 DOI: 10.1016/j.jhep.2011.09.013]

49 **Cheng YL**, Wang YJ, Kao WY, Chen PH, Huo TI, Huang YH, Lan KH, Su CW, Chan WL, Lin HC, Lee FY, Wu JC. Inverse association between hepatitis B virus infection and fatty liver disease: a large-scale study in populations seeking for check-up. *PLoS One* 2013; **8**: e72049 [PMID: 23991037 DOI: 10.1371/journal.pone.0072049]

50 **Hui RWH**, Seto WK, Cheung KS, Mak LY, Liu KSH, Fung J, Wong DK, Lai CL, Yuen MF. Inverse relationship between hepatic steatosis and hepatitis B viremia: Results of a large case-control study. *J Viral Hepat* 2018; **25**: 97-104 [PMID: 28772340 DOI: 10.1111/jvh.12766]

51 **Joo EJ**, Chang Y, Yeom JS, Ryu S. Hepatitis B virus infection and decreased risk of nonalcoholic fatty liver disease: A cohort study. *Hepatology* 2017; **65**: 828-835 [PMID: 28035771 DOI: 10.1002/hep.28917]

52 **Nguyen KA**, Peer N, Mills EJ, Kengne AP. A Meta-Analysis of the Metabolic Syndrome Prevalence in the Global HIV-Infected Population. *PLoS One* 2016; **11**: e0150970 [PMID: 27008536 DOI: 10.1371/journal.pone.0150970]

53 **Adébayo A**, Albert DC, Ericie S, Angelo AC, Jules G, Armand W, Séraphin A, Léopold C, Gabriel A. [Prevalence, associated and predisposing factors of metabolic syndrome among people living with HIV on antiretroviral treatment in Porto Novo in 2014]. *Pan Afr Med J* 2015; **22**: 296 [PMID: 26966492 DOI: 10.11604/pamj.2015.22.296.7923]

54 **Ayodele OE**, Akinboro AO, Akinyemi SO, Adepeju AA, Akinremi OA, Alao CA, Popoola AA. Prevalence and clinical correlates of metabolic syndrome in Nigerians living with human immunodeficiency virus/acquired immunodeficiency syndrome. *Metab Syndr Relat Disord* 2012; **10**: 373-379 [PMID: 22799758 DOI: 10.1089/met.2012.0050]

55 **Berhane T**, Yami A, Alemseged F, Yemane T, Hamza L, Kassim M, Deribe K. Prevalence of lipodystrophy and metabolic syndrome among HIV positive individuals on Highly Active Anti-Retroviral treatment in Jimma, South West Ethiopia. *Pan Afr Med J* 2012; **13**: 43 [PMID: 23330034]

56 **Bosho DD**, Dube L, Mega TA, Adare DA, Tesfaye MG, Eshetie TC. Prevalence and predictors of metabolic syndrome among people living with human immunodeficiency virus (PLWHIV). *Diabetol Metab Syndr* 2018; **10**: 10 [PMID: 29483948 DOI: 10.1186/s13098-018-0312-y]

57 **Dimodi H**, Etame L, Nguimkeng B, Mbappe F, Ndoe N, Tchinda J, Ebene J, Ntentié F, Kingue G, Angie M, Paka G, Kouanfack C, Ngondi J, Enyong J. Prevalence of metabolic syndrome in HIV-infected Cameroonian patients. *World J AIDS* 2014; **4**: 85-92 [DOI: 10.4236/wja.2014.41011]

58 **Guira O**, Tiéno H, Diendéré AE, Sagna Y, Diallo I, Yaméogo B, Zoungrana L, Yaméogo TM, Bognounou R, Drabo JY. Features of metabolic syndrome and its associated factors during Highly Active Antiretroviral Therapy in Ouagadougou (Burkina Faso). *J Int Assoc Provid AIDS Care* 2016; **15**: 159-163 [PMID: 26307211 DOI: 10.1177/2325957415601503]

59 **Hirigo AT**, Tesfaye DY. Influences of gender in metabolic syndrome and its components among people living with HIV virus using antiretroviral treatment in Hawassa, southern Ethiopia. *BMC Res Notes* 2016; **9**: 145 [PMID: 26945987 DOI: 10.1186/s13104-016-1953-2]

60 **Mbunkah HA**, Meriki HD, Kukwah AT, Nfor O, Nkuo-Akenji T. Prevalence of metabolic syndrome in human immunodeficiency virus - infected patients from the South-West region of Cameroon, using the adult treatment panel III criteria. *Diabetol Metab Syndr* 2014; **6**: 92 [PMID: 25197324 DOI: 10.1186/1758-5996-6-92]

61 **Muhammad S**, Sani MU, Okeahialam BN. Cardiovascular disease risk factors among HIV-infected Nigerians receiving highly active antiretroviral therapy. *Niger Med J* 2013; **54**: 185-190 [PMID: 23901181 DOI: 10.4103/0300-1652.114591]

62 **Muyanja D**, Muzoora C, Muyingo A, Muyindike W, Siedner MJ. High prevalence of metabolic

syndrome and cardiovascular disease risk among people with HIV on stable ART in Southwestern Uganda. *AIDS Patient Care STDS* 2016; **30**: 4-10 [PMID: 26683587 DOI: 10.1089/apc.2015.0213]

63 **Ngatchou W**, Lemogoum D, Ndobo P, Yagnigni E, Tiogou E, Nga E, Kouanfack C, van de Borne P, Hermans MP. Increased burden and severity of metabolic syndrome and arterial stiffness in treatment-naïve HIV+ patients from Cameroon. *Vasc Health Risk Manag* 2013; **9**: 509-516 [PMID: 24043942 DOI: 10.2147/VHRM.S42350]

64 **Nguyen KA**, Peer N, de Villiers A, Mukasa B, Matsha TE, Mills EJ, Kengne AP. Metabolic syndrome in people living with Human Immunodeficiency Virus: An assessment of the prevalence and the agreement between diagnostic criteria. *Int J Endocrinol* 2017; **2017**: 1613657 [PMID: 28392801 DOI: 10.1155/2017/1613657]

65 **Obirikorang C**, Quaye L, Osei-Yeboah J, Odame EA, Asare I. Prevalence of metabolic syndrome among HIV-infected patients in Ghana: A cross-sectional study. *Niger Med J* 2016; **57**: 86-90 [PMID: 27226681 DOI: 10.4103/0300-1652.182082]

66 **Sobieszczyk ME**, Werner L, Mlisana K, Naicker N, Feinstein A, Gray CM, Masson L, Passmore JS, Williamson C, Abdool Karim Q, Abdool Karim SS, Garrett NJ. Metabolic syndrome After HIV acquisition in South African women. *J Acquir Immune Defic Syndr* 2016; **73**: 438-445 [PMID: 27391387 DOI: 10.1097/QAI.0000000000001123]

67 **Tesfaye DY**, Kinde S, Medhin G, Megerssa YC, Tadewos A, Tadesse E, Shimelis T. Burden of metabolic syndrome among HIV-infected patients in Southern Ethiopia. *Diabetes Metab Syndr* 2014; **8**: 102-107 [PMID: 24907175 DOI: 10.1016/j.dsx.2014.04.008]

68 **Alvarez C**, Salazar R, Galindez J, Rangel F, Castañeda ML, Lopardo G, Cuhna CA, Roldan Y, Sussman O, Gutierrez G, Cure-Bolt N, Seas C, Carcamo C, Castrillo M. Metabolic syndrome in HIV-infected patients receiving antiretroviral therapy in Latin America. *Braz J Infect Dis* 2010; **14**: 256-263 [PMID: 20835509 DOI: 10.1016/S1413-8670(10)70053-2]

69 **Bonfanti P**, De Socio GL, Marconi P, Franzetti M, Martinelli C, Vichi F, Penco G, Madeddu G, Orofino G, Valsecchi L, Vitiello P, Menzaghi B, Magni C, Ricci E. Is metabolic syndrome associated to HIV infection per se? *Curr HIV Res* 2010; **8**: 165-171 [PMID: 20163341 DOI: 10.2174/157016210790442731]

70 **Calza L**, Masetti G, Piergentili B, Trapani F, Cascavilla A, Manfredi R, Colangeli V, Viale P. Prevalence of diabetes mellitus, hyperinsulinaemia and metabolic syndrome among 755 adult patients with HIV-1 infection. *Int J STD AIDS* 2011; **22**: 43-45 [PMID: 21364066 DOI: 10.1258/ijsa.2010.010256]

71 **Biron A**, Bobin-Dubigeon C, Volteau C, Piroth L, Perré P, Leport C, Prazuck T, Jovelin T, Billard M, Sébille V, Bard JM, Raffi F, Biron C. Metabolic syndrome in French HIV-infected patients: Prevalence and predictive factors after 3 years of antiretroviral therapy. *AIDS Res Hum Retroviruses* 2012; **28**: 1672-1678 [PMID: 22731114 DOI: 10.1089/AID.2012.0048]

72 **Bonfanti P**, De Socio GV, Ricci E, Antinori A, Martinelli C, Vichi F, Penco G, Madeddu G, Orofino G, Valsecchi L, Rusconi S, Menzaghi B, Pocaterra D, Quirino T. The feature of Metabolic Syndrome in HIV naive patients is not the same of those treated: Results from a prospective study. *Biomed Pharmacother* 2012; **66**: 348-353 [PMID: 22705335 DOI: 10.1016/j.biopha.2012.01.005]

73 **Rivero-Juárez A**, Camacho A, Merchante N, Pérez-Camacho I, Macias J, Ortiz-Garcia C, Cifuentes C, Torre-Cisneros J, Peña J, Pineda JA, Rivero A; Grupo para el estudio de las hepatitis vı´ricas (HEPAVIR) de la Sociedad Andaluza de Enfermedades Infecciosas (SAEI). Incidence of liver damage of uncertain origin in HIV patients not co-infected with HCV/HBV. *PLoS One* 2013; **8**: e68953 [PMID: 23874824 DOI: 10.1371/journal.pone.0068953]

74 **Maurice JB**, Patel A, Scott AJ, Patel K, Thursz M, Lemoine M. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. *AIDS* 2017; **31**: 1621-1632 [PMID: 28398960 DOI: 10.1097/QAD.0000000000001504]

75 **van Welzen BJ**, Mudrikova T, El Idrissi A, Hoepelman AIM, Arends JE. A review of Non-Alcoholic Fatty Liver Disease in HIV-infected patients: The next big thing? *Infect Dis Ther* 2019; **8**: 33-50 [PMID: 30607807 DOI: 10.1007/s40121-018-0229-7]

76 **Paruk IM**, Pirie FJ, Motala AA. Non-alcoholic fatty liver disease in Africa: A hidden danger. *Glob Health Epidemiol Genom* 2019; **4**: e3 [PMID: 31019719 DOI: 10.1017/gheg.2019.2]

77 **Hoffmann CJ**, Hoffmann JD, Kensler C, van der Watt M, Omar T, Chaisson RE, Martinson NA, Variava E. Tuberculosis and hepatic steatosis are prevalent liver pathology findings among HIV-infected patients in South Africa. *PLoS One* 2015; **10**: e0117813 [PMID: 25668620 DOI: 10.1371/journal.pone.0117813]

78 **Nishijima T**, Gatanaga H, Shimbo T, Komatsu H, Nozaki Y, Nagata N, Kikuchi Y, Yanase M, Oka S. Traditional but not HIV-related factors are associated with nonalcoholic fatty liver disease in Asian patients with HIV-1 infection. *PLoS One* 2014; **9**: e87596 [PMID: 24498148 DOI: 10.1371/journal.pone.0087596]

79 **Benmassaoud A**, Ghali P, Cox J, Wong P, Szabo J, Deschenes M, Osikowicz M, Lebouche B, Klein MB, Sebastiani G. Screening for nonalcoholic steatohepatitis by using cytokeratin 18 and transient elastography in HIV mono-infection. *PLoS One* 2018; **13**: e0191985 [PMID: 29381754 DOI: 10.1371/journal.pone.0191985]

80 **Lombardi R**, Sambatakou H, Mariolis I, Cokkinos D, Papatheodoridis GV, Tsochatzis EA. Prevalence and predictors of liver steatosis and fibrosis in unselected patients with HIV mono-infection. *Dig Liver Dis* 2016; **48**: 1471-1477 [PMID: 27623186 DOI: 10.1016/j.dld.2016.08.117]

81 **Morse CG**, McLaughlin M, Matthews L, Proschan M, Thomas F, Gharib AM, Abu-Asab M,

Orenstein A, Engle RE, Hu X, Lempicki R, Hadigan C, Kleiner DE, Heller T, Kovacs JA. Nonalcoholic Steatohepatitis and Hepatic Fibrosis in HIV-1-Monoinfected Adults With Elevated Aminotransferase Levels on Antiretroviral Therapy. *Clin Infect Dis* 2015; **60**: 1569-1578 [PMID: 25681381 DOI: 10.1093/cid/civ101]

82 **Husain NE**, Noor SK, Elmadhoun WM, Almobarak AO, Awadalla H, Woodward CL, Mital D, Ahmed MH. Diabetes, metabolic syndrome and dyslipidemia in people living with HIV in Africa: Re-emerging challenges not to be forgotten. *HIV AIDS (Auckl)* 2017; **9**: 193-202 [PMID: 29184449 DOI: 10.2147/HIV.S137974]

