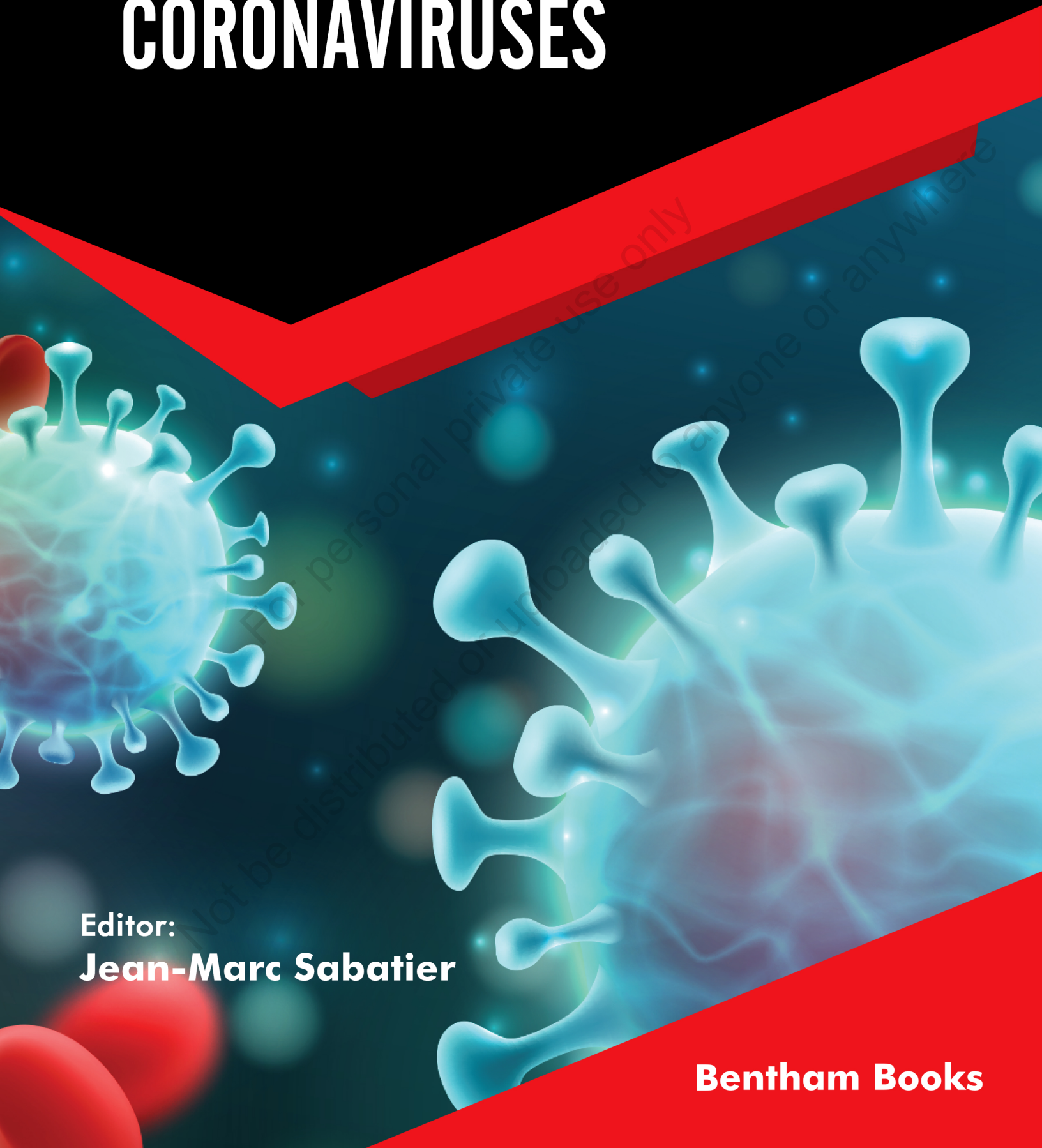
CORONAVIRUSES



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(Volume 3)

Edited by

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PREFACE

In this third year of the SARS-CoV-2 pandemic responsible for Covid-19 diseases worldwide, the scientific studies and reviews focused on this virus and related variants are still crucial. This book, corresponding to the third volume of the e-book series on 'Coronaviruses', brings together some essential data regarding the origin, pathology and chemotherapeutic drugs to treat coronavirus infections. It consists of six chapters concerning (1) the bat's coronaviruses in the world (chapter 1 by Karin Correa Scheffer *et al*.), (2) the hospital challenges during the Covid-19 pandemic (Chapter 2 by Salman Zarka *et al*.), (3) the pro-inflammatory and thrombotic manifestations and the therapeutic options of Covid-19 (chapter 3 by Mradul Kumar Daga *et al*.), (4) the common and rare dermatologic manifestations registered in Covid-19 patients (Chapter 4 by Amin Daemi *et al*.), (5) the circulating biomarkers of cardiopulmonary disturbances in Covid-19 (chapter 5 by Amin Daemi *et al*.), and (6) the aspects of pathology and pathogenesis of coronavirus infection (Chapter 6 by V.A. Zinserling *et al*.). This new volume actually compiles the most important data/information on SARS-CoV-2 and associated Covid-19 diseases. It is therefore of clear value for all the researchers working in these research fields, and for the clinicians dealing with a growing number of persons with Covid-19 and/or suffering from post-Covid sequelae, referred to as long Covid (data from 23rd November 2022: 644 million cases of SARS-CoV-2 infection worldwide, with 6.6 million deaths).

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CHAPTER 1

Bat Coronaviruses in the World

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Abstract: Bats belong to the second-largest order in a number of species diversity within the Mammalia class, containing 21 families and more than 1300 species. It is estimated that more than 200 viruses from 28 families have been isolated or detected in 37 different bat genera, many of them related to emerging infectious diseases with the potential to cross species barriers and infect other animals. The group of coronaviruses (CoV) is one of these viruses, which includes CoVs that can cause serious diseases in humans and animals, such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), porcine epidemic diarrhea (PED), swine acute diarrheal syndrome (SADS) and coronavirus disease 2019 (COVID-19). Some of the human and animal coronaviruses appear to be originated from bats. With the advent of new generation molecular techniques and increased surveillance of wild animal species, many new coronaviruses have been identified. The coronaviruses belong to the Nidovirales order and *Coronaviridae* family. The subfamily *Coronavirinae* is divided into four genera, Alphacoronavirus (alphaCoV), Betacoronavirus (betaCoV), Deltacoronavirus, and Gammacoronavirus. Since the first report of coronavirus in a Miniopterus pusillus bat, the coronavirus occurrence in a bats population of different regions in the world has been studied and, until now, both alphaCoV and betaCoV have been detected. The aim of this study was to perform a literature review regarding the detection of coronavirus (alphaCoV or betaCoV) in different bat species around the world and their immune response against coronavirus. This review reinforces the importance of these animals as hosts, reservoirs, or sources of viruses, including emerging viruses.

Keywords: Alphacoronavirus, Bats, Betacoronavirus, Chiroptera, Coronaviridae, Reservoirs, Viruses.

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Jean-Marc Sabatier (Ed.)

INTRODUCTION

Bats belong to the order *Chiroptera*, which represents the second-largest order in a number of species diversity within the class *Mammalia*. This diversity is demonstrated in over 1300 species already identified, which are grouped in 21 families, distributed in two suborders, *Yinpterochiroptera* (*Pteropodiformes*) and *Yangochiroptera* (*Vespertilioniformes*). The suborder *Yinpterochiroptera* is formed by the family *Pteropodidae* (former *Megachiroptera* suborder) and the superfamily *Rhinolophoidea* (formed by the families *Rhinolophidae*, *Hipposideridae*, *Rhinonycteridae*, *Rhinopomatidae*, *Megadermatidae* and *Craseonycteridae*). Three superfamilies belong to the suborder *Yangochiroptera*, *Noctilionoidea* (families *Mystacinidae*, *Furipteridae*, *Noctilionidae*, *Thyropteridae*, *Mormoopidae*, and *Phyllostomidae*), *Emballonuroidea* (families *Emballonuridae, Nycteridae* and *Myzopodidae*) and *Vespertilionoidea* (families *Molossidae*, *Vespertilionidae*, *Miniopteridae*, *Natalidae* e *Cistugidae*) [1, 2].

The feature that groups and distinguishes bats from other mammals is the ability to fly. Genetic studies have shown that this singularity may have influenced some aspects of the innate immune system evolution of these animals, generating hypotheses that bats can control viral replication differently from other mammals. In addition, another theory would be that the high metabolic rate and the increase in body temperature during flight, similar to a febrile response, make it difficult to replicate temperature-sensitive infectious agents [3, 4].

The first suspicion that bats were reservoirs of viral zoonoses was suggested by Carini [5], who suggested that the transmission of rabies to herbivores was by the bite of the blood-sucking bat. This hypothesis was proven a few years later when Negri's corpuscles were first identified in a hematophagous bat [6], and since then, bats have been considered reservoirs for rabies and other lyssaviruses.

For a long time, the search for new viruses in bats has been neglected due to the tendency of researchers to search only for viruses that cause pathologies [7]. It is estimated that more than 200 viruses from 28 families have been isolated or detected in 37 different bat genera, many of them related to emerging infectious diseases with the potential to cross species barriers and infect other animals [7 - 10]. Therefore, these animals play an important role in the dynamics of viruses in the environment, acting as reservoirs and probable sources of infections for other animals [3].

Researchers point out that bats have certain characteristics that make them ideal for hosting and spreading a greater number of viruses than most animals [11, 12]. Among these characteristics, the ability to fly allows them to have greater contact

with other different species in different locations when compared to other terrestrial mammals. In addition, this ability can also confer greater transmission of viruses between species [12].

The long life expectancy in relation to their body size can facilitate viral persistence through the transmission of chronic infections, besides the ability of bats to enter into prolonged torpor, with immunity decrease caused by the drop in body temperature. The gregarious way of life is also pointed out as an important characteristic, as it allows inter and intra species contact, which facilitates the transmission of pathogens. Lastly, another characteristic is the fact that different species have different diets [8, 11 - 14].

Environmental alteration by human activities has caused an enormous impact on the ecology, inducing the circulation of different wild animals species from their natural habitat to urban or rural areas [15], increasing the chances of human beings and domestic animals contact with wild animals. Consequently, several problems are arriving, mainly regarding viral zoonosis dissemination [16].

One of those viral zoonosis is the coronavirus, a disease caused by a virus belonging to order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*, which is divided into four genus, *Alphacoronavirus* (alphaCoV), *Betacoronavirus* (betaCoV), *Deltacoronavirus* and *Gammacoronavirus*. It was believed that alphaCoV and betaCoV groups were only found in some mammals, while the Gammacoronavirus group would be restricted to birds [17]. However, recent research pointed out the presence of Gammacoronavirus in mammals and Deltacoronavirus common to birds and mammals [18].

SARS (Severe Acute Respiratory Syndrome) was the first human pandemic of the XXI century, and a coronavirus causal agent of the disease, namely SARS-CoV, was discovered in 2002 [19, 20]. This pandemic generated the appearance of theories about the transmission and interspecies adaptation capacity of the coronavirus [21]. A virus similar to SARS-CoV in small mammals, masked palm civet (*Paguma larvata*), was detected and initially, it was thought to be the source of infection [22]. However, in 2005, a coronavirus related to SARS and a coronavirus isolated from *P. larvata* was isolated from bats, evidencing that bats could act as natural reservoirs of a SARS-CoV ancestral virus [23].

In 2012, MERS (Middle East Respiratory Syndrome) emerged caused by a new betaCoV, more related to bats HKU4 and HKU5 coronavirus [24]. Although the natural reservoir of MERS has not been identified yet, the high similarity with a nucleotide sequence of coronavirus implies that the origin is present in the bat [25].

A different coronavirus was reported in 2019 in registered cases in China. The virus was isolated and it showed to be genetically similar to SARS-CoV (nucleotide identity about 79,0%) and MERS (nucleotide identity about 51,8%) [26 - 28], therefore, it was denominated SARS-CoV2. The dissemination of the pandemic, called COVID-19, was observed in all continents and it was suspected that the origin of this new coronavirus are bats.

Woo *et al.* [29] pointed out that bats are carriers of a set of genes from alphaCoV and betaCoV. They also suggested that coronavirus from birds show a set of genes from gammacoronavirus, and the ancestral of all these viruses was present in a bat and it was transmitted to a bird, or vice versa. This phenomenon occurs due to the ability of these animals to migrate for long distances, allowing the exchange of the virus genetic material between hosts of different species. This predisposes to the high diversity of coronavirus in bats and birds, as well as the dissemination of this viral agent to other animal species.

Since the emergence of the first report of coronavirus in *Miniopterus pusillus* (bats) [30], the presence of coronavirus in bats population all over the world have been studied, and until now both alphaCoV and betaCoV were detected (Fig. **1**). Within these two genera, new virus species obtained from bats are being determined, showing the importance of those animals as coronavirus hosts [31]. Next, the classification of coronavirus species detected in different bat species is presented in Table **1**.

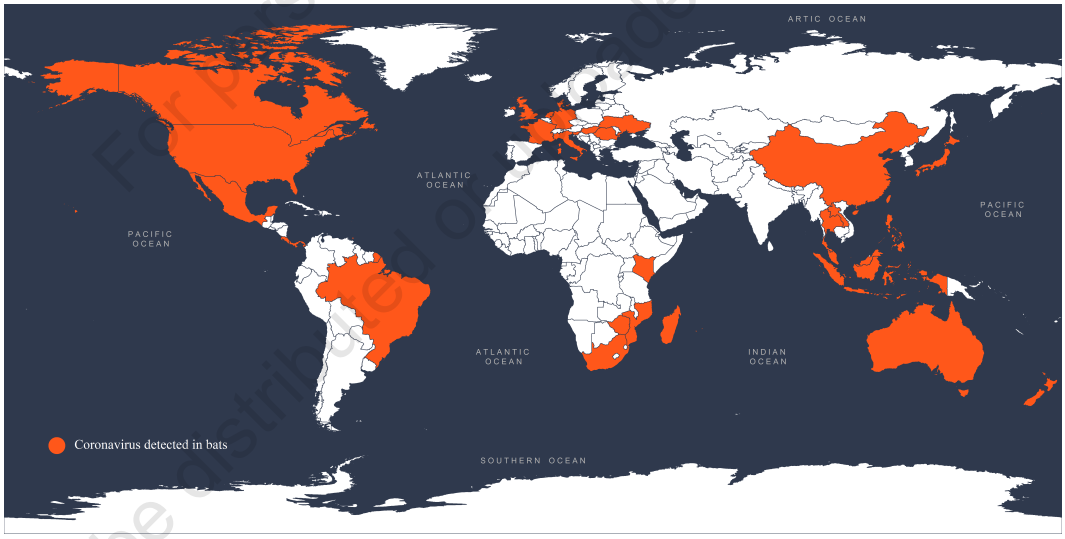

Fig. (1). Distribution of alphaCov and betaCoV cases described in bats worldwide.

In some hosts, a disease may not be caused by intraspecific transmission, however, the transmission can occur from another reservoir species that maintains a relatively high pathogen population. In such a case, the pathogen typically reaches high prevalence in the reservoir and then spills over into the other host; a process called "the spillover effect" or "pathogen spillover" [32].

As spillover from reservoirs occurs, the proportion of new virus emergences seems to be increasing [33], and studies show that some virus families are more prevalent in bats with spillover potential for other species of animals [8, 34].

The proliferation of bat-associated viruses demands a combination of factors, such as an opportunity for contact between reservoirs and hosts, molecular and cellular compatibility between virus and host, and a flexible immune response [35].