83 **Prioreschi A**, Munthali RJ, Soepnel L, Goldstein JA, Micklesfield LK, Aronoff DM, Norris SA. Incidence and prevalence of type 2 diabetes mellitus with HIV infection in Africa: A systematic review and meta-analysis. *BMJ Open* 2017; **7**: e013953 [PMID: 28360243 DOI: 10.1136/bmjopen-2016-013953]

84 **Njuguna B**, Kiplagat J, Bloomfield GS, Pastakia SD, Vedanthan R, Koethe JR. Prevalence, Risk Factors, and Pathophysiology of Dysglycemia among People Living with HIV in Sub-Saharan Africa. *J Diabetes Res* 2018; **2018**: 6916497 [PMID: 30009182 DOI: 10.1155/2018/6916497]

85 **Tshikuka JG**, Magafu MGMD, Rankgoane-Pono G, Mwita JC, Masupe T, Hamda SG, Tapera R, Molefi M, Tshibangu J, Tlhakanelo JT. Overweight and Obesity among Recipients of Antiretroviral Therapy at HIV Clinics in Gaborone, Botswana: Factors Associated with Change in Body Mass Index. *AIDS Res Treat* 2020; **2020**: 8016791 [PMID: 32395339 DOI: 10.1155/2020/8016791]

86 **Lacombe K**, Bottero J, Lemoine M, Boyd A, Girard PM. HIV/hepatitis B virus co-infection: current challenges and new strategies. *J Antimicrob Chemother* 2010; **65**: 10-17 [PMID: 19900950 DOI: 10.1093/jac/dkp414]

87 **Singh KP**, Crane M, Audsley J, Avihingsanon A, Sasadeusz J, Lewin SR. HIV-hepatitis B virus coinfection: epidemiology, pathogenesis, and treatment. *AIDS* 2017; **31**: 2035-2052 [PMID: 28692539 DOI: 10.1097/QAD.0000000000001574]

88 **Lien K**, Mayer W, Herrera R, Rosbe K, Tugizov SM. HIV-1 proteins gp120 and tat induce the epithelial-mesenchymal transition in oral and genital mucosal epithelial cells. *PLoS One* 2019; **14**: e0226343 [PMID: 31869348 DOI: 10.1371/journal.pone.0226343]

89 **Nagu TJ**, Kanyangarara M, Hawkins C, Hertmark E, Chalamila G, Spiegelman D, Mugusi F, Fawzi W. Elevated alanine aminotransferase in antiretroviral-naïve HIV-infected African patients: magnitude and risk factors. *HIV Med* 2012; **13**: 541-548 [PMID: 22416813 DOI: 10.1111/j.1468-1293.2012.01006.x]

90 **Mohr R**, Schierwagen R, Schwarze-Zander C, Boesecke C, Wasmuth JC, Trebicka J, Rockstroh JK. Liver Fibrosis in HIV Patients Receiving a Modern cART: Which Factors Play a Role? *Medicine (Baltimore)* 2015; **94**: e2127 [PMID: 26683921 DOI: 10.1097/MD.0000000000002127]

91 **Rajbhandari R**, Jun T, Khalili H, Chung RT, Ananthakrishnan AN. HBV/HIV coinfection is associated with poorer outcomes in hospitalized patients with HBV or HIV. *J Viral Hepat* 2016; **23**: 820-829 [PMID: 27291562 DOI: 10.1111/jvh.12555]

92 **Seto WK**, Fung J, Cheung KS, Mak LY, Hui RW, Liu KS, Lai CL, Yuen MF. Body-mass index is associated with fibrosis regression during long-term nucleoside analogue therapy in chronic hepatitis B. *Aliment Pharmacol Ther* 2016; **44**: 1071-1079 [PMID: 27659292 DOI: 10.1111/apt.13804]

93 **Shaheen AA**, AlMattooq M, Yazdanfar S, Burak KW, Swain MG, Congly SE, Borman MA, Lee SS, Myers RP, Coffin CS. Tenofovir disoproxil fumarate significantly decreases serum lipoprotein levels compared with entecavir nucleos(t)ide analogue therapy in chronic hepatitis B carriers. *Aliment Pharmacol Ther* 2017; **46**: 599-604 [PMID: 28707319 DOI: 10.1111/apt.14218]

94 **Aberg JA**, Sponseller CA, Ward DJ, Kryzhanovski VA, Campbell SE, Thompson MA. Pitavastatin *versus* pravastatin in adults with HIV-1 infection and dyslipidaemia (INTREPID): 12 week and 52 week results of a phase 4, multicentre, randomised, double-blind, superiority trial. *Lancet HIV* 2017; **4**: e284-e294 [PMID: 28416195 DOI: 10.1016/S2352-3018(17)30075-9]

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MINIREVIEWS

Thymosin alpha 1: A comprehensive review of the literature

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Abstract

Thymosin alpha 1 is a peptide naturally occurring in the thymus that has long been recognized for modifying, enhancing, and restoring immune function. Thymosin alpha 1 has been utilized in the treatment of immunocompromised states and malignancies, as an enhancer of vaccine response, and as a means of curbing morbidity and mortality in sepsis and numerous infections. Studies have postulated that thymosin alpha 1 could help improve the outcome in severely ill corona virus disease 2019 patients by repairing damage caused by overactivation of lymphocytic immunity and how thymosin alpha 1 could prevent the excessive activation of T cells. In this review, we discuss key literature on the background knowledge and current clinical uses of thymosin alpha 1. Considering the known biochemical properties including antibacterial and antiviral properties, time-honored applications, and the new promising findings regarding the use of thymosin, we believe that thymosin alpha 1 deserves further investigation into its antiviral properties and possible repurposing as a treatment against severe acute respiratory syndrome coronavirus-2.

Key Words: Thymosin alpha 1; Thymalfasin; Immunomodulating; T lymphocytes; Infectious diseases; Immune deficiency; Oxidative damage

Core Tip: Thymosin alpha 1 is a naturally occurring peptide in the human thymus, which has long been recognized for its immune-modulating properties. The synthetic

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analog of thymosin alpha 1 has various clinical applications, such as in infectious diseases, malignancies and in immunocompromised states. There is emerging data postulating that this peptide could be of benefit in the treatment of severe acute respiratory syndrome coronavirus-2 infection. We herein discuss the underlying knowledge, current clinical uses and results of recent studies of thymosin alpha 1.

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INTRODUCTION

Thymosin alpha 1 is a 28 amino acid peptide originally isolated from the thymus[1], which has been extensively studied in terms of its functions in the immune system. Thymosin alpha 1 has long been recognized as an immune enhancing, immune modulating, as well as an immune restoring agent[2], and as such it has been utilized in several clinical and research settings. The synthetic form of thymosin alpha 1, thymalfasin, is approved in more than 35 countries for the treatment of hepatitis B and C and as an immune enhancer in several other diseases[1]. More specifically, it has been of benefit as a means of augmenting immune response in immune deficiencies[3], psoriatic arthritis[4], aging[3], as well as in increasing response to vaccines[3] and decreasing chemotherapy-induced toxicity[5]. It has additionally been of value in treating oncologic patients, especially those with hepatocellular carcinoma, renal cell carcinoma and non-small cell lung cancer[5]. Last but not least, it has been used in the fight of numerous infections, such as human immunodeficiency virus (HIV)[6], pseudomonas[1], and mold toxicity[7], as well as sepsis[8], and recently in severely ill coronavirus disease 2019 (COVID-19) patients[9]. In light of the current pandemic situation, efforts are being made worldwide to understand the impact of infection caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) on the immune system in the hopes of getting closer to an effective treatment. To this end, it would be worthwhile to further investigate thymosin alpha 1 through the relevant published literature. In this review, we aim to understand the characteristics of thymosin alpha 1, from its chemical structure and biological properties all the way to its clinical applications, their safety and efficacy which would provide an insight on whether it could be used as a therapeutic option to help curb mortality and improve outcomes in severely ill COVID-19 patients.

BIOCHEMISTRY

Thymosin proteins are short, positively charged, and inherently unregulated peptides. Induction of thymosin protein configuration *via* organic reagents, such as trifluoroethanol, hexafluoroisopropanol, dodecyl trimethylammonium bromide, and Zn^{2+} ions, charges the proteins neutralizing them at a low PH to potentiate their absolute effects[10]. The nuclear magnetic resonance structure of thymosin alpha 1 has been determined by mixing in 40% trifluoroethanol/60% water (v/v) solvent. The study has determined 800 MHz of a polypeptide chain consisting of 28 residues. To comprehend its distinct structure, multiple molecular trials with solvent composed of 40% trifluoroethanol/60% transferable intermolecular potential with 3 points water (v/v) were utilized to create a three-dimensional configuration of the peptide. Ultimately, it was able to depict a distorted helical configuration with two stable regions: Alpha-helix site from 14-26 residues and two double turns in the β region in the N-terminal site consisting of 12 residues[11].

Thymalfasin, the synthetic analog of thymosin alpha 1, induces interleukin (IL)-2 production, differentiation of immature cord blood lymphocytes, production of B cell growth factors, and increased macrophage antigen presentation efficiency. It is used to

treat chemotherapy-induced immunosuppression and to enhance the efficacy of influenza and hepatitis B vaccines in immunocompromised patients[12]. Thymosin alpha 1 therapy modulates and partially normalizes T-lymphocyte numbers and function. T cell rosette percentages have been shown to increase in patients with T cell lymphopenia. Thymalfasin may benefit conditions such as T cell lymphopenia, immunosuppression, and immune dysregulation seen in COVID-19 due to SARS-CoV-2 induced cytokine storm and the immunosuppressive effects of its viral envelope proteins[13]. This may be why Thymalfasin has been used in China for general treatment of COVID-19 patients since April 2020[14].

EXTRACTION AND ANALYSIS

Thymosin alpha 1 is a peptide hormone that is endogenously produced by the thymus gland and potentiates T cell-mediated immune responses *via* differentiation and maturation of T-cell progenitor cells, activation of dendritic and natural killer cells, and stimulation of cytokine-mediated inflammation[15]. Since first isolated from a preparation of bovine thymuses, named the thymosin fraction 5 in 1977, thymosin alpha 1 has been widely recognized for its immune-enhancing properties. Therefore, various efforts have been made towards finding the most efficient method for its production and purification. There are currently three distinct ways bioactive thymosin alpha 1 can be obtained. The first method of extraction is *via* isolation from calf thymuses. It can also be extracted from thymosin fraction 5, which was first isolated from calf thymuses using the technique described in 1975[12]. The second method is through solid-phase synthesis, which is a purely chemical way of peptide synthesis and is nowadays the only method accepted for production of thymosin alpha 1 for clinical use. Lastly, genetic engineering expression makes use of the advances in biotechnology to produce purified recombinant thymosin alpha 1 from either prokaryotic organisms such as *Escherichia coli*, or eukaryotic organisms such as yeast, plants or Pichia Pastoris. Regarding thymosin alpha 1 expression in *Escherichia coli*, numerous expression systems have been developed based on the insertion of the recombinant gene for human thymosin alpha 1 in different vectors, such as pGEX-2T, pThioHis B, pBV222. According to Antachopoulos *et al*[7] (2012), the most promising results came from the BL21/pET-28a system, with thymosin alpha 1 being 70% of total bacterial protein production. The protein can then be analyzed *via* sodium dodecyl sulfate-polyacrylamide gel electrophoresis or by measuring ultraviolet light absorbance at 215 nm. For purification, the primary methods proposed are nickel affinity chromatography, thermal denaturation, and high-performance liquid chromatography. Thymosin alpha 1 expression in yeast is an attractive alternative because post-translational modifications and the development of stable cell lines are made possible[6].

Chen *et al*[16] describe their own yeast-based expression system for thymosin alpha 1, which proved to be effective in producing thymosin alpha 1 capable of increasing CD8+ counts in mice pre-treated with cyclophosphamide. An example of thymosin alpha 1 expression in plants (transgenic tomato Solanum Lycopersicum) is described by Chen *et al*[17]. As promising as it may seem, the genetic engineering method for thymosin alpha 1 production has not yet been introduced into clinical practice, primarily due to difficulties pertaining to extraction and purification. Other than direct extraction from calf thymus, which can only produce trace amounts of the peptide, thymosin alpha 1 used for therapeutic purposes comes from chemical synthesis.

Enzyme-linked immunosorbent assay and radioimmunoassay are the most commonly used methods for quantitative analysis of the peptide. Tuthill *et al*[18] also suggest liquid chromatography with tandem mass spectrometry, which has proven to be accurate, precise, and sensitive for measurement of thymosin alpha 1 in the serum[18].

STORAGE OF THYMOSIN ALPHA 1

Thymosin alpha 1 should be stored at -20 degrees Celsius. Lyophilized thymosin alpha 1 may remain stable for up to three weeks at room temperature; however, for long term storage, it should be kept below -180 degrees Celsius and stored in the desiccated form. When ready to use, it may be reconstituted and subsequently stored at 40 degrees Celsius for a period of two to seven days. If the intention is to store thymosin alpha 1 for a longer period of time, then it is advised to store it in combination with a

carrier protein such as 0.1% human serum albumin or bovine serum albumin. It is recommended to avoid repeated freezing and thawing[19].

BIOLOGICAL ACTIVITIES AND HEALTH BENEFITS

Thymosin alpha 1 functions as a toll-like receptor (TLR)-9 and TLR-2 agonist in both myeloid and dendritic cells, the professional antigen-presenting cells[20]. By targeting TLRs, thymosin alpha 1 can stimulate the adaptive immune response, which is essential for fighting viral, bacterial, and fungal infections and cancers, as well as stimulation of posterior humoral immunity[20-22]. Additionally, thymosin alpha 1 can increase levels of IL-2, IL-10, IL-12, interferon (IFN)-α, and IFN-γ[23]. The role of thymosin alpha 1 in stimulating T-cell dependent antibody production is also the reason why it has been considered as a vaccine adjuvant for enhancing response to vaccines[24].