Research has shown that SARS-CoV-2, the etiologic agent of Covid-19, is likely to originate from bats of the genus *Rhinolophus* [36], which is similar to the origin of others coronavirus species that also cause human diseases, SARS-CoV-1 and MERS-CoV [37]. Furthermore, SARS-CoV was identified in palm civets and MERS-CoV in dromedary camels (*Camelus dromedarius*), which would act as intermediate hosts before crossing the species barrier to infect humans. In addition, Malaysian pangolins (*Manis javanica*) could act as intermediate hosts for SARS-CoV-2 [38].

The aim of this study was to perform a literature review about the detection of coronaviruses (alphaCoV or betaCoV) in different bat species throughout the world and their immune response against coronavirus.

CORONAVIRUSES IN BATS FROM NORTH AMERICA

In the Americas, the first occurrence of coronavirus in bats was in 2007, in the Rock Mountains region (Colorado-USA) [39]. Seven different bat species were tested, and new alphacoronaviruses were detected in two species (*Myotis occultus* and *Epitesicus fuscus*), different from those known in Asia. In this study, a high detection frequency of the virus was observed, 50% in *M. occultus* and 17% in *E. fuscus*.

In 2010, two studies using metagenomics confirmed the presence of alphaCoV in bats in the USA. Li *et al.* [40] identified alphaCoV in bat guanos collected in a cave inhabited by *Tadarida brasiliensis*, *Myotis velifer*, *Nycticeus humeralis* and *Perimyotis subflavus*. Donaldson *et al.* [41] identified new species of *alphaCoV* in *Epitesicus fuscus*, namely Appalachian Ridge CoV (ARCoV).

From 2007 to 2009, a large surveillance study was conducted in Colorado, where alphaCoV was detected in four different species of bats, *Eptesicus fuscus* (prevalence of 10%), *Myotis volans* (prevalence of 8%), *Myotis lucifugus* (prevalence of 3%) and *Myotis evotis* (2% prevalence). There was a higher prevalence of the virus in young animals and the presence of coronavirus positive colonies in shelters in an urban area close to human residences suggested a risk of potential transmission of the virus [42].

Huynh *et al.* [43] showed by molecular clock analysis that alphaCoV sequences derived from the North American tricolored bat (*Perimyotis subflavus*) are predicted to share common ancestry with human CoV (HCoV)-NL63.

In Florida, alphacoronaviruses were detected in *Tadarida brasiliensis,* similar to those detected in *T. brasiliensis* and *Molossus molossus* in Brazil. This fact suggests that similar coronaviruses may be present in bats of the same species even in very different regions and that viruses can evolve according to the bat species [44].

In Canada, there are few studies on coronavirus in bats. The first report of coronavirus was described by Misra *et al.* [45] in *M. lucifugus*. This alphaCoV showed genetic similarity to the coronavirus found in *M. occultus* in the Rock Mountains region [39].

Subudhi *et al.* [46] detected the presence of coronavirus in 30% of the hibernating *M. lucifugus* and observed that the infection persisted for at least 4 months. In addition, the authors performed immunohistochemistry of the lungs on infected animals, which detected the presence of viral antigen, however, without a consistent inflammatory reaction. A low level of neutrophilic infiltration in the infected lungs reinforces the fact that bats are unique in the way they respond to coronaviruses.

Davy *et al.* [47] demonstrated that the coronavirus elimination by bats can be increased when they are co-infected with Pseudogymnoascus destructans, the fungus that causes White Nose Syndrome (WNS). The systemic effects of WNS can down-regulate the antiviral response in M. lucifugus infected with coronavirus, increasing viral replication and, consequently, viral elimination.

Two works on *Coronavirus* present in bats has been published in Mexico so far, and as a result, both alphaCoV and betaCoV have been detected. The first description was made in the region of Campeche, Chiapas and Mexico City, where 13 different coronaviruses were found, 9 classified as *Alphacoronavirus* and 4 as *Betacoronavirus*, demonstrating a great diversity. Of the 42 species tested, 11 were positive for coronavirus, *Artibeus lituratus, Artibeus phaeotis,*

Artibeus jamaicensis, Carollia sowelli, Carollia perspicillata, Lonchorhina aurita, Pteronotus parnelli, Nyctinomops laticaudatus, Tadarida brasiliensis, Myotis fusifercus and *Epitesicus fuscus*. In the same year, a new betaCoV was detected in a *Pteronotus davyi* in La Huerta, Jalisco State [48].

Analyses of these viruses in the context of their hosts and ecological habitat indicated that host species or genus is a strong selective driver in CoV evolution, even in allopatric populations separated by significant geographical distance, and that a single species/genus of bat can contain multiple CoVs [49].

Fig. (**2**) shows the distribution of cases in North America, considering alphaCoV and betaCoV and Table **1** shows coronaviruses family or genus, continent and country, identification of bats, number of animals, samples from where viruses were isolated, and references.

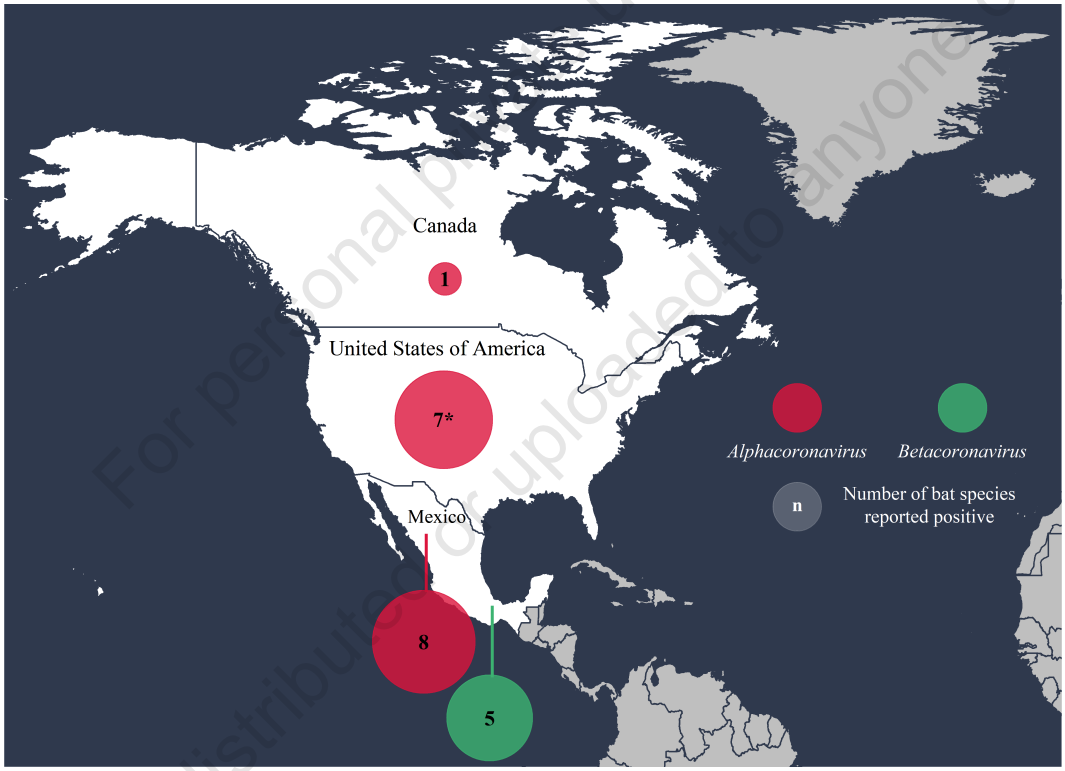

Fig. (2). Distribution of alphaCoV and betaCoV cases described in North American countries and the number of bats described.

CORONAVIRUSES IN BATS FROM CENTRAL AMERICA

Central America can be divided into two parts, one located between North America and South America, known as continental Central America, including Panama, Costa Rica, Nicaragua, Honduras, El Salvador, Guatemala and Belize. The other part comprises the islands of the Caribbean Sea, the Bahamas and the Turks and Caicos Islands. The region is one of the most diverse hotspots, home to approximately 7% of the world's plant and animal species [50]. The bats constitute an important group in the Central American mammals, with nine families, predominantly the *Phyllostomidae* bat family, more than 80 genera and about 200 species [51]. Similar to South America, both alphaCoV and betaCoV occur in the Central American bats (Fig. **3**) and Table **1**.

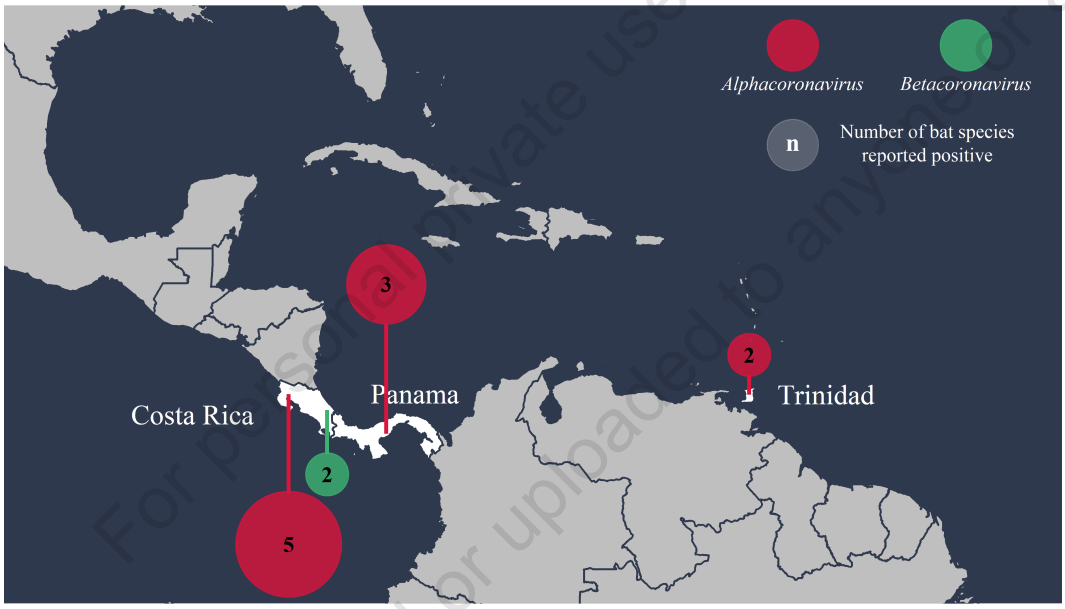

Fig. (3). Distribution of alphaCoV and betaCoV cases described in Central American countries and the number of bats described.

The first coronavirus description in Central American bat species was the alphaCoV in 2008. Two alphacoronaviruses were reported in two species, *Glossophaga soricina* and *Carollia perspicillata,* from Trinidad, an island of the Caribbean archipelago. Even though both coronaviruses clustered in the same clade, relatively a high divergence found was from the adaptations of the virus to different host species [52].

In 2013, extensive research discovered other alphacoronaviruses in Central American countries. Panama presented three different alphaCoVs in three bat species, *Artibeus jamaicensis*, *Artibeus lituratus* and *Phyllostomus discolor*. Two bat species in Costa Rica tested positive for two alphacoronaviruses, *Carollia perspicillata and Anoura geoffroyi.* The same study reported only the beta coronaviruses detection in Costa Rica until now. Two beta coronaviruses were detected in *Pteronotus parnellii* and *Carollia perspicillata* bat species, not found in South America [53]. Finally, the latest study detected four alphacoronaviruses in four bat species, *Artibeus jamaicensis, Carollia perspicillata, Carollia castanea* and *Glossophaga soricina* from Costa Rica. All alphaCoV sequences clustered with preliminary published data from South and Central American countries, except the *G. soricina* sequence. This divergent sequence indicates that the diversity of coronaviruses in particular bat species could be more significant than formerly known [54].

CORONAVIRUSES IN BATS FROM SOUTH AMERICA

South America is the fourth largest continent, with a total area of 17.840.000 km^2. The continent includes the following 13 countries: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Guyana, Paraguay, Peru, Suriname, Uruguay, Venezuela and the overseas territory of French Guinea. Due to the richness of hydrological, geological, geomorphological and climatic variety, South America possesses a notable diversity for the most existing biological groups, including vertebrates [55]. It is estimated that South America has about 30% of existing mammal species [56], among which bats can be highlighted with 9 families, approximately 80 genera and 300 species [51].

Bats are known to be hosts or reservoirs to various viruses, including coronaviruses. Naturally, as a result of the richness and diversity of bat species present in South America, coronaviruses were reported in some countries. Unfortunately, the scarce information regarding bat coronaviruses disabled the knowledge of the real situation in all South American countries. Of the coronaviruses reported, both alphaCoV and betaCoV are present in South American bats [37].

Overall, alphaCoV appears to be more prevalent, with a higher detection rate worldwide compared to betaCoV [37]. Despite the lower detection rate of betacoronaviruses, it was the first genus discovered around 2008 in South America, Brazil. A group of betaCoV was detected in the *Desmodus rotundus* (vampire bat). Until that year, coronavirus had only been described in insectivorous bats. In addition, the authors already supported the hypothesis of

reservoirs for exclusive coronavirus lineages, emphasizing the importance of this bat species in the coronavirus area [57].

However, the prevalence of alphacoronaviruses is considerably higher. So far, in South America, only Brazil reported the occurrence of alphaCoV in 17 different bat species (Fig. (**4**) and Table (**1**)).

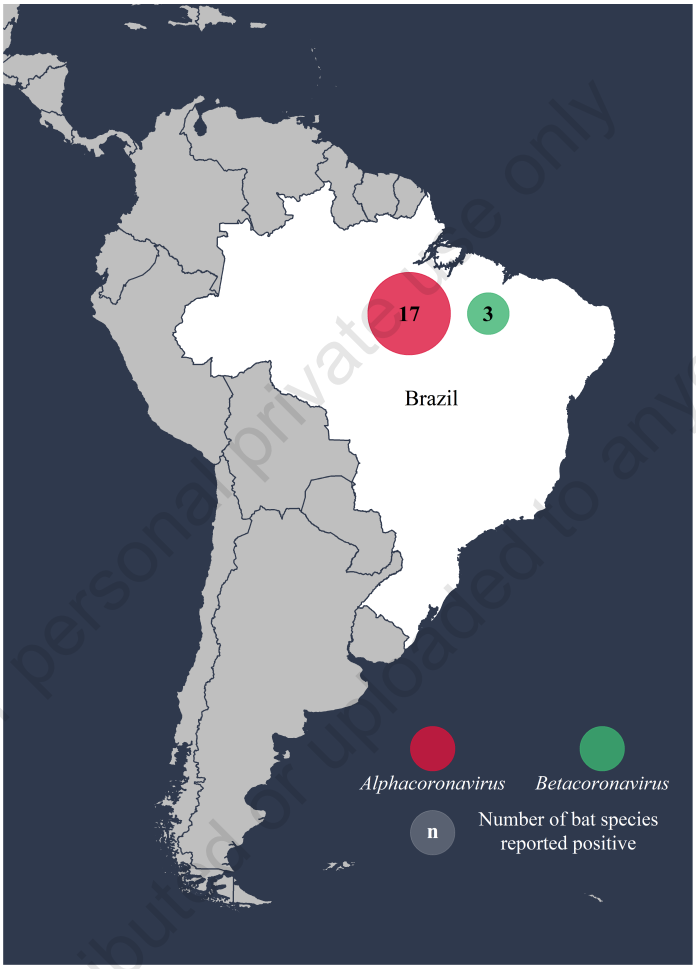

Fig. (4). Distribution of alphaCoV and betaCoV cases described in South American countries and the number of bats described.