Thymosin alpha 1 has a wide range of biological activities that range from anti-tumor to immune-modulating properties (Figure 1). The immune response of thymosin alpha 1 is due to its action in elevating the activity of T cell maturation into CD4+/CD8+ T cells. It works to directly activate natural killer cells as well as CD8+ T cells through which it kills virally infected cells. Thymosin alpha 1 has a negative effect on IL-1β and tumor necrosis factor-α, which in turn leads to a decreased inflammatory response and is quite beneficial in conditions such as chronic hepatitis and acute pancreatitis. Not only does it play a role in enhancing cytokine expression, but it also increases the prominence of major histocompatibility complex I/viral antigens on their respective target infected cells and decreases viral replication[6]. Naylor and his associates pointed out that thymosin alpha 1 does not only have one but rather a varied range of targets for its immune-enhancing activity[25].

Thymosin alpha 1 has exhibited the ability to restrain tumor growth, hence its use in the treatment of various cancers. It has anti-proliferative properties which have been exhibited in lung and liver tumor metastases. According to studies conducted by Moody *et al*[25], the anti-tumor activity of thymosin alpha 1 worked best with small tumor size. Overall, thymosin alpha 1 works *via* two main mechanisms: Either stimulating the immune system or employing its anti-proliferative activities on tumor cells. The protective action of thymosin alpha 1 against oxidative damage as a result of its effect on liver superoxide dismutase and glutathione peroxidase has been explored by Armutcu *et al*[26].

Since thymosin alpha 1 is a polypeptide naturally present in the thymus, it plays a fundamental role in the control of inflammation, immunity, and tolerance. Thymosin alpha 1 has an immune-modulating action through its interaction with toll-like receptors. Due to the action of thymosin alpha 1 on other cell types, it is used as a therapeutic agent for diseases with evident immune dysfunction[4]. Clinical trials with thymosin alpha 1 for diseases like DiGeorge syndrome, non-small cell lung cancer, hepatocellular carcinoma, hepatitis B and C, HIV, and melanoma have been conducted and yielded promising results[27,28]. FDA approved the orphan drug thymalfasin (Zadaxin) for treatment of malignant melanoma, chronic active hepatitis B, DiGeorge anomaly with immune defects, and hepatocellular carcinoma due to its immunomodulatory and anti-tumor effect.

CLINICAL AND COMMERCIAL APPLICATIONS

Thymosin alpha 1 has been extensively tested and its synthetic form, thymalfasin, is widely used in the clinical field (Figure 2). Some of its applications are as follows.

Hepatitis B

The safety and efficacy of thymosin alpha 1 in patients with chronic hepatitis B have been tested through clinical trials. Thymosin alpha 1 has been tested as monotherapy as well as in combination with interferon-alpha and other nucleoside analogs. There has been found a complete virological response rate [clearance of serum hepatitis B virus deoxyribonucleic acid and hepatitis B e antigen] of 40.6% in patients given 1.6 mg subcutaneous injection twice a week and 26.5% in patients given the same regimen for 52 wk[29]. However, it is important to note that treatment of Hepatitis B using thymosin alpha 1 was only used in the era of interferon and is now obsolete in the era post-discovery of direct antiviral agents.

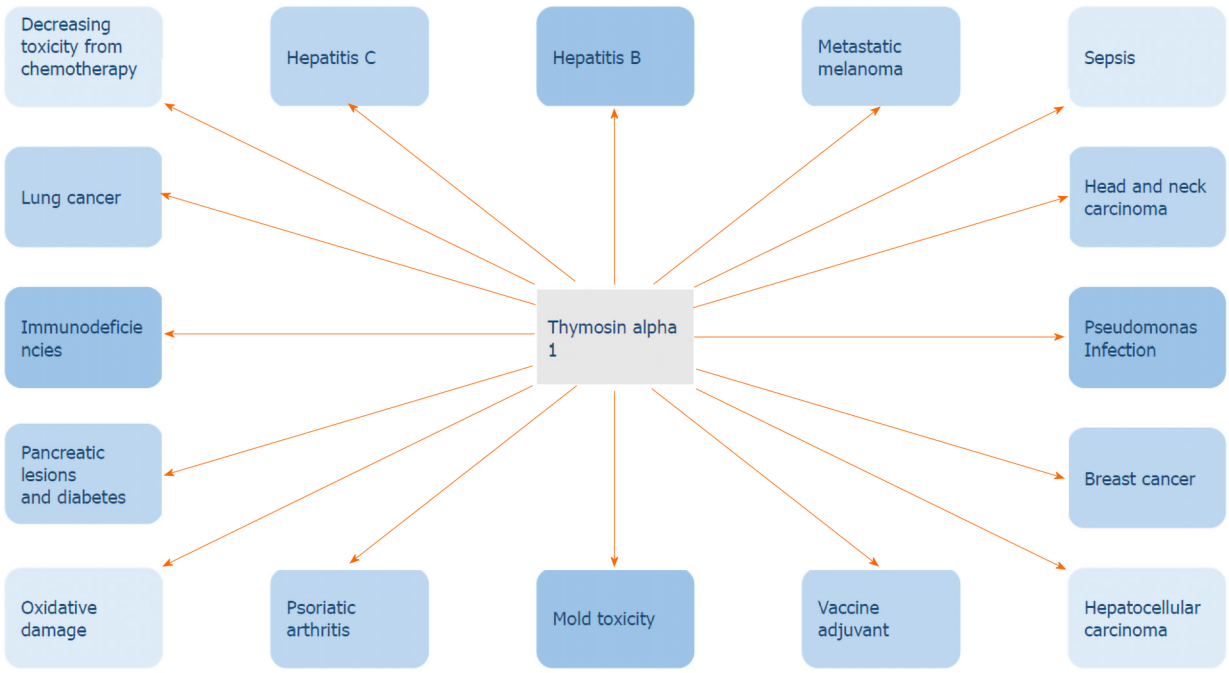

Figure 1 Thymosin alpha 1 has a wide range of biological activities. IL: Interleukin; IFN: Interferon; TLR: Toll-like receptors.

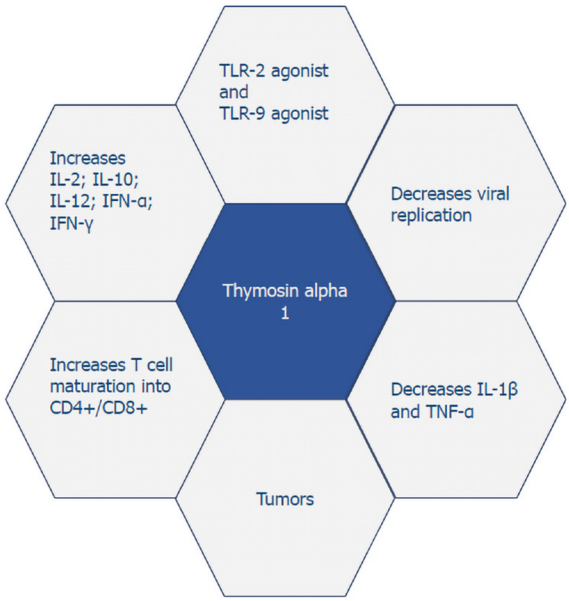

Figure 2 Clinical applications of thymosin alpha 1.

Hepatitis C

Thymosin alpha 1 as a monotherapy does not seem to be useful in treating hepatitis C infection. However, combination therapy of thymosin alpha 1 and pegylated interferon alpha 2a could effectively repress viral replication in hepatitis C patients. Thymosin alpha 1, in combination with interferon-alpha 1 has also been tested for treatment in patients with chronic hepatitis C. Moreover, thymosin alpha 1 is well tolerated, with no significant adverse effects observed. A meta-analysis conducted by Sherman, included many trials showing the superiority of the combination of thymosin alpha 1 and interferon alpha 1 compared to interferon alpha mono-therapy[30]. It remains important to note that similar to Hepatitis B, the treatment of Hepatitis C with thymosin alpha 1 has been discontinued in favor of direct antiviral agents.

Sepsis

The use of thymosin alpha 1 in patients with sepsis has shown a significant decrease in mortality due to multiple-organ failure, which is the primary cause of death in sepsis[8].

HIV infection

Thymosin alpha 1, interferon alpha 1, and zidovudine combination therapy has been well-tolerated in HIV patients. Thymosin alpha 1 enhances the function and increases the number of CD4+ T cells, while it also decreases viral load. Thymosin alpha 1 influences thymic T-cell output. The safety and efficacy of thymosin alpha 1 in combination with highly-active antiretroviral therapy in stimulating immune reconstitution has been proven[6]. It has been shown that thymosin alpha 1 is well tolerated, and could dramatically increase the levels of signal joint T cell receptor excision circles in patients with advanced HIV disease. Prolonged use of high-dose thymosin alpha 1 is more effective[2].

Pseudomonas - bone marrow transplant patients

Thymosin alpha 1 is also used in other infections like pseudomonas or infections following bone marrow transplant[1].

Mold toxicity

This thymic peptide has the ability to prime dendritic cells and to enhance Th1 and Treg cells so that inflammation is balanced, and an antifungal response is generated. Th1 response will activate the production of Th2 cytokines (IFN-γ, IL-2, IL-12, IL-18), stimulating phagocytic activity. Therefore, cytotoxic CD4+, CD8+, and T cells and opsonizing antibodies will be produced, generating a protective effect against fungal pathogens[7].

Immune deficiency

Treatment with thymosin alpha 1 serves as a stimulus for IL-2 receptor expression and IL-2 internalization. It also has a restoring effect on patients with a suppressed lymphokine-activated killer cell activity and with immunodeficiency[29]. Acting through Toll-like receptors in both myeloid and plasmacytoid dendritic cells, thymosin alpha 1 stimulates the signaling pathways and initiates the production of immune-related cytokines. Thus, thymosin alpha 1 is anticipated to bring about encouraging results in the treatment of immunocompromised patients. Overall, it improves immune system function without causing adverse events[13].

Psoriatic arthritis

Thymosin alpha 1 is a potent modulator of immunity and inflammation. Evidence is growing that diseases characterized by deregulation of the immune system and inflammation, such as psoriatic arthritis, are associated with serum levels of thymosin alpha 1 significantly lower than those of healthy individuals. The data is consistent with the role of thymosin alpha 1 as a regulator of immunity, tolerance and inflammation in patients with psoriatic arthritis[4].

Vaccine adjuvant

The use of thymosin alpha 1 as an adjuvant to the influenza vaccine has shown promising results, especially among elderly and immunocompromised patients[31]. Thymosin alpha 1 has also been shown to improve the immunogenicity of the influenza vaccine[3].

Decreasing toxicity from chemotherapy

Clinical studies show that thymosin alpha 1 has been utilized in patients with different malignancies, reducing the toxicity of chemotherapy, and improving the quality of life. An increase in the numbers and functions of immune cells and the decrease of toxicity from chemotherapy was also an effect of utilizing this medication. In general, fewer infections occurred during chemotherapy, neurotoxicity decreased, and the quality of life improved[5].

Oxidative damage, pancreatic lesions and diabetes

Many studies have shown that thymosin alpha 1 has protective effects against oxidative damage. By remarkably amplifying the activity of catalase, superoxide dismutase, and glutathione peroxidase, thymosin alpha 1 reduces the production of reactive oxygen species and prevents oxidative damage to hepatic tissue. Thymosin

alpha 1 has well-established antiproliferative properties seen with various human malignancies and this is a result of its capacity to decrease oxidative stress[6]. It also helps ameliorate pancreatic damage and the resulting diabetes by reducing the production of malondialdehyde and by improving the function of superoxide dismutase and catalase. The antioxidant properties of thymosin alpha 1 are considered to be of great benefit in the treatment of pancreatic lesions[32].

Applications in oncologic patients

Multiple studies have shown promising results for the use of thymosin alpha 1 in patients with metastatic melanoma, head and neck carcinoma, lung cancer, breast cancer, and hepatocellular carcinoma[33]. Thymosin alpha 1 is indicated as adjuvant for chemotherapy-induced immune depression, immune insufficiency, and immune suppression in patients[5]. In addition, it has been shown that thymosin alpha 1 in combination with chemotherapy or radiation improves survival rate in patients with non-small cell lung cancer, which accounts for 85% of all lung cancers and is known for its low responsiveness to chemotherapy[5].

SAFETY AND DOSES

Thymosin alpha 1 is usually found in an injection form and is commonly prescribed by a primary care physician. Thymosin alpha 1 is usually administered twice a week *via* a subcutaneous route. The standard single dosage ranges from 0.8 to 6.4 mg, while multiple doses range from 1.6 to 16 mg for five to seven days. Utilized in various illnesses such as liver disease, cancer, and autoimmune diseases, thymosin alpha 1 has been shown to be well-tolerated and safe[34].

ADVERSE EFFECTS AND CONTRAINDICATIONS

Thymalfasin, the synthetic form of thymosin alpha 1, is usually well tolerated. The most common adverse effects include local irritation, redness, or discomfort at the site of injection. In clinical trials, the combination of thymalfasin with interferon 2b was reported to have rare side effects such as fever, fatigue, muscle aches, nausea, vomiting, and neutropenia when compared to interferon-alpha 2b alone or with placebo[34]. Thymalfasin is contraindicated in patients with hypersensitivity to thymosin alpha 1 or any of the components of the injection. Due to the immunomodulatory action of thymalfasin, it is also contraindicated in immunosuppressed patients, such as organ transplant recipients, unless the benefits of the treatment exceed the risks[35].