One of the first studies completed in Brazil (2013) reported the presence of alphacoronaviruses in two bat species: *Molossus molossus* and *Tadarida brasiliensis*. In this study, the samples also clustered with other alphacoronaviruses were found in distinct bat species located in Asia and North

America, indicating a low level of host restriction for these coronaviruses in bat populations [58]. In the same year, another alphaCoV was described in two bat species from Brazil, *Carollia perspicillata* and *Carollia brevicauda*. Moreover, the authors emphasized the possibility of another alphaCoV clade in the same country, in *Molossus rufus and Molossus correntium* bat species [53]. In the same year, an identical alphaCoV was also detected in *Molossus rufus* and *Molossus molossus* in Brazil [48].

In 2016, additional five bat species were tested positive for alphacoronaviruses in Brazil. *Cynomops abrasus* and *Cynomops planirostris* composed the genus with the higher occurrence of alphaCoV in the research, suggesting a significant role in the alphaCoV maintenance in this area. *Desmodus rotundus,* a hematophagous species exclusive to the Americas, was also reported, for the first time, positive for alphaCoV, demonstrating the importance of the species as a carrier of coronaviruses. Glossophaga soricina and Platyrrhinus lineatus were two other bat species that tested positive for alphaCoV [59]. Another Brazilian research also described 13 alphacoronaviruses in eight bat species from the Atlantic Forest Biome, a region with 9% of the world's bat variety. The positive bat species were *Artibeus lituratus*, *Carollia perspicillata*, *Glossophaga soricina*, *Molossus rufus*, *Myotis nigricans*, *Myotis riparius* and *Sturnira lillium*. The sequences acquired from bats of the same genus also showed strong similarity with other sequences obtained in the previously shown studies from bats of distant locations, suggesting co-evolution of the coronavirus genotypes and specific host genera. The authors also reported additional betacoronaviruses. Two betacoronaviruses were detected in *Artibeus lituratus* and *Eumops glaucinus* species. The betaCoV found in the *E. glaucinus* bat clustered within the lineage C of the genus, where MERS-CoV also belongs. It was the first time that the lineage C detection occurred in South American bats, highlighting possible virus transmission to humans and the role of domestic animal-human dynamics, considering that the positive *Eumops* bat was predated in an urban area by a domestic cat [60].

Finally, the most recent article about alphacoronaviruses was published in 2019, also developed in Brazil. Alphacoronaviruses were detected in six bat species, *Molossus rufus*, *Eptesicus sp*, *Phyllostomus discolor*, *Glossophaga soricina*, *Molossus molossus* and *Artibeus literatus*. This study corroborates the hypothesis of species-specific bat coronaviruses since similar sequences were found in geographically distant regions [61].

CORONAVIRUSES IN BATS FROM EUROPE

Chiroptera corresponds to approximately one-fifth of mammals, consisting of over 1300 species known, of which 44 are present in Europe and 34 are found in

Italy and 35 in France [62, 63]. Bat populations are considered to be reservoirs of emerging viruses, such as SARS (Severe Acute Respiratory Syndrome), MERS (Middle East Respiratory Syndrome), PED (Porcine Epidemic Diarrhea) and SADS (Severe Acute Diarrhea Syndrome) [64], as well as for alpha (alpha CoVs) and beta-coronavirus (beta CoVs) [17]. However, it cannot be said which species host certain CoVs, as well as which strains or genera are circulating in Europe [65].

Bats are host to several CoVs, however, only alpha CoVs and beta CoVs were found in the animals, being detected in Europe, North and South America, Asia, Australasia, and Africa. Alpha CoV has a higher detection rate and is more widespread. Beta CoVs, on the other hand, are commonly found in bats, the subgenera *Nobecovirus*, *Sarbecovirus*, *Merbecovirus* and *Hibecovirus*. It is observed that horseshoe bats are the reservoir for beta CoVs and SARS-CoV [37].

Alpha CoVs were detected in *Pipistrellus sp.* in France, Hungary, Romania, Germany, Spain, Italy, and the Netherlands; in *Myotis sp*. in Hungary, Germany, Spain, the United Kingdom and the Netherlands; in *Rhinolophus sp*. in Bulgaria and Hungary; and in *Nyctalus sp*. in Spain and Bulgaria [66, 67].

In France, although there are 35 species of bats, only four have been studied for Coronaviridae [66]. From 2013 to 2015, intestinal samples were obtained from carcasses collected, 12 of which had coronavirus infection; five sequences of *Pipistrellus pipistrellus* and two of *Pipistrellus sp.* They had *94% to 99% of the nucleic acid sequence with* alpha CoVs from *Pipistrellus* from France and Germany; two *Myotis emarginatus* showed 100% identity with *Myotis emarginatus* alpha CoV sequences in Luxembourg; one *Myotis nattereri* with 95% identity with an unclassified coronavirus of *Myotis nattereri* in Hungary and two *Miniopterus schreibersii* have 98% to 99% identity with the same species found in Bulgaria [67].

In 2010 in Italy, CoV in bats was notified and it was identified that beta CoVs was similar to SARS found in *Rhinolophus* [68], with CoV sequences being detected only in the species *Eptesicus serotinus, Myotis blythii*, fecal samples of *R. hipposideros* and *Hypsugo savii, Nyctalus noctula* and *Pipistrellus kuhlii* [69, 70]. In northeastern Italy and Liguria, from 2013 to 2016, CoVs and PMVs (Paramyxoviruses) were investigated using 19 different species of bats, with 302 individuals. 32 animals were positive using PCR and it was also possible to detect CoV RNA in 36 bats and PMV RNA in three animals. After genetic characterization, 15 alpha CoVs, 5 beta CoVs, and three PMVs were observed, as well as a specimen *P. pipistrellus* co-infected with CoV and PMV [71].

In the island of Sardinia (Italy), in 2019, a survey and molecular characterization of beta CoVs were carried out, using oral, fecal and skin samples from 15 species of bats, with 46 animals. The viral presence was detected in *Rhinolophus ferrumequinum, Plecotus auritus,* and *Tadarida teniotis*. After phylogenetic analysis, it was noted that bat CoVs were similar to the SARS previously reported [72]. Although there are few studies on the CoV ecology of bats in Italy, it has been observed that horseshoe bats have higher detection and viral prevalence, whereas alpha CoVs and beta CoVs have been detected in several species of *Kuhl's pipistrelle, Lesser horseshoe bats, and Serotine bats* [69, 73]; two MERS-related beta CoVs were also detected in *Kuhl's pipistrelle* and *Savi's bat* [70, 74].

In Slovenia, in 2008, a study by Rihtaric *et al.* [75] observed the presence of SARS in 38.8% (14/36), belonging to group 2 of the coronaviruses, *Rhinolophus hipposideros*. In the Netherlands, in 2010, the presence of Group 2 CoV was detected for the first time in Europe in bats *Pipistrellus pipistrellus* and the presence of Group 1 CoV in *Myotis daubentonii*, *M. dasycneme, P. pipistrellus* and *Nyctalus noctula* [76]. In Hungary, from 2012 to 2013, the 1.79% detection rate for CoV was low when compared to European studies [77], but a large number of animals infected with CoV were identified in the United Kingdom, Germany, Holland, Bulgaria and Slovenia [75 - 79].

In Germany, Holland, Romania, and Ukraine and Ghana, Annan *et al.* [80], detected the presence of human beta CoV EMC / 2012 in 24.9% (46/185) of *Nycteris* bats and 14.7% (40/272) of *Pipistrellus* bats,. In Denmark, from 2013 to 2017, Lazov *et al.* [81] collected 271 fecal samples of bats, where 10 were positive for alpha Cov, obtained from *M. daubentonii, P. pygmaeus, M. dasycneme, M. nattereri* and *E. serotinus*.

Fig. (**5**) shows the distribution of cases described in Europe, considering the alphaCoV and betaCoV (Table **1**).

CORONAVIRUSES IN BATS FROM ÁFRICA

Several studies have identified coronavirus infecting bats in countries on the African continent. In Zimbabwe, Bourgarel *et al.* [82] reported the circulation of alphaCoV and betaCoV in bat species of the genus *Hipposideros*. Geldenhuys *et al.* [83] reported the presence of coronavirus belonging to the alphaCoV group in bats of the species *Neoromicia capensis* (*Neo-BtCoV 167/SA/07*), *Miniopterus spp.* (*Miniopterus-BtCoV Irene/SA/09*) and *Mops midas* (*Mops-BtCoV 1364/SA/11*) in South Africa. In Kenya, Tao *et al.* [84] identified coronaviruses related to human coronaviruses NL63 and 229E in bats of the species *Cardioderma cor, Eidolon helvum, Epomophorus labiatus, Hipposideros sp.,*

Miniopterus minor, Otomops martiensseni, Rhinolophus hildebrandtii, Rhinolophus sp. and *Triaenops afer*. Waruhiu *et al.* [85] detected alphaCoV RNA in bats of the genus *Hipposideros*, and through phylogenetic analyzes, it was identified that the CoVs sequences were related to the CoV 229E. In the group of beta coronaviruses, CoVs related to HKU-9 and SARS-L CoVs were detected. In addition, Razanajatovo *et al.* [86] reported betaCoV of subgroup D in two species of fruit bats (*Eidolon dupreanum* and *Pteropus rufus*) in Madagascar.

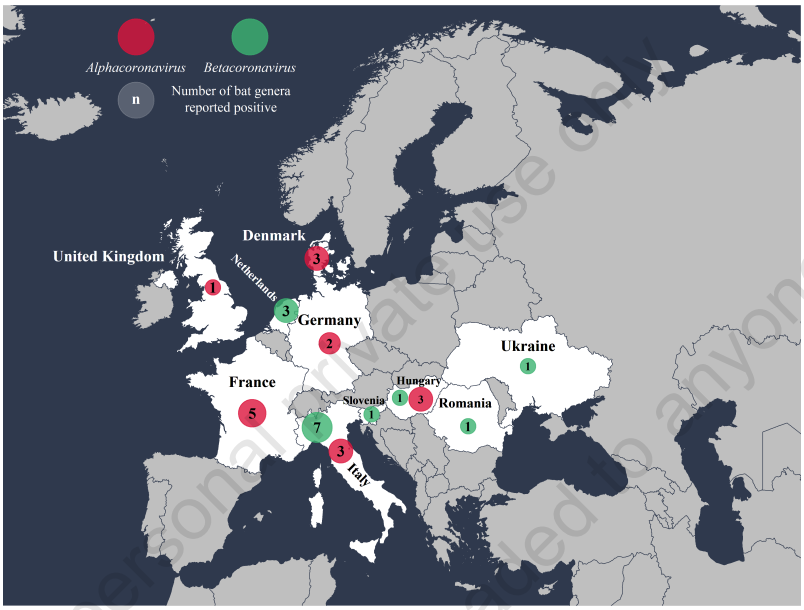

Fig. (5). Distribution of alphaCoV and betaCoV cases described in European countries and the number of bats described.

Joffrin *et al.* [87] found bats positive for CoVs in Mozambique, Mayotte, Reunion Island, Seychelles, Mauritius and Madagascar, with a higher prevalence in animals from the *Nycteridae* and *Rhinolophidae* families. The results of the prevalence of CoVs in bats presented by the authors corroborate the results of studies carried out in continental Africa and the islands of the Australasian region [88, 89]. Phylogenetic analysis showed that 25 sequences were classified as alphaCoV and three as betaCoV. Several of the coronaviruses identified in bats from the *Rhinonycteridae* and *Hipposideridae* families in Mozambique had high nucleotide sequence similarity with human CoVs NL63 and human CoVs 229E.

Bat species of the genus *Neoromicia* are widely distributed in Africa. *N. capensis* has been identified as a potential host for coronaviruses related to Middle East respiratory syndrome (MERS). In South Africa, coronavirus of the genera alphaCoV and betaCoV have been isolated from *N. capensis*. A betaCoV of strain

C, called NeoCoV/PML-PHE1 and grouped taxonomically within the same species as MERS-CoV, was detected in this same species of bat [83, 88, 90]. Likewise, Geldenhuys *et al.* [91] detected, by genetic sequencing, viruses belonging to the families *Coronaviridae, Adenoviridae, Herpesviridae, Papillomaviridae, Parvoviridae, Phenuiviridae* and *Picornaviridae* in bats of the genus *Neoromy*, from South Africa, in addition to identifying a new species of virus belonging to the family *Circoviridae*.

Fig. (**6**) and Table **1** show the distribution of the reported cases in Africa, considering the alphaCoV and betaCoV.

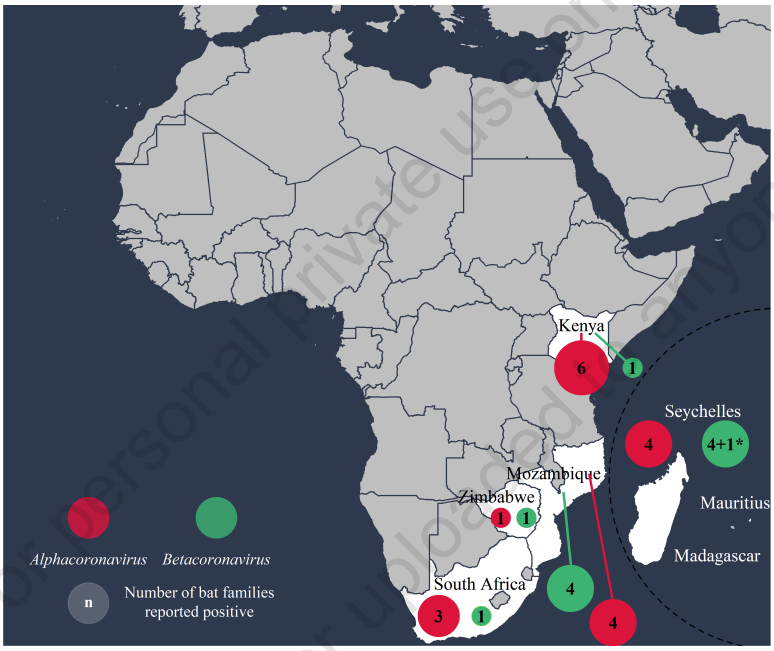

Fig. (6). Distribution of alphaCoV and betaCoV cases described in African countries and the number of families of bats described.

CORONAVIRUSES IN BATS FROM ASIA

Geographically, Asia is divided into five different parts (East Asia, Central Asia, Middle East, South Asia and Southeast Asia) that present 51 countries, China, India, Indonesia, Pakistan, Bangladesh, Japan, Philippines, Vietnam, Turkey, Iran, Thailand, Myanmar, South Korea, Iraq, Afghanistan, Saudi Arabia, Uzbekistan, Malaysia, Yemen, Nepal, North Korea, Taiwan, Sri Lanka, Kazakhstan, Syria, Cambodia, Jordan, Azerbaijan, United Arab Emirates, Tajikistan, Israel, Hong Kong, Laos, Lebanon, Kyrgyzstan, Turkmenistan, Singapore, State of Palestine, Oman, Kuwait, Georgia, Mongolia, Armenia, Qatar, Bahrain, Timor-Leste, Cyprus, Bhutan, Macao, Maldives and Brunei [92].

In Asia, there are different families of bats, such as *Rhinopomatidae*, *Emballonuridae*, *Megadermatidae*, *Rhinolophidae*, *Hipposideridae*, *Molossidae*, *Vespertilionidae*, *Miniopteridae*, *Craseonycteridae*, *Nycteridae* and *Pteropodidae* [93, 94].