EVIDENCE FROM PREVIOUS HUMAN CLINICAL STUDIES

Thymosin alpha 1 has been utilized in various cases to enhance cell-mediated immunity and for the treatment of a multitude of different diseases (Table 1). A study was performed to demonstrate the effect of thymosin alpha 1 in the human breast cancer lines ZR-75-1, MCF-7, MDA-MB-231, MCF-10A and BT-549. For this experiment, thymosin alpha 1 was dissolved in sterile water and stored in 2 mL plastic tubes at -20 °C. Results showed that thymosin alpha 1 inhibited cell proliferation and induced apoptosis in human leukemia, non-small cell lung cancer, melanoma, and other cancers. Apoptosis was significantly induced in human breast cancer and leukemia cell lines with a thymosin alpha 1 concentration of 100 to 160 IM. Additionally, data exhibited that ZR-75-1 and MCF-7 cells display different sensitivities to thymosin alpha 1. In general, the study revealed that thymosin alpha 1 could be a possible approach to breast cancer treatment[36]. Other studies demonstrate that thymosin alpha 1 could be a promising therapy for severe sepsis. Various small-scale studies as well as a large-scale, multicenter, single-blinded, and randomized control trial were conducted in six tertiary teaching hospitals in China, with the purpose of demonstrating the vital role thymosin alpha 1 plays in sepsis therapy. Patients admitted to the intensive care unit with severe sepsis were distributed randomly among the control group and the thymosin alpha 1 group. Hypodermic injections of 1.6 mg of thymosin alpha-1 or normal saline were distributed to all individuals two times a day for five days; afterwards, the dose was reduced to once

Table 1 Summarizing pre-clinical and clinical studies

Pre-clinical studies		
Ref.	**Year**	**Application of thymosin alpha 1**
Guo *et al*[36]	2015	The anti-tumor effect of thymosin alpha 1 was studied on human cancer cell lines. The study concluded that thymosin alpha 1 can decrease proliferation and induce apoptosis in human leukemia, non-small cell lung cancer, melanoma, and other cancers. The study concluded that thymosin alpha 1 could be an approach to breast cancer treatment
Clinical studies		
Sherman *et al*[29]	2010	Thymosin alpha 1 was tested as monotherapy and in combination with interferon-alpha for the treatment of chronic hepatitis B. It was also shown to stimulate IL-2 receptor expression and IL-2 internalization and to enhance immune response in patients with immunodeficiency
Eckert *et al*[30]	1994	Combination therapy of thymosin alpha 1 and pegylated interferon alpha 2a preferred over interferon monotherapy for the treatment of chronic hepatitis C
Li *et al*[8]	2015	Significant decrease in mortality due to multiple organ failure in patients with sepsis
Li *et al*[6]	2010	Thymosin alpha 1 can be safely used as an adjuvant to antiretroviral therapy in HIV patients. It helps increase CD4+ count, stimulates the function of CD4+ cells, and helps decrease viral load. By amplifying the activity of catalase, superoxide dismutase, and glutathione peroxidase, it decreases oxidative damage to tissues. Thymosin alpha 1 reduces tumor cell proliferation in human malignancies by decreasing oxidative stress
Matteucci *et al*[2]	2017	Thymosin alpha 1 significantly increases levels of sjTREC in patients with advanced HIV disease
Camerini *et al*[1]	2015	Thymosin alpha 1 can be used in pseudomonas infections or infections following bone marrow transplant
Antachopoulos *et al*[7]	2012	Thymosin alpha 1 might be effective against mold toxicity
King *et al*[13]	2016	Thymosin alpha 1 increases cytokine production and is expected to be beneficial in immunocompromised patients
Pica *et al*[4]	2018	It has been postulated that thymosin alpha 1 can help regulate immunity and reduce inflammation in patients with psoriatic arthritis
Panatto *et al*[31]	2011	Thymosin alpha 1 has shown promising results as an adjuvant to the influenza vaccine
Carraro *et al*[3]	2012	Thymosin alpha 1 improves immunogenicity of the influenza vaccine
Qin *et al*[32]	2009	Thymosin alpha 1 can reduce oxidative damage to the pancreas and mitigate the risk of resulting diabetes
Costantini *et al*[33]	2019	Thymosin alpha 1 has shown promising results in patients with malignancies, such as metastatic melanoma, head and neck carcinoma, lung cancer, breast cancer, and hepatocellular carcinoma
Romani *et al*[21]	2007	A single-blind randomized control trial was conducted in six tertiary hospitals in China to study the beneficial effects of thymosin alpha 1 on patients with sepsis. The results showed 9% lower mortality in the treatment group compared to the control group
Sugahara *et al*[37]	2002	Patients with chronic hepatitis B who were treated with thymosin alpha 1 showed an overall improvement in serum ALT levels. ALT levels were reduced to normal in 42.9%. A total disappearance of serum HBV DNA was noted in 28.6% of patients

IL: Interleukin; sjTREC: Signal joint T cell receptor excision circles; HIV: Human immunodeficiency virus; ALT: Glutamic-pyruvic transaminase; HBV: Hepatitis B virus; DNA: Deoxyribonucleic acid.

per day. Results showed that the thymosin alpha 1 group was 9.0% lower in mortality rate than the control group[21].

As discussed above, one of the strongest properties of thymosin alpha 1 is its role in the activation of T cell responses in the body. A study in seven patients with chronic hepatitis caused by hepatitis B virus tried to identify the immunomodulatory properties of thymosin alpha 1. Each individual was treated for a total of 24 wk with a hypodermic injection at a dose of 1.29/0.4 mg/body/day six times weekly for the first 2 wk and then twice weekly for an additional 22 wk. Subsequently, liver biopsies were performed to gather data. The serum alanine transaminase levels improved to 47.39/17.0 IU/L and normalized in 42.9% of patients after 48 wk of treatment. However, complete disappearance of serum hepatitis B virus deoxyribonucleic acid was seen in 28.6%. Thymosin alpha 1 also affected maturation of T-cells, demonstrating its high immunomodulatory properties. Overall, it has been reported that combination therapy with thymosin alpha 1 and IFN-α has demonstrable biological activity in patients with viral hepatitis[37].

THYMOSIN ALPHA 1/THYMALFASIN *VS* THYMOSIN BETA 4/TIMBETASIN

Thymosin alpha 1 and thymosin beta 4 are two hormone peptides that are secreted from the thymus and have vastly different chemical compositions and immunological actions. These proteins are separated from thymosin fraction 5 and have the potential to change a variety of immune functions in mammals. Thymosin alpha 1 is thought to be responsible for rebuilding the immune system by enhancing cell-mediated immunity in animals without a thymus gland. Thymosin beta 4 is in the family of actin monomer-sequestering proteins which essentially regulate unpolymerized actin and have an active role in maintaining the free G-actin monomers in the cytoplasm. Thymosin alpha 1 is clinically relevant in various types of cancer, specifically hepatocellular carcinoma, lung cancer and melanomas. Thymosin beta 4, has a strong response to virally infected cells. It is currently being tested as a possible therapy against influenza, HIV, and acquired immune deficiency syndrome[38-41].

COULD THYMOSIN ALPHA 1 IMPROVE THE OUTCOMES IN COVID-19 PATIENTS?

The COVID-19 pandemic has had a worldwide impact and multiple studies have shown the immunological effects of this disease. All countries affected by SARS-CoV-2 are focused on searching for an effective treatment. Thymosin alpha 1 has a very prominent role in both immunity control and inflammation (Table 2). So far, it has been used in various pathologic conditions: Infections, sepsis, immune deficiencies and malignancies, just to name a few. It has also been found to curb mortality in several of them, such as sepsis and HIV infection. Although clinical studies on the efficiency of thymosin alpha 1 in treating COVID-19 are still limited, it would be of great value to further explore the potential benefits that this drug can bring about in mitigating the devastating effects of the current pandemic.

A recent study in COVID-19 patients demonstrated how thymosin alpha 1 significantly promoted the proliferation of activated T cells and this led to a critical prevention of lymphopenia in infected patients. In total, there were 25 severely and critically ill patients who participated in the study. Eleven of them received daily treatment of thymosin alpha 1 for one week, while the rest of the patients remained untreated. Data illustrates that patients in the thymosin alpha 1 treatment group had a higher number of lymphocytes than patients without treatment[42]. In another retrospective study conducted in China, patients in the treatment group received subcutaneous injections of 10 mg thymosin alpha 1 once per day for at least seven consecutive days. Thymosin alpha 1 supplementation showed improvement and restoration of T cell counts in COVID-19 patients with severe lymphocytopenia and, in the end, thymosin alpha 1 supplementation reduced mortality in patients severely ill with COVID-19[30].

In COVID-19 treatment, it has been postulated to administer thymosin alpha 1 as an intramuscular injection for 7 d for patients who have CD8 cells less than 400/μL and CD4 cells less than 650/μL. This is postulated on the understanding that thymosin alpha 1 induction showed improvement in T cell number in elderly patients with comorbidities like hypertension and cardiovascular diseases. Healthy people who are older than 60 years of age should receive thymosin alpha 1 as a supplement to prevent COVID-19 infection[43]. It has also been suggested that thymosin alpha 1 taken before administration of methylprednisolone in COVID-19 patients may prevent steroid-induced death of thymocytes[44]. The National Health Commission of China included thymosin alpha 1 as an alternative treatment option for patients with lymphocytopenia or immunodeficiency.

Currently, there are various ongoing clinical trials registered on clinicaltrials.gov of thymosin in COVID-19 patients (Table 2).

CONCLUSION

Thymosin alpha 1 is a thymus peptide with recognized immune modulating capacity and biochemical properties. The synthetic analogue of thymosin alpha 1, thymalfasin, induces IL-2 and B cell growth factor production, differentiation of immature cord blood lymphocytes, raises efficiency of macrophage antigen presentation, and the modulation and partial normalization of function and number of T-lymphocytes. The

Table 2 Summary of ongoing clinical trials of thymosin and coronavirus disease 2019

Clinical trial number	Location	Status	Condition	Intervention	Results
NCT04428008	United States	Not yet recruiting	COVID-19	Drug: Thymalfasin	Not yet available
NCT04487444	United States	Recruiting	COVID-19	Drug: Thymalfasin	Not yet available
NCT04268537	China	Not yet recruiting	COVID-19	Drug: PD-1 blocking antibody + standard treatment; Drug: Thymosin + Standard treatment; Other: Standard treatment	Not yet available
NCT04320238	China	Recruiting	COVID-19	Drug: Recombinant human interferon Alpha-1b; Drug: Thymosin alpha 1	Not yet available

Data retrieved from clinicaltrials.gov on October 5th, 2020. COVID-19: Coronavirus disease 2019.

effects of immune stimulation occur through TLR action in both the myeloid and plasmacytoid dendritic cells with production of cytokines. The immunosuppressive effects of the SARS-CoV-2 viral envelope in inducing cytokine storm may be modulated with thymosin alpha 1 therapy. This would be especially beneficial in preventing catastrophic events such as cytokine storm in more severe cases. Thymosin alpha 1 and its synthetic analogue thymalfasin have well-studied safety profiles and are well-tolerated with only minor side effects. Clinical studies have demonstrated the significant role of thymosin alpha 1 in immune and inflammatory responses, and extensive research has shown its effective use in a myriad of diseases ranging from hepatitis and HIV to immune deficiencies and cancers, as well as its use as a vaccine adjuvant.

Within the context of COVID-19 infection, it has been shown to reduce mortality in those with severe disease and aid in restoring some immune function through increasing thymic activity. Further study would be highly beneficial in determining if thymosin alpha 1 could serve as a therapeutic agent or in combination with other treatments to mitigate the progression and severity of the disease. For this purpose, we can conclude that further studies are mandated for using thymosin alpha 1 in these patients.

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REFERENCES

1 **Camerini R**, Garaci E. Historical review of thymosin α 1 in infectious diseases. *Expert Opin Biol Ther* 2015; **15** Suppl 1: S117-S127 [PMID: 26098768 DOI: 10.1517/14712598.2015.1033393]
2 **Matteucci C**, Grelli S, Balestrieri E, Minutolo A, Argaw-Denboba A, Macchi B, Sinibaldi-Vallebona P, Perno CF, Mastino A, Garaci E. Thymosin alpha 1 and HIV-1: recent advances and future perspectives. *Future Microbiol* 2017; **12**: 141-155 [PMID: 28106477 DOI: 10.2217/fmb-2016-0125]
3 **Carraro G**, Naso A, Montomoli E, Gasparini R, Camerini R, Panatto D, Tineo MC, De Giorgi L, Piccirella S, Khadang B, Ceracchi M, De Rosa A. Thymosin-alpha 1 (Zadaxin) enhances the immunogenicity of an adjuvated pandemic H1N1v influenza vaccine (Focetria) in hemodialyzed patients: a pilot study. *Vaccine* 2012; **30**: 1170-1180 [PMID: 22178096 DOI: 10.1016/j.vaccine.2011.12.014]
4 **Pica F**, Gaziano R, Casalinuovo IA, Moroni G, Buè C, Limongi D, D'Agostini C, Tomino C, Perricone R, Palamara AT, Sinibaldi Vallebona P, Garaci E. Serum thymosin alpha 1 Levels in normal and pathological conditions. *Expert Opin Biol Ther* 2018; **18**: 13-21 [PMID: 30063864 DOI: 10.1080/14712598.2018.1474197]
5 Zadaxin (Thymalfasin): Uses, Dosage, Side Effects, Interactions, Warning [Internet]. RxList. 2009. [cited 2020 September 27]. Available from: https://www.rxlist.com/zadaxin-drug.htm#description
6 **Li J**, Liu CH, Wang FS. Thymosin alpha 1: biological activities, applications and genetic engineering production. *Peptides* 2010; **31**: 2151-2158 [PMID: 20699109 DOI: 10.1016/j.peptides.2010.07.026]
7 **Antachopoulos C**, Katragkou A, Roilides E. Immunotherapy against invasive mold infections. *Immunotherapy* 2012; **4**: 107-120 [PMID: 22150004 DOI: 10.2217/imt.11.159]

8 **Li C**, Bo L, Liu Q, Jin F. Thymosin alpha1 based immunomodulatory therapy for sepsis: a systematic review and meta-analysis. *Int J Infect Dis* 2015; **33**: 90-96 [PMID: 25532482 DOI: 10.1016/j.ijid.2014.12.032]

9 **Romani L**, Tomino C, Puccetti P, Garaci E. Off-label therapy targeting pathogenic inflammation in COVID-19. *Cell Death Discov* 2020; **6**: 49 [PMID: 32547788 DOI: 10.1038/s41420-020-0283-2]

10 **Hoch K**, Volk DE. Structures of Thymosin Proteins. Vitam Horm. Epub 2016; 102: 1-24 [DOI: 10.1016/bs.vh.2016.04.009]

11 **Elizondo-Riojas MA**, Chamow SM, Tuthill CW, Gorenstein DG, Volk DE. NMR structure of human thymosin alpha-1. *Biochem Biophys Res Commun* 2011; **416**: 356-361 [PMID: 22115779 DOI: 10.1016/j.bbrc.2011.11.041]

12 Thymalfasin [Internet]. Pubchem.ncbi.nlm.nih.gov. [cited 2020 September 27]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Thymalfasin

13 **King R**, Tuthill C. Immune Modulation with Thymosin Alpha 1 Treatment. *Vitam Horm* 2016; **102**: 151-178 [PMID: 27450734 DOI: 10.1016/bs.vh.2016.04.003]

14 **Liu XH**, Lu SH, Chen J, Xia L, Yang ZG, Charles S, Yang Y, Lin Y, Lu HZ. Clinical characteristics of foreign-imported COVID-19 cases in Shanghai, China. *Emerg Microbes Infect* 2020; **9**: 1230-1232 [PMID: 32515651 DOI: 10.1080/22221751.2020.1766383]

15 **Poo JL**, Sánchez-Avila F, Kershenobich D, García-Samper X, Gongora J, Uribe M. Triple combination of thymalfasin, peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior interferon and ribavirin treatment: 24-week interim results of a pilot study. *J Gastroenterol Hepatol* 2004; **19** Suppl 6: S79-S81 [PMID: 15546256 DOI: 10.1111/j.1440-1746.2004.03634.x]

16 **Chen F**, Chen XM, Chen Z, Jiang HL, Pan XP, Hu ZR, Liu RH, Chen XM. Construction and application of a yeast expression system for thymosin alpha1. *Biocell* 2005; **29**: 253-259 [PMID: 16524246]

17 **Chen Y**, Wang A, Zhao L, Shen G, Cui L, Tang K. Expression of thymosin alpha1 concatemer in transgenic tomato (Solanum lycopersicum) fruits. *Biotechnol Appl Biochem* 2009; **52**: 303-312 [PMID: 18752465 DOI: 10.1042/BA20080054]

18 **Tuthill CW**, Rudolph A, Li Y, Tan B, Fitzgerald TJ, Beck SR, Li YX. Quantitative analysis of thymosin alpha1 in human serum by LC-MS/MS. *AAPS PharmSciTech* 2000; **1**: E11 [PMID: 14727844 DOI: 10.1208/pt010211]

19 Thymosin alpha 1 steroid [Internet]. Mybiosource.com. [cited 2020 September 27]. Available from: https://www.mybiosource.com/steroid/thymosin-alpha-1/639043

20 **Romani L**, Bistoni F, Gaziano R, Bozza S, Montagnoli C, Perruccio K, Pitzurra L, Bellocchio S, Velardi A, Rasi G, Di Francesco P, Garaci E. Thymosin alpha 1 activates dendritic cells for antifungal Th1 resistance through toll-like receptor signaling. *Blood* 2004; **103**: 4232-4239 [PMID: 14982877 DOI: 10.1182/blood-2003-11-4036]

21 **Romani L**, Bistoni F, Montagnoli C, Gaziano R, Bozza S, Bonifazi P, Zelante T, Moretti S, Rasi G, Garaci E, Puccetti P. Thymosin alpha1: an endogenous regulator of inflammation, immunity, and tolerance. *Ann N Y Acad Sci* 2007; **1112**: 326-338 [PMID: 17495242 DOI: 10.1196/annals.1415.002]

22 **Wu J**, Zhou L, Liu J, Ma G, Kou Q, He Z, Chen J, Ou-Yang B, Chen M, Li Y, Wu X, Gu B, Chen L, Zou Z, Qiang X, Chen Y, Lin A, Zhang G, Guan X. The efficacy of thymosin alpha 1 for severe sepsis (ETASS): a multicenter, single-blind, randomized and controlled trial. *Crit Care* 2013; **17**: R8 [PMID: 23327199 DOI: 10.1186/cc11932]

23 **Tuthill C**, Rios I, De Rosa A, Camerini R. Thymosin α1 continues to show promise as an enhancer for vaccine response. *Ann N Y Acad Sci* 2012; **1270**: 21-27 [PMID: 23050813 DOI: 10.1111/j.1749-6632.2012.06680.x]

24 **Naylor PH**, Quadrini K, Garaci E, Rasi G, Hadden JW. Immunopharmacology of thymosin alpha1 and cytokine synergy. *Ann N Y Acad Sci* 2007; **1112**: 235-244 [PMID: 17567942 DOI: 10.1196/annals.1415.036]

25 **Moody TW**. Thymosin alpha1 as a chemopreventive agent in lung and breast cancer. *Ann N Y Acad Sci* 2007; **1112**: 297-304 [PMID: 17567944 DOI: 10.1196/annals.1415.040]

26 **Armutcu F**, Coskun O, Gürel A, Kanter M, Can M, Ucar F, Unalacak M. Thymosin alpha 1 attenuates lipid peroxidation and improves fructose-induced steatohepatitis in rats. *Clin Biochem* 2005; **38**: 540-547 [PMID: 15885234 DOI: 10.1016/j.clinbiochem.2005.01.013]

27 **Tuthill C**, Rios I, McBeath R. Thymosin alpha 1: past clinical experience and future promise. *Ann N Y Acad Sci* 2010; **1194**: 130-135 [PMID: 20536460 DOI: 10.1111/j.1749-6632.2010.05482.x]

28 **Chien RN**, Liaw YF, Chen TC, Yeh CT, Sheen IS. Efficacy of thymosin alpha1 in patients with chronic hepatitis B: a randomized, controlled trial. *Hepatology* 1998; **27**: 1383-1387 [PMID: 9581695 DOI: 10.1002/hep.510270527]

29 **Sherman KE**. Thymosin alpha 1 for treatment of hepatitis C virus: promise and proof. *Ann N Y Acad Sci* 2010; **1194**: 136-140 [PMID: 20536461 DOI: 10.1111/j.1749-6632.2010.05460.x]

30 **Eckert K**, Schmitt M, Garbin F, Wahn U, Maurer HR. Thymosin alpha 1 effects, in vitro, on lymphokine-activated killer cells from patients with primary immunodeficiencies: preliminary results. *Int J Immunopharmacol* 1994; **16**: 1019-1025 [PMID: 7705963 DOI: 10.1016/0192-0561(94)90081-7]

31 **Panatto D**, Amicizia D, Lai PL, Camerini R, De Rosa A, Gasparini R. Utility of thymosin alpha-1 (Zadaxin) as a co-adjuvant in influenza vaccines: a review. *J Prev Med Hyg* 2011; **52**: 111-115 [PMID: 22010537]

32 **Qin Y**, Chen FD, Zhou L, Gong XG, Han QF. Proliferative and anti-proliferative effects of thymosin alpha1 on cells are associated with manipulation of cellular ROS levels. *Chem Biol Interact* 2009; **180**: 383-388 [PMID: 19442654 DOI: 10.1016/j.cbi.2009.05.006]

33 **Costantini C**, Bellet MM, Pariano M, Renga G, Stincardini C, Goldstein AL, Garaci E, Romani L. A Reappraisal of Thymosin Alpha1 in Cancer Therapy. *Front Oncol* 2019; **9**: 873 [PMID: 31555601 DOI: 10.3389/fonc.2019.00873]

34 **Ancell CD**, Phipps J, Young L. Thymosin alpha-1. *Am J Health Syst Pharm* 2001; **58**: 879-85; quiz 886 [PMID: 11381492 DOI: 10.1093/ajhp/58.10.886]

35 **Rekdal M**, Pai A, Bs M. Experimental data of co-crystals of Etravirine and L-tartaric acid. *Data Brief* 2018; **16**: 135-140 [PMID: 29201980 DOI: 10.1016/j.dib.2017.11.019]

36 **Guo Y**, Chang H, Li J, Xu XY, Shen L, Yu ZB, Liu WC. Thymosin alpha 1 suppresses proliferation and induces apoptosis in breast cancer cells through PTEN-mediated inhibition of PI3K/Akt/mTOR signaling pathway. *Apoptosis* 2015; **20**: 1109-1121 [PMID: 26002438 DOI: 10.1007/s10495-015-1138-9]

37 **Sugahara S**, Ichida T, Yamagiwa S, Ishikawa T, Uehara K, Yoshida Y, Yang XH, Nomoto M, Watanabe H, Abo T, Asakura H. Thymosin-alpha1 increases intrahepatic NKT cells and CTLs in patients with chronic hepatitis B. *Hepatol Res* 2002; **24**: 346-354 [PMID: 12479932 DOI: 10.1016/s1386-6346(02)00145-6]

38 **Renault L**. Intrinsic, Functional, and Structural Properties of β-Thymosins and β-Thymosin/WH2 Domains in the Regulation and Coordination of Actin Self-Assembly Dynamics and Cytoskeleton Remodeling. *Vitam Horm* 2016; **102**: 25-54 [PMID: 27450729 DOI: 10.1016/bs.vh.2016.04.006]

39 **Kim J**, Jung Y. Thymosin Beta 4 Is a Potential Regulator of Hepatic Stellate Cells. *Vitam Horm* 2016; **102**: 121-149 [PMID: 27450733 DOI: 10.1016/bs.vh.2016.04.011]

40 **Xue B**, Robinson RC. Actin-Induced Structure in the Beta-Thymosin Family of Intrinsically Disordered Proteins. *Vitam Horm* 2016; **102**: 55-71 [PMID: 27450730 DOI: 10.1016/bs.vh.2016.04.007]

41 **Hsia J**, Sztein MB, Naylor PH, Simon GL, Goldstein AL, Hayden FG. Modulation of thymosin alpha 1 and thymosin beta 4 Levels and peripheral blood mononuclear cell subsets during experimental rhinovirus colds. *Lymphokine Res* 1989; **8**: 383-391 [PMID: 2572736]

42 **Yu K**, He J, Wu Y, Xie B, Liu X, Wei B, Zhou H, Lin B, Zuo Z, Wen W, Xu W, Zou B, Wei L, Huang X, Zhou P. Dysregulated adaptive immune response contributes to severe COVID-19. *Cell Res* 2020; **30**: 814-816 [PMID: 32759967 DOI: 10.1038/s41422-020-0391-9]

43 **Bergstrom DJ**, Kotb R, Louzada ML, Sutherland HJ, Tavoularis S, Venner CP; Myeloma Canada Research Network Consensus Guideline Consortium. Consensus Guidelines on the Diagnosis of Multiple Myeloma and Related Disorders: Recommendations of the Myeloma Canada Research Network Consensus Guideline Consortium. *Clin Lymphoma Myeloma Leuk* 2020; **20**: e352-e367 [PMID: 32249195 DOI: 10.1016/j.clml.2020.01.017]

44 **Zhang L**, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. *J Med Virol* 2020; **92**: 479-490 [PMID: 32052466 DOI: 10.1002/jmv.25707]

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SYSTEMATIC REVIEWS

Reinfection risk of novel coronavirus (COVID-19): A systematic review of current evidence

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Abstract

BACKGROUND

There is recently a concern regarding the reinfection and reactivation of previously reCoVered coronavirus disease 2019 (CoVID-19) patients.

AIM

To summarize the recent findings and reports of CoVID-19 reinfection in patients previously reCoVered from the disease.

METHODS

This study was a systematic review of current evidence conducted in August 2020. The authors studied the probable reinfection risk of novel coronavirus (CoVID-19). We performed a systematic search using the keywords in online databases. The investigation adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist to ensure the reliability and validity of this study and results.

RESULTS

We reviewed 31 studies. Eight studies described reCoVered patients with reinfection. Only one study reported reinfected patients who died. In 26 studies, there was no information about the status of the patients. Several studies indicated that reinfection is not probable and that post-infection immunity is at least temporary and short.

CONCLUSION

Based on our review, we concluded that a positive polymerase chain reaction retest could be due to several reasons and should not always be considered as reinfection or reactivation of the disease. Most relevant studies in positive retest patients have shown relative and probably temporary immunity after the reCoVery of the disease.