Eight different species of alphaCoV and betaCoV were identified in bats in China, Miniopterus bat coronavirus 1 (BtCoV 1), Miniopterus bat coronavirus HKU8 (BtCoV HKU8), Rhinolophus bat coronavirus HKU2 (BtCoV HKU2), Scotophilus bat coronavirus 512 (BtCoV 512), SARS-related or SARS-like CoVs (SL-CoV; WIV1, Rp3, HKU3, *etc*.), Pipistrellus bat coronavirus HKU5 (BtCoV HKU5), Rousettus bat coronavirus HKU9 (BtCoV HKU9), and Tylonycteris bat coronavirus HKU4 (BtCoV HKU4) [95]. In general, alphaCoVs seem to be more widespread than betaCoVs, and their detection rate is also higher [37].

The diversity present in Merbecovirus (MERS-CoV) may be related to the high variety of bats in the family *Vespertilionidae*. MERS-CoV is able to infect different genus of bats belonging to the *Vespertiliionidae* family, giving the idea that this virus is highly widespread geographically [37].

Phylogenetically, MERS-CoV belongs to the betaCoV lineage C, being closely related to Tylonycteris bat CoV HKU4 (BtTyCoV-HKU4) and Pipistrellus bat CoV HKU5 (BtPiCoV-HKU5), previously discovered in lesser bamboo bat (*Tylonycteris pachypus*) and Japanese pipistrelle (*Pipistrellus abramus*), respectively, in Hong Kong, China [96]. In addition, several MERS-related CoVs (MERSr-CoVs) have been described in China from various *Vespertilionidae* bat species, including pipistrelle bats (*P. abramus* and *Pipistrellus pipistrellus*), Chinese pipistrelle (*Hypsugo pulveratus*), great evening bats (*Ia io*), and particolored bats (*Vespertilio superans*) [97 - 99].

BetaCoV has been predominantly found in *Hipposideridae*, *Rhinolophidae*, *Pteropodidae*, *Craseonycteridae*, *Megadermatidae* and *Rhinopomatidae* [89].

Studies show that *Rhinolophidae* family, *Rhinolophus* genus, species, *R. sinicus*, *R. ferrumequinum*, *R. macrotis*, *R. pearsoni*, and *R. pusillus* are the most frequent SARSr-CoV carriers in China [100]; Rhinolophus bat species discovered in mainland China and Hong Kong, host SARS-related coronaviruses (SARSr-CoV) variants (betacoronaviruses of lineage b), Chinese rufous horseshoe bat (*R. sinicus*), Pearson's horseshoe bat (*R. pearsonii*), greater horseshoe bat (*R. ferrumequinum*), big-eared horseshoe bat (*R. macrotis*), and least horseshoe bat (*R. pusillus*) [23, 101 - 103].

Zhou *et al.* [104] recently reported a closely related bat coronavirus detected in Intermediate horseshoe bat (*Rhinolophus affinis*) from Yunnan province, China,

with high sequence identity to SARS-CoV-2 in a short region of RNA-dependent RNA polymerase (RdRp) gene. Rhinolophus bats are natural reservoirs of SARS-CoV-2-related viruses and may likely be sources of the first SARS-CoV-2 infections in humans.

The transmission of alphaCoV BatCoV HKU10 between *Rousettus leschenaulti*, family *Rhinolophidae*, and *Hipposideros pomona*, the family *Hipposideridae*, was described in Hong Kong. *Rousettus leschenaulti* is very widespread throughout countryside areas and roost in colonies with up to several hundred individuals. Biological features, such as the ability to long flying distances, may explain the fact of Leschenault's rousettes to acquire various viruses and transmit them to other bat species [105].

AlphaCoV and betaCoV, which are related to coronavirus found in Africa and Europe, were detected in two bat species in Southeast Asia. Those bats belong to the *Hipposideridae* family, *Hipposideros armiger* (alphaCoV) and *Hipposideros larvatus* (betaCoV) [106].

Coronavirus may undergo recombination of genetic material with viruses from other families, such as Nobecovirus, Marburg virus, Hendra virus and Nipah viruses [37]. Furthermore, it is hard to describe the CoV diversity in bats due to both the number of species and geographical distribution [100].

Fig. (**7**) shows the distribution of cases described in Asia, considering the alphaCoV and betaCoV and Fig. (**8**) shows cases described in Asia countries, considering the family coronaviridae (Table **1**).

CORONAVIRUSES IN BATS FROM OCEANIA

Despite SARS outbreaks in 2003 and MERS outbreaks in 2012, and the emerging concern about bat-transmitted zoonoses, until 2016, there were no reports of studies regarding the occurrence of coronavirus in bats in the Australasian region. Smith *et al.* [89] carried out a study with insectivorous bats from this region (Australia, Papua New Guinea, East Timor, Indonesia, Malaysia and Taiwan) belonging to eight families and 22 genera (*Hipposideros, Rhinonicteris, Macroderma, Acerodon, Cynopterus, Dobsonia, Eonycteris, Macroglossus, Pteropus, Rousettus, Rhinolophus, Saccolaimus, Taphozous, Miniopterus, Chaerephon, Mormopterus, Chalinolobus, Myotis, Nyctophilus, Scotophilus, Scotorepens* and *Vespadelus*) [89]. Coronaviruses were detected in samples of bats from seven species and antibodies anticoronavirus were detected in 23 species. Phylogenetic analyses identified four different coronavirus genotypes, two of them grouped in the genus Alphacoronavirus and two in the genus

Betacoronavirus. Notably, three of these four genotypes shared less than 90% of the nucleotide sequence identity with the most well-known coronaviruses. In terms of host species and geographic location, the most widespread genotype was an alphacoronavirus identified in *Miniopterus australis, M. schreibersii* and *Rhinolophus megaphyllus*, in Queensland - Australia. A second and new alphacoronavirus genotype was identified in *Myotis macropus* and *Vespadelus pumilus* (Queensland - Australia). As for the two new betacoronaviruses identified, one was detected in *Rhinonicteris aurantia* (Northern Territory - Australia) and the other in *Pteropus alecto* (Queensland - Australia). Although rhinolophid bats are considered putative natural hosts for the SARS coronavirus, SARS-like betacoronavirus was not found in this study [89].

Prada *et al.* [107] conducted a study with eleven insectivorous bat species from the families *Vespertilionidae* and *Molossidae* in southwestern Western Australia. Five alphacoronavirus sequences were identified in the following bat species, *Chalinolobus gouldii, Vespadelus regulus, Chalinolobus morio, Vespadelus baverstocki, Falsistrellus mackenziei* and *Ozimops sp*. The authors reported that the viral load (prevalence and richness) is not homogeneous between species and that multiple viral strains circulate simultaneously in specific populations of bats. To date, host-pathogen associations, potential risks to humans, or the implications of conserving virus diversity in Australian bats remain relatively unknown.

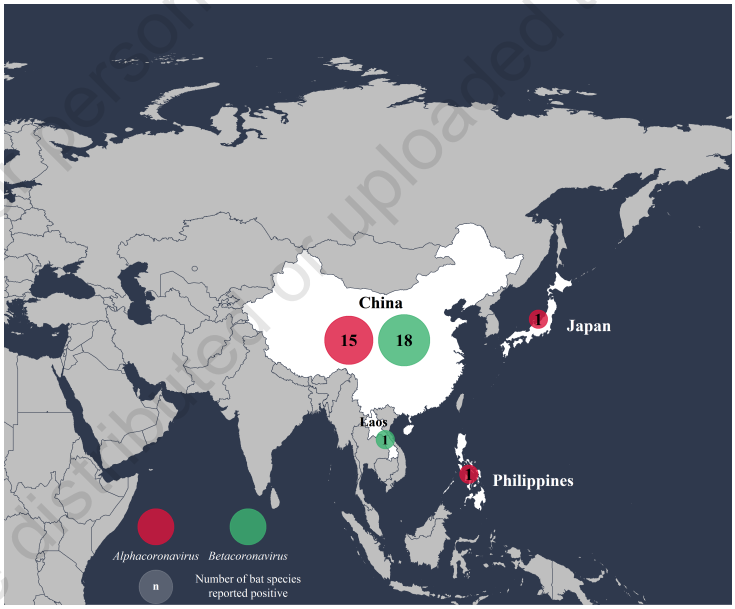

Fig. (7). Distribution of alphaCoV and betaCoV cases described in Asia countries and the number of bats described.