Key Words: Reactivation; Reinfection; Postinfection; Coronavirus; CoVID-19; SARS-CoV-2

Core Tip: The reinfection in patients reCoVered from coronavirus disease 2019 (CoVID-19) could create a serious challenge in tackling the CoVID-19 pandemic as the reCoVered patients could be a source of virus spread in society. Previous studies have found a positive viral ribonucleic acid test in some of the discharged CoVID-19 patients 10 to 27 d after reCoVery. Recurrence of CoVID-19 after reCoVery should be differentiated from secondary medical conditions such as super infection, pulmonary embolism, or persistent ribonucleic acid virus that can be disCoVered in respiratory specimens in clinically cured CoVID-19 patients. This review aims to assist a systematic compilation of severe acute respiratory syndrome coronavirus 2 reactivation in reCoVered CoVID-19 patients.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new strain of coronavirus, causes coronavirus disease 2019 (CoVID-19), which was first reported in

China in late 2019 and then spread rapidly worldwide[1-5]. The symptoms of CoVID-19 are high temperature, dry cough, shortness of breath, headache, tiredness, loss of taste or smell, and gastrointestinal symptoms such as diarrhea, anorexia, nausea, and abdominal pain[6-8]. Increased liver enzyme and low counts of lymphocytes (lymphocytopenia) along with increased C-reactive protein (CRP) levels are often present in CoVD-19 patients[9]. It could eventually lead to acute respiratory distress syndrome (ARDS) and death[1,10,11]. Although there is currently no certainty in virus biological behavior and risk of recurrence in the human body, recent studies reported evidence of the virus reactivation following an asymptomatic CoVID-19 infection in a small group of patients[1,12,13].

The risk factors of SARS-CoV-2 reactivation are related to the type of immunosuppressive therapies, factors in the host such as older age, gender, underlying diseases such as diabetes, heart disease, obesity, cancer, and virologic factors[1,14]. Some viruses such as varicella-zoster can remain dormant in host cells for some time, not causing any illness and then reactivate and cause the disease. Recent evidence indicates that SARS-CoV-2 could present similar behavior and reactivate in patients with previously confirmed CoVID-19 infection and cause illness and person-to-person transmission[15].

Recent studies reported that some reCoVered CoVID-19 patients tested positive for virus nucleic acid again[16,17]. Elderly people with comorbidities are more likely to present with CoVID-19 reinfection[18]. Studies suggested that there are three major mechanisms for the reinfection of CoVID-19, including short-lived, ineffective, and strain-specific immune response[19,20].

The gold standard test for diagnosing SARS-CoV-2 infection is nasopharyngeal swab. Swabs from patients who reCoVered from CoVID-19 infection are negative, indicating full reCoVery from CoVID-19 infection. However, a certain number of individuals could be a false negative[17,18], because the samples for identifying SARS-CoV-2 viral load depend on the result of reverse transcription polymerase chain reaction (RT-PCR). SARS-CoV-2 uses angiotensin-converting enzyme-2 (ACE-2) as the receptor for cellular entry. The expression of ACE2 protein in the lungs is more than that in the upper respiratory tract. Therefore, it is important from which site the sample was taken in a patient with CoVID-19, as it may cause false-negative RT-PCR results[21].

In recent studies, SARS-CoV-2 was detected in fecal and sputum specimens of patients who were discharged from the hospital with a negative pharyngeal swab after a couple of weeks[17,22]. In other coronavirus pandemics such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS), immunoglobulin levels in patients lasted for a minimum of 2 years, indicating that patients could be vulnerable to reinfection after 3 years[23,24]. The tests that detect SARS-CoV-2 genetic material are very sensitive; however, in patients who have reCoVered from CoVID-19, virus fragments can persist in the body and can be detected by the test. This should not be considered as a new infection[23].

The reinfection in patients reCoVered from CoVID-19 could create a serious challenge in tackling the CoVID-19 pandemic as the reCoVered patients could be a source of virus spread in society[19]. Previous studies have found a positive viral ribonucleic acid (RNA) test in some discharged CoVID-19 patients 10 to 27 d after reCoVery[1,19]. Recurrence of CoVID-19 after reCoVery should be differentiated from secondary medical conditions such as super infection, pulmonary embolism, or persistent RNA virus that can be disCoVered in respiratory specimens in clinically cured CoVID-19 patients[25]. This review aims to provide a systematic compilation of SARS-CoV-2 reactivation in reCoVered CoVID-19 patients.

MATERIALS AND METHODS

This study was a systematic literature review of current evidence conducted in August 2020. The authors studied the probable reinfection risk of novel coronavirus (CoVID-19). Our study adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist to ensure the reliability and validity of this study and results.

Data sources

By application of a systematic search and using the keywords in the online databases including PubMed, Scopus, Web of Science, and Science Direct, we extracted all the relevant papers and reports published in English from December 2019 through August

2020. We included several combinations of keywords in the following orders to conduct the search strategy: (1) "Coronavirus" or "CoVID-19" or "SARS-CoV-2" or "Novel Coronavirus" or "2019-nCoV" [Title/Abstract]; (2) "Reactivation" or "Reinfection" or "Postinfection" [Title/Abstract]; and (1) and (2).

Study selection

Three independent investigators retrieved the studies that were the most relevant by titles and abstracts. Subsequently, the full text of the retrieved papers was reviewed and the most relevant papers were chosen according to the eligibility criteria. Then, we extracted the relevant data and organized them in Tables. The original papers that were peer-reviewed and published in English and fulfilled the eligibility criteria were included in the final report.

We considered the exclusion criteria for this study as follows: (1) Papers conveying non-human studies including *in vitro* observations or articles focusing on animal experiments, or discussing CoVID-19 as a whole subject, without citation of the keywords of this study; (2) Papers in which their full text were out of access; and (3) Any suspicious and duplicated results in the databases.

Data extraction

After summarizing, we transferred the information of the authors, type of article (*e.g.*, case reports), publication date, country of origin, sample size, age, gender, and clinical symptoms to a data extraction sheet. Two independent investigators collected this information and subsequently organized them in the Tables. Finally, to ensure no duplications or overlap exist in the content, all the selected articles were cross-checked by other authors.

Quality assessment

As aforementioned, we applied the PRISMA checklist to ensure the quality and reliability of selected articles. Two independent researchers evaluated the consistency and quality of the articles and the bias risk. In either case of discrepancy in viewpoints, a third independent researcher resolved the issue. The full text of selected articles was fully read, and the key findings were extracted.

RESULTS

In this study, 981 documents were identified using a systematic search strategy. After a primary review of retrieved articles, 498 duplicates were removed, and the title and abstract of the remaining 483 resources were reviewed. After applying the selection criteria, 552 articles were excluded, and only 31 articles met the inclusion criteria and were included in the final review (Figure 1).

We have reviewed 35 studies. Eight studies described reCoVered patients with reinfection. Only one study reported reinfected patients who died. In 26 studies, there was no information about the status of the patients (Table 1)[2,10,16,17,20,25-28,30-53].

Several studies indicated that reinfection is not probable and that postinfection immunity is at least temporarily and short; however, other studies, particularly from South Korea and China, reported some reinfection cases. South Korea reported that 116 reCoVered cases of CoVID-19 were found to be positive again[16]. Another study from South Korea reported that up to 163 patients who were presumed to have reCoVered from SARS-CoV-2 ended up testing positive again[20]. Several studies from China do not support reinfection[26-29]. There is only one study from China that reported five cases of reactivation[5].

The results of the present study showed that there are many factors that we need to take into account about reinfection. Some cases may have resulted in a false negative at discharge or patients did not completely meet discharge criteria. Although we should not forget that reinfection could be possible, because some studies have shown humoral immunity weakens over time.

DISCUSSION

Due to the widespread expansion of the CoVID-19 epidemic around the world, there are more and more infected cases, and of course, many people have reCoVered from this viral infection. However, there is recently a concern regarding the reinfection in

Table 1 Identified reinfection risk of novel coronavirus

ID	Ref.	Type of study	Country	Study population	Reinfection outcome			
					ReCoVery	Death	Unknown	Other findings
1	Alizargar *et al*[16]	Letter to the editor	South Korea	CoVID-19 patients	No	No	Yes	South Korea reported that 116 reCoVered cases of CoVID-19 were found positive again
2	Gousseff *et al*[25]	Letter to the editor	France	CoVID-19 patients	Yes	Yes	No	Between April 6 and May 14, 2020, 11 patients were identified (sex ratio M/F 1.2, median age 55, range 19-91 yr). The median duration of symptoms was 18 (13-41) d for the first episode and 10 d for the second one for the 7 patients who eventually reCoVered
3	Chaturvedi *et al*[20]	Review	South Korea	CoVID-19 patients	No	No	Yes	Concerning reports released from the Korea Centers for Disease Control and Prevention (KCDC) have noted that up to 163 patients who were presumed to have reCoVered from SARS-CoV-2 infection ended up testing positive with PCR testing yet again
4	Gomez-Mayordomo *et al*[30]	Short communication	Spain	A case study in a patient with relapsing-remitting MS treated with fingolimod	No	No	Yes	This case suggests that discontinuation of fingolimod during CoVID-19 could imply a worsening of SARS-CoV-2 infection. No information about reinfection
5	Hageman *et al*[31]	Editorial	United States	CoVID-19 in children	Yes	No	No	Limited data suggest that reCoVery might confer immunity
6	Hoang *et al*[32]	Letter to the editor	France	Patients reCoVered from CoVID-19	No	No	Yes	Recurrence of SARS-CoV-2 in patients who had reCoVered from CoVID-19 has been described. However, it is possible that recurrences could actually be persistent infections in which the PCR resulted falsely negative at discharge
7	Inamo *et al*[33]	Letter of biomedical and clinical research	Japan	CoVID-19 patients	No	No	Yes	-
8	Islam *et al*[34]	Review article	Bangladesh	CoVID-19 patients	No	No	Yes	There is a possibility of reinfection as the humoral immunity weakens over time
9	Kang *et al*[26]	Commentary	China	CoVID-19 patients	No	No	Yes	ReCoVered patients become retest positive due to false-negative PCR or patients did not completely meet discharge criteria or due to dead viruses
10	Kannan *et al*[35]	Review article	India	Gene study between SARS-CoV-2 and SARS-CoV-1 and batCoV and MERS-CoV	No	No	Yes	Many researchers observed that there is SARS-CoV-2 reinfection in the same treated patients
11	Karimi *et al*[36]	Letter to the editor	Iran	CoVID-19 patients	Yes	No	No	-
12	Kassa *et al*[37]	Analytic article	Botswana	CoVID-19 patients	No	No	Yes	Not related to our topic but it is said "reinfection" by the family of coronavirus is possible
13	Kellam *et al*[38]	Review article	United Kingdom	Patients with coronavirus infection	No	No	Yes	Immediate reinfection is not possible but reinfection of previously mild SARS-CoV-2 cases is a realistic possibility
14	Kirkcaldy *et al*[39]	Viewpoint	United States	CoVID-19 Patients	No	No	Yes	ReCoVery from CoVID-19 might confer immunity against reinfection, at least temporarily

15	Koks *et al*[40]	Commentary	Australia	CoVID-19 patients	No	No	Yes	No information related to our study except "the testing needs to be repeated several times as persons with negative tests could become positive the next day as a result of a new infection or there plication of the virus"
16	Law *et al*[27]	Letter to the editor	China/Hong Kong	Patients reCoVered from CoVID-19	No	No	Yes	There is currently no supporting evidence for CoVID-19 reinfection after reCoVery but retest can be positive due to several reasons
17	Laxminarayan *et al*[41]	Perspective	India	CoVID-19 in children	No	No	Yes	Reinfection is not probable
18	Leslie *et al*[42]	Letter	United States	SARS-CoV-2 patients	No	No	Yes	Patients with past infection with other coronaviruses that cause common cold may have some immunity to SARS-CoV-2
19	Luo *et al*[43]	Case report	China	Woman with CoVID-19	Yes	No	No	-
20	Meca-Lallana *et al*[44]	Correspondence	Spain	CoVID-19 patients with MS	No	No	Yes	-
21	Okhuese *et al*[45]	Statistical	Nigeria	CoVID-19 patients	No	No	Yes	There is no secondary reinfection in reCoVered patients. However, some reports have shown there have been a few rare cases of reinfection
22	Omer *et al*[46]	Viewpoint	United States	CoVID-19 patients in the United States	No	No	Yes	True reinfection is unlikely
23	Ota *et al*[47]	In brief	United States	Rhesus monkeys	No	No	Yes	-
24	Ozdinc *et al*[48]	Statistical	Turkey	Turkish people infected with CoVID-19	No	No	Yes	There is short term immunity
25	Roy *et al*[17]	Review	India	CoVID-19 patients	No	No	Yes	Reinfection with SARS-CoV-2 seems unlikely taking into consideration our knowledge. We must maintain vigilance during the convalescence period and must take into consideration the probability of genetic mutations, as observed, rather than reinfection by the same strain
26	Steinchen *et al*[49]	Case report	Germany	A case of rheumatoid arthritis and CoVID-19 patient	Yes	No	No	A case of rheumatoid arthritis and insufficient compensation is reported under long-term combination therapy with methotrexate and leflunomide. After going through CoVID-19 infection, a new adjustment was made to a tumor necrosis factor (TNF) blocker. No reactivation of the infection has occurred in the short period of time initiated by the initiated bDMARD (biologic disease-modifying antirheumatic drug) therapy after surviving CoVID-19 infection with positive antibody status. Biologic therapy without mandatory medical indication should not be performed to protect against SARS-CoV-2 infection
27	Ueffing *et al*[50]	Review	Germany	CoVID-19 patients	No	No	Yes	Seven human pathogenic coronaviruses have already been detected in humans, most of which can cause respiratory diseases, but occasionally also conjunctivitis and middle ear infections. Four of the previously known coronaviruses (229E, NL63, OC43, and HKU1) typically cause relatively minor symptoms in the context of human infection of the upper respiratory tract. SARS-CoV and the 2012 MERS-CoV lead to severe respiratory diseases and have a significant mortality rate. Experiences with other coronavirus infections (SARS and MERS) indicate that the immunity could persist for several years. Based on animal experiments, already acquired data on other coronavirus types and plausibility, it can be assumed that seroconverted patients have the immunity of limited duration and only a very low risk of reinfection
28	Verhagen *et al*[51]	Research study	England and Wales	CoVID-19 patients	No	No	Yes	Areas face disproportionate risks for CoVID-19 hospitalization pressures due to their socioeconomic differences and the demographic composition of their populations. Our flexible online dashboard allows policymakers and health officials to monitor and evaluate potential health care demand at a granular level as the infection rate and hospital capacity changes throughout the course of this pandemic. This agile knowledge is invaluable to tackle the enormous logistical challenges to re-allocate resources and target susceptible areas for aggressive testing and tracing to mitigate

								transmission
29	Waltuch *et al*[52]	Case reports	United States	Children with CoVID-19 infection	No	No	Yes	Patients presenting with CoVID-19 associated post-infectious cytokine release syndrome appear to present with prolonged fever (5 d or greater) and GI symptoms with or without rash. This syndrome may overlap with features of Kawasaki Disease and Toxic Shock Syndrome. Patients who present with this clinical picture should have frequent vital signs and will require admission due to the potential for rapid deterioration
30	Tao *et al*[28]	Research study	China	CoVID-19 patients	Yes	No	No	These results implied that the positive result is unlikely caused by the reinfection from others or the remained virus. Rather, it may derive from the remained virus transferred from the lower respiratory tract to the throat or nose with coughing. Accordingly, it is suggested that the specimen detection of bronchoalveolar lavage fluid from the lower respiratory tract should be used as the discharge criteria
31	Zhou *et al*[53]	Review	China	CoVID-19 patients	No	No	Yes	Re-fever and positive nucleic acid test after discharge from the hospital might be due to the biological characteristics of 2019-nCoV, and might also be related to the basic disease, clinical status, glucocorticoid use, sampling, processing, and detecting of patients, and some even related to the reinfection or secondary bacterial virus infection

CoVID-19: Coronavirus disease 2019; F: Female; GI: Gastrointestinal; HBV: Hepatitis B virus; M: Male; MERS-CoV: Middle East respiratory syndrome-coronavirus; MS: Multiple sclerosis; PCR: Polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

previously reCoVered SARS-CoV-2 patients. In the present review, we summarized the recent findings and reports of CoVID-19 reinfection in patients previously reCoVered from the disease. This is important to inform the public regarding the possible risk of reinfection to restrain the transmission of SARS-CoV-2 and control the current epidemic[25].

The findings from the current review of existing evidence suggest two possible scenarios for new infection in patients who were previously reCoVered from CoVD-19, including reinfection and reactivation. Studies have shown some cases of symptom recurrence such as fever, malaise, myalgia, and cough after discharge. The positive PCR test confirmed the infection and suggested reinfection. Although this has been attributed to the biological characteristics of CoVID-19 and other factors, such as underlying diseases, clinical status, glucocorticoid use, sample collection, patient detection, follow-up, and even secondary bacterial infection, it could be due to reinfection with CoVID-19[53,54]. Positive follow-up tests may also derive from the remained virus transferred from the lower respiratory tract to the throat and nose with coughing. Therefore, it is suggested that the fluid collected in the bronchoalveolar lavage of the lower respiratory tract should be tested and used as the discharge criteria in SARS-CoV-2 patients[28]. In fact, a retest can be positive due to several reasons; thus, it is difficult to distinguish between reinfection, reactivation, or other causes.

Among the reviewed studies, six studies emphasized short-term immunity following reCoVery[18,19,25,26,33,35]. One study indicated that the antibodies and the immunity could last about 40 d and that there is a possibility of reinfection or reactivation of latent infection after this period. Therefore, reCoVery from CoVID-19 might not confer immunity against reinfection forever[38,39]. Furthermore, previous studies related to other human coronavirus types suggested the possibility of reinfection by other members of the coronavirus family following reCoVery from a

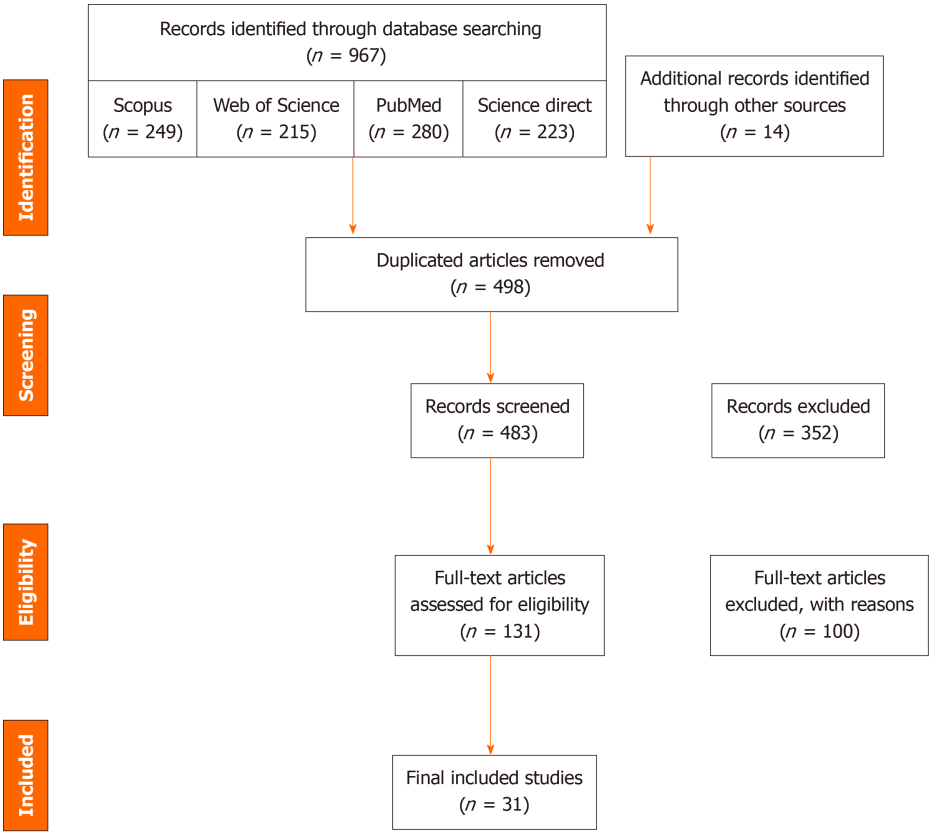

Figure 1 Flow diagram for the selection process of identified articles.

particular type[24]. Although there are previous studies that suggest the reinfection with SARS-CoV-2 is unlikely, we must maintain vigilance during the convalescence period and consider the probability of genetic mutations as observed rather than reinfection by the same strain[6,29,33,34].

The results of the present study showed that there are many factors that we need to take into account about reinfection. Some cases may have resulted in false negative at discharge or patients did not completely meet discharge criteria. We should not forget, however, that reinfection could be possible because some studies have shown humoral immunity weakens over time. The certainty regarding the reinfection in CoVID-19 patients is limited, and we strongly recommend further studies to explore the virological, immunological, and epidemiologic characteristics of SARS-CoV-2 to determine the biological behavior of the virus and describe the potential mechanisms of disease recurrences.

CONCLUSION

In conclusion, positive PCR retest results could be due to several reasons such as the type of specimen collection and technical errors associated with each component of swab testing, the methods used before discharging patients, prolonged viral shedding, and infection by mutated SARS-CoV-2. Thus, it should not always be considered as a reinfection or reactivation of the disease. Furthermore, most relevant studies on symptomatic and positive retest patients have shown relative and probably temporary immunity after the reCoVery of the disease, which means that immunity acquired following primary infection with SARS-CoV-2 may protect from subsequent exposure to the virus at least for a limited period.

ARTICLE HIGHLIGHTS

Research background

Due to the high rate of transmission of coronavirus disease 2019 (CoVID-19), a large number of people around the world became infected with the virus. There is evidence of reinfection with this virus. Therefore, people who get the disease once may be reinfected after reCoVery. Further investigation of reinfection by CoVID-19 is one of the necessities for better management of current conditions.

Research motivation

There have been reports of reCoVered individuals who have a second positive coronary test. This has raised concerns that there is no guarantee that the body will be safe after corona disease, even in the short term.

Research objectives

The aim of the present study was to investigate the available evidence of reinfection in patients with CoVID-19 who have reCoVered.

Research methods

This is a review study of different research types. Since there are myriads of publications released each and every day, with each trying to shed light on this pandemic from different perspectives, we aimed to summarize the very recent and of course the most trustworthy studies regarding the possibility of reinfection of CoVID-19 in this review in order to provide health care professionals and researchers imminent access to a multitude of these studies *via* a concise resource to save their invaluable time for other yet to do tasks.

Research results

The results have shown that there is a slight chance of reinfection. Though the duration of immunity is still unknown and needs to be determined; there is no guarantee that infected patients will not be infected again according to our results. These reinfections can be related to immunity system problems in cases of immunosuppressive disease or drugs that can misdirect our results, but there were many cases that got reinfected without any sign of the problems mentioned above.

Research conclusions

Based on the available evidence, reinfection in improved patients has been proven. Still, there is not enough data to definitely distinguish reinfection, reactivation, or infection with a new mutated severe acute respiratory syndrome coronavirus 2. So, further studies are necessary to understand if a CoVID-19 recurrence is possible and whether it could be considered a real threat.

Research perspectives

We strongly suggest further studies to follow up discharged CoVID-19 patients, check their course of symptoms periodically, and analyze related antibody levels; widespread virological studies are necessary to understand better this new global predicament.

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REFERENCES

1 **Sekhavati E**, Jafari F, SeyedAlinaghi S, Jamalimoghadamsiahkali S, Sadr S, Tabarestani M, Pirhayati M, Zendehdel A, Manafi N, Hajiabdolbaghi M, Ahmadinejad Z, Kouchak HE, Jafari S, Khalili H, Salehi M, Seifi A, Golestan FS, Ghiasvand F. Safety and effectiveness of azithromycin in patients with CoVID-19: An open-label randomised trial. *Int J Antimicrob Agents* 2020; **56**: 106143 [PMID:

32853672 DOI: 10.1016/j.ijantimicag.2020.106143]
2 **Ye G**, Pan Z, Pan Y, Deng Q, Chen L, Li J, Li Y, Wang X. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. *J Infect* 2020; **80**: e14-e17 [PMID: 32171867 DOI: 10.1016/j.jinf.2020.03.001]
3 **Mehraeen E**, Behnezhad F, Salehi MA, Noori T, Harandi H, SeyedAlinaghi S. Olfactory and gustatory dysfunctions due to the coronavirus disease (CoVID-19): a review of current evidence. *Eur Arch Otorhinolaryngol* 2020; **17**: 1-6 [PMID: 32556781 DOI: 10.1007/s00405-020-06120-6]
4 **SeyedAlinaghi S**, Ghadimi M, Hajiabdolbaghi M, Rasoolinejad M, Abbasian L, Nezhad MH, Manshadi SD, Ghadimi F, Ahmadinejad Z. Prevalence of CoVID-19-like Symptoms among People Living with HIV, and Using Antiretroviral Therapy for Prevention and Treatment. *Curr HIV Res* 2020; **18**: 373-380 [PMID: 32652912 DOI: 10.2174/1570162X18666200712175535]
5 **Zhao W**, Wang Y, Tang Y, Zhao W, Fan Y, Liu G, Chen R, Song R, Zhou W, Liu Y, Zhang F. Characteristics of Children With Reactivation of SARS-CoV-2 Infection After Hospital Discharge. *Clin Pediatr (Phila)* 2020; **59**: 929-932 [PMID: 32462940 DOI: 10.1177/0009922820928057]
6 **Sadr S**, SeyedAlinaghi S, Ghiasvand F, Hassan Nezhad M, Javadian N, Hossienzade R, Jafari F. Isolated severe thrombocytopenia in a patient with CoVID-19: A case report. *IDCases* 2020; **21**: e00820 [PMID: 32483524 DOI: 10.1016/j.idcr.2020.e00820]
7 **Ghiasvand F**, SeyedAlinaghi S. Isolated Anosmia as a Presentation of CoVID-19: An Experience in a Referral Hospital. *Infect Disord Drug Targets* 2020; **20**: 350 [PMID: 32436835 DOI: 10.2174/1871526520999200520173216]
8 **Ghiasvand F**, Miandoab SZ, Harandi H, Golestan FS, Alinaghi SAS. A Patient with CoVID-19 Disease in a Referral Hospital in Iran: A Typical Case. *Infect Disord Drug Targets* 2020; **20**: 559-562 [PMID: 32348232 DOI: 10.2174/1871526520666200429115535]
9 **Mahmoudi S**, Mehdizadeh M, Shervin Badv R, Navaeian A, Pourakbari B, Rostamyan M, Sharifzadeh Ekbatani M, Eshaghi H, Abdolsalehi MR, Alimadadi H, Movahedi Z, Mamishi S. The Coronavirus Disease 2019 (CoVID-19) in Children: A Study in an Iranian Children's Referral Hospital. *Infect Drug Resist* 2020; **13**: 2649-2655 [PMID: 32801803 DOI: 10.2147/IDR.S259064]
10 **Aldhaleei WA**, Alnuaimi A, Bhagavathula AS. CoVID-19 Induced Hepatitis B Virus Reactivation: A Novel Case From the United Arab Emirates. *Cureus* 2020; **12**: e8645 [PMID: 32550096 DOI: 10.7759/cureus.8645]
11 **Ghiasvand F**, Ghadimi M, Ghadimi F, Safarpour S, Hosseinzadeh R, SeyedAlinaghi S. Symmetrical polyneuropathy in coronavirus disease 2019 (CoVID-19). *IDCases* 2020; **21**: e00815 [PMID: 32514394 DOI: 10.1016/j.idcr.2020.e00815]
12 **Ravioli S**, Ochsner H, Lindner G. Reactivation of CoVID-19 pneumonia: A report of two cases. *J Infect* 2020; **81**: e72-e73 [PMID: 32389787 DOI: 10.1016/j.jinf.2020.05.008]
13 **Asadollahi-Amin A**, Hasibi M, Ghadimi F, Rezaei H, SeyedAlinaghi S. Lung Involvement Found on Chest CT Scan in a Pre-Symptomatic Person with SARS-CoV-2 Infection: A Case Report. *Trop Med Infect Dis* 2020; **5** [PMID: 32272630 DOI: 10.3390/tropicalmed5020056]
14 **Mehraeen E**, Hayati B, Saeidi S, Heydari M, Seyed Alinaghi S. Self-Care Instructions for People Not Requiring Hospitalization for Coronavirus Disease 2019 (CoVID-19). *Arch Clin Infect Dis* 2020; **15**
15 **Shah VK**, Firmal P, Alam A, Ganguly D, Chattopadhyay S. Overview of Immune Response During SARS-CoV-2 Infection: Lessons From the Past. *Front Immunol* 2020; **11**: 1949 [PMID: 32849654 DOI: 10.3389/fimmu.2020.01949]
16 **Alizargar J**. Risk of reactivation or reinfection of novel coronavirus (CoVID-19). *J Formos Med Assoc* 2020; **119**: 1123 [PMID: 32340768 DOI: 10.1016/j.jfma.2020.04.013]
17 **Roy S**. CoVID-19 Reinfection: Myth or Truth? *SN Compr Clin Med* 2020; 1-4 [PMID: 32838134]
18 **Bongiovanni M**, Basile F. Re-infection by CoVID-19: a real threat for the future management of pandemia? *Infect Dis (Lond)* 2020; **52**: 581-582 [PMID: 32434442 DOI: 10.1080/23744235.2020.1769177]
19 **Elrashdy F**, Aljaddawi AA, Redwan EM, Uversky VN. On the potential role of exosomes in the CoVID-19 reinfection/reactivation opportunity. *J Biomol Struct Dyn* 2020; 1-12 [PMID: 32643586 DOI: 10.1080/07391102.2020.1790426]
20 **Chaturvedi R**, Naidu R, Sheth S, Chakravarthy K. Efficacy of Serology Testing in Predicting Reinfection in Patients With SARS-CoV-2. *Disaster Med Public Health Prep* 2020; 1-3 [PMID: 32576315]
21 **Jahromi R**, Avazpour A, Jahromi M, Alavi JJIJoCR. CoVID-19 with positive bronchoalveolar lavage fluid but negative nasopharyngeal and oropharyngeal swabs: A case report and insights. *Indian J Case Rep* 2020; 380-382 [DOI: 10.32677/IJCR.2020.v06.i07.010]
22 **Wu Y**, Guo C, Tang L, Hong Z, Zhou J, Dong X, Yin H, Xiao Q, Tang Y, Qu X, Kuang L, Fang X, Mishra N, Lu J, Shan H, Jiang G, Huang X. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 2020; **5**: 434-435 [PMID: 32199469 DOI: 10.1016/S2468-1253(20)30083-2]
23 **Villamil JFP**, Olivera MJ. CoVID-19: Is Reinfection a Threat or Not? *Iran J Public Health* 2020; **49**: 112-3 [DOI: 10.18502/ijph.v49iS1.3678]
24 **Wu LP**, Wang NC, Chang YH, Tian XY, Na DY, Zhang LY, Zheng L, Lan T, Wang LF, Liang GD. Duration of antibody responses after severe acute respiratory syndrome. *Emerg Infect Dis* 2007; **13**: 1562-1564 [PMID: 18258008 DOI: 10.3201/eid1310.070576]
25 **Gousseff M**, Penot P, Gallay L, Batisse D, Benech N, Bouiller K, Collarino R, Conrad A, Slama D, Joseph C, Lemaignen A, Lescure FX, Levy B, Mahevas M, Pozzetto B, Vignier N, Wyplosz B,

Salmon D, Goehringer F, Botelho-Nevers E; in behalf of the COCOREC study group. Clinical recurrences of CoVID-19 symptoms after reCoVery: Viral relapse, reinfection or inflammatory rebound? *J Infect* 2020; **81**: 816-846 [PMID: 32619697 DOI: 10.1016/j.jinf.2020.06.073]

26 **Kang H**, Wang Y, Tong Z, Liu X. Retest positive for SARS-CoV-2 RNA of "reCoVered" patients with CoVID-19: Persistence, sampling issues, or re-infection? *J Med Virol*2020 [PMID: 32492212 DOI: 10.1002/jmv.26114]

27 **Law SK**, Leung AWN, Xu C. Is reinfection possible after reCoVery from CoVID-19? *Hong Kong Med J* 2020; **26**: 264-265 [PMID: 32536614 DOI: 10.12809/hkmj208601]

28 **Tao JJ**, Hu Z, Liu J, Pang P, Fu G, Qian A, Chen S, Lin L, Cao G, Sun H, Yang Y, Wang M. Positive RT-PCR Test Results in Discharged CoVID-19 Patients: Reinfection or Residual? 2020 [DOI: 10.21203/rs.3.rs-18042/v1]

29 **Zhang L**, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. *J Med Virol* 2020; **92**: 479-490 [PMID: 32052466 DOI: 10.1002/jmv.25707]

30 **Gomez-Mayordomo V**, Montero-Escribano P, Matías-Guiu JA, González-García N, Porta-Etessam J, Matías-Guiu J. Clinical exacerbation of SARS-CoV-2 infection after fingolimod withdrawal. *J Med Virol* 2020 [PMID: 32644205 DOI: 10.1002/jmv.26279]

31 **Hageman JR**. What Are the Newest Effects of CoVID-19 in Children? *Pediatr Ann* 2020; **49**: e242-e243 [PMID: 32520362 DOI: 10.3928/19382359-20200520-02]

32 **Hoang VT**, Dao TL, Gautret P. Recurrence of positive SARS-CoV-2 in patients reCoVered from CoVID-19. *J Med Virol* 2020 [PMID: 32449789 DOI: 10.1002/jmv.26056]

33 **Inamo J**. How should we overcome the threat by the pandemic of 2019-nCoV? *Clin Rheumatol* 2020; **39**: 2471-2473 [PMID: 32388750 DOI: 10.1007/s10067-020-05083-7]

34 **Islam H**, Rahman A, Masud J, Shweta DS, Araf Y, Ullah MA, Sium SM, Sarkar B. A Generalized Overview of SARS-CoV-2: Where Does the Current Knowledge Stand? *Electron J Gen Med* 2020; **17**: em251 [DOI: 10.29333/ejgm/8258]

35 **Kannan S**, Subbaram K, Ali S, Kannan H. Molecular characterization and amino acid homology of nucleocapsid (N) Protein in SARS-CoV-1, SARS-CoV-2, MERS-CoV, and bat coronavirus. *J Pure Appl Microbio* 2020; **23**: 14

36 **Karimi A**, Shirvani F, Seifi K. Reinfection or relapse in sars-CoV-2-infected patients; does it occur? *Arch Pediatr Infecti Dis* 2020; **8**: e103466 [DOI: 10.5812/archcid.103466]

37 **Kassa SM**, Njagarah JBH, Terefe YA. Analysis of the mitigation strategies for COVID-19: From mathematical modelling perspective. *Chaos Solitons Fractals* 2020; 109968 [PMID: 32536760 DOI: 10.1016/j.chaos.2020.109968]

38 **Kellam P**, Barclay W. The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection. *J Gen Virol* 2020; **101**: 791-797 [PMID: 32430094 DOI: 10.1099/jgv.0.001439]

39 **Kirkcaldy RD**, King BA, Brooks JT. CoVID-19 and Postinfection Immunity: Limited Evidence, Many Remaining Questions. *JAMA* 2020; **323**: 2245-2246 [PMID: 32391855 DOI: 10.1001/jama.2020.7869]

40 **Koks S**, Williams RW, Quinn J, Farzaneh F, Conran N, Tsai SJ, Awandare G, Goodman SR. CoVID-19: Time for precision epidemiology. *Exp Biol Med (Maywood)* 2020; **245**: 677-679 [PMID: 32301338 DOI: 10.1177/1535370220919349]

41 **Laxminarayan R**, John TJ. Is Gradual and Controlled Approach to Herd Protection a Valid Strategy to Curb the CoVID-19 Pandemic? *Indian Pediatr* 2020; **57**: 505-507 [PMID: 32376793 DOI: 10.1007/s13312-020-1844-4]

42 **Leslie M**. T cells found in coronavirus patients 'bode well' for long-term immunity. *Science* 2020; **368**: 809-810 [PMID: 32439770 DOI: 10.1126/science.368.6493.809]

43 **Luo A**. Positive SARS-CoV-2 test in a woman with CoVID-19 at 22 days after hospital discharge: A case report. *Zhongyi Kexue Zazhi (English)* 2020; 1-5 [DOI: 10.1016/j.jtcms.2020.04.001]

44 **Meca-Lallana V**, Aguirre C, Cardeñoso L, Alarcon T, Figuerola-Tejerina A, Del Río B, Álvarez MR, Vivancos J. Establishment of a safety protocol for the administration of treatments in multiple sclerosis during the SARS-CoV-2 pandemic. *Mult Scler Relat Disord* 2020; **44**: 102244 [PMID: 32544863 DOI: 10.1016/j.msard.2020.102244]

45 **Okhuese AV**. Estimation of the Probability of Reinfection With CoVID-19 by the Susceptible-Exposed-Infectious-Removed-Undetectable-Susceptible Model. *JMIR Public Health Surveill* 2020; **6**: e19097 [DOI: 10.2196/19097]

46 **Omer SB**, Malani P, Del Rio C. The CoVID-19 Pandemic in the US: A Clinical Update. *JAMA* 2020; **323**: 1767-1768 [PMID: 32250388 DOI: 10.1001/jama.2020.5788]

47 **Ota M**. Will we see protection or reinfection in CoVID-19? *Nat Rev Immunol* 2020; **20**: 351 [PMID: 32303697 DOI: 10.1038/s41577-020-0316-3]

48 **Ozdinc M**, Senel K, Ozturkcan S, Akgul A. Predicting the progress of CoVID-19: the case for Turkey. *Turkiye Klinikleri J Med Sci* 2020 [DOI: 10.5336/medsci.2020-75741]

49 **Steinchen N**, Müller-Ladner U, Lange U. [Biological therapy after CoVID-19 infection : No reactivation of a CoVID-19 infection with positive SARS-CoV-2 antibody status under biological therapy]. *Z Rheumatol* 2020; **79**: 574-577 [PMID: 32514854 DOI: 10.1007/s00393-020-00824-0]

50 **Ueffing M**, Bayyoud T, Schindler M, Ziemssen F. [Basic principles of replication and immunology of SARS-CoV-2]. *Ophthalmologe* 2020; **117**: 609-614 [PMID: 32613257 DOI: 10.1007/s00347-020-01155-w]

51 **Verhagen MD**, Brazel DM, Dowd JB, Kashnitsky I, Mills MC. Forecasting spatial, socioeconomic

and demographic variation in CoVID-19 health care demand in England and Wales. *BMC Med* 2020; **18**: 203 [PMID: 32594909 DOI: 10.1186/s12916-020-01646-2]

52 **Waltuch T**, Gill P, Zinns LE, Whitney R, Tokarski J, Tsung JW, Sanders JE. Features of CoVID-19 post-infectious cytokine release syndrome in children presenting to the emergency department. *Am J Emerg Med* 2020; **38**: 2246.e3-2246. e6 [PMID: 32471782 DOI: 10.1016/j.ajem.2020.05.058]

53 **Zhou L**, Liu K, Liu HG. [Cause analysis and treatment strategies of "recurrence" with novel coronavirus pneumonia (CoVID-19) patients after discharge from hospital]. *Zhonghua Jiehe He Huxi Zazhi* 2020; **43**: 281-284 [PMID: 32118391 DOI: 10.3760/cma.j.cn112147-20200229-00219]

54 **Arafkas M**, Khosrawipour T, Kocbach P, Zielinski K, Schubert J, Mikolajczyk A, Celinska M, Khosrawipour V. Current meta-analysis does not support the possibility of CoVID-19 reinfections. *J Med Virol* 2020 [PMID: 32897549 DOI: 10.1002/jmv.26496]

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