Fig. (8). Distribution of coronaviridae cases described in Asia countries and the number of genera bats described.

Likewise, in a study performed by Hall *et al.* [108], investigating the presence of different viruses, coronavirus was found in guano from bats of the species *Mystacina tuberculata* in a pristine indigenous forest on a remote offshore island named Codfish Island, New Zealand. The virus was classified as a novel alphacoronavirus.

Fig. (**9**) indicates the distribution of the reported cases in Oceania, considering the alphaCoV and betaCoV (Table **1**).

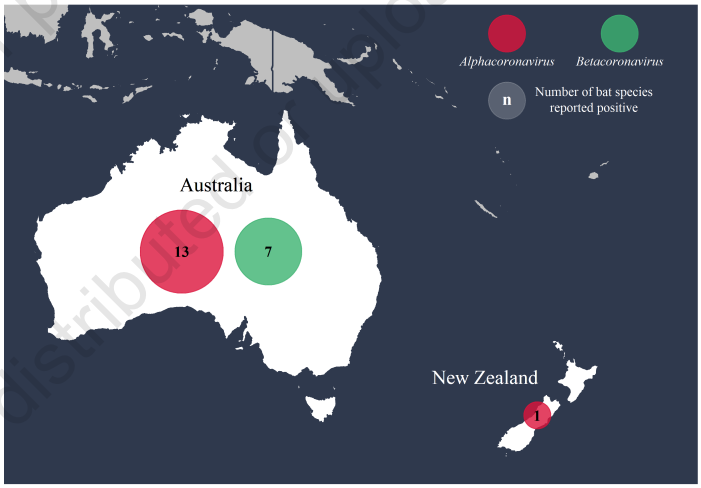

Fig. (9). Distribution of alphaCoV and betaCoV cases described in Oceania countries and the number of bats described.

BAT IMMUNE RESPONSE TO CORONAVIRUSES

A wide variety of pathogenic viruses for humans can be housed in bats since they limit the development of the disease after the infection. Studies to define these mechanisms and the immune response to viral infection are difficult to perform, as reagents with limited access and appropriate *in vitro* and *in vivo* techniques are needed. It is noteworthy that studies related to coronavirus infection in bats are scarce, with a focus on henipavirus and phylovirus [64].

Table 1. Genus, subgenus and species of coronaviruses found in different bat species, with Genbank accession number and reference.

Genus	Subgenus	CoV Species	Bat Species	Genbank Accession Number	Country	References
AlphaCoV	*Colacovirus*	*Bat coronavirus CDPHE15*	*Myotis lucifugus*	NC_022103.1	USA	[143] ICTV, 2021
	Decacovirus	*Bat coronavirus HKU10*	*Rousettus leschenaulti*	NC_018871.1	China	[105] Lau *et al*, 2012
			Hipposideros pomona	JQ989273.1	China	[105] Lau *et al*, 2012
	Decacovirus	*Rhinolophus ferrumequinum alphacoronavirus HuB-2013*	*Rhinolophus ferrumequinum*	NC_028814.1	China	[135] Wu *et al*., 2016[a]
	Minunacovirus	*Miniopterus bat coronavirus 1*	*Miniopterus spp*	NC_010437.1	Hong Kong	[136] Chu *et al*., 2008
		Miniopterus bat coronavirus HKU8	*Miniopterus spp*	NC_010438.1	Hong Kong	[136] Chu *et al*., 2008
	Myotacovirus	*Myotis ricketti alphacoronavirus Sax-2011*	*Myotis ricketti*	NC_028811.1	China	[135] Wu *et al*, 2016[a]

(Table 1) cont.....

Genus	Subgenus	CoV Species	Bat Species	Genbank Accession Number	Country	References
	Nyctacovirus	*Nyctalus velutinus alphacoronavirus SC-2013*	*Nyctalus velutinus*	NC_028833.1	China	[135] Wu *et al*., 2016[a]
	Nyctacovirus	*Pipistrellus kuhlii coronavirus 3398*	*Pipistrellus kuhlii*	NC_046964.1	Italy	[144] De Sabato *et al*., 2018
	Pedacovirus	*Scotophilus bat coronavirus 512*	*Scotophilus kuhlii*	NC_009657.1	China	[138] Tang *et al*. 2006
	Rhinacovirus	*Rhinolophus bat coronavirus HKU2*	*Rhinolophus sinicus*	NC_009988.1	China	[137] Lau *et al*., 2007.
	Setracovirus	*NL63-related bat coronavirus strain BtKYNL63-9b*	*Triaenops afer*	NC_048216.1	Kenya	[84] Tao *et al*., 2017
BetaCoV	*Hibecovirus*	*Bat Hp-betacoronavirus Zhejiang2013*	*Hipposideros pratti*	NC_025217.1	China	[145] Wu *et al*. 2016b
	Merbecovirus	*Middle East respiratory syndrome-related coronavirus*	*Hypsugo savii*	MG596802.1	Italy	[74] Moreno *et al*, 2017
			Pipistrellus kuhlii	MG596803.1	Italy	[74] Moreno *et al*, 2017
	Merbecovirus	*Pipistrellus bat coronavirus HKU5*	*Pipistrellus abramus*	NC_009020.1	China	[146] Lau *et al*., 2013
	Merbecovirus	*Tylonycteris bat coronavirus HKU4*	*Tylonycteris pachypus*	NC_009019.1	China	[146] Lau *et al*., 2013
	Nobecovirus	*Eidolon bat coronavirus C704*	*Eidolon helvum*	NC_048212.1	Cameroon	[147] Yinda *et al*., 2018
		Rousettus bat coronavirus GCCDC1	*Rousettus leschenaulti*	NC_030886.1	China	[148] Huang *et al*., 2016
		Rousettus bat coronavirus HKU9	*Rousettus lechenaulti*	NC_009021.1	China	[149] Woo *et al*., 2007
	Sarbecovirus	*Severe acute respiratory syndrome-related coronavirus*	*Rhinolophus pearsoni*	DQ071615.1	China	[23] Li *et al*, 2005
			Rhinolophus sinicus	FJ588686.1	China	[150] Yuan *et al*. 2010

In the *in vitro* research model, using cell culture, studies have shown that bat cells showed unique adaptations for antiviral responses. The species studied were *Artibeus jamaicensis* (Jamaican frugivorous bat), *Pteropusalecto* (flying black fox) and *Rousettusaegyptiacus* (*Egyptian rousette*) [109]. The sequence of approximately 500 genes analyzed identified proteins linked to the immune response, such as protein 5 associated with melanoma differentiation (MDA 5), the retinoic acid-inducible gene I (RIG -I) and the toll-like receptor (TLRs) 1 -10, together with canonical pattern recognition receivers. T cell receptors (TCRs), cytokines and chemokines, genes related to interferon and genes from different subsets of immune cells have also been observed. However, there was an absence of genes that encode natural killer cell receptors (NK) [109 - 114].

The interferon-mediated immune response (IFN) showed that baseline expression of type I IFNs can occur in a specific manner according to the species, but the molecular mechanisms that generate the differential expression pattern are not known [110, 115 - 118]. When compared to the immune response in human cells, bats have positive regulation of basal expression of interferon-alpha/beta receptors, IFNAR1 and IFNAR 2, and varied genes that are stimulated by interferons [119 - 122].

Studies carried out using the Sendai virus, the avian Paramyxovirus serotype virus type 1 (APMV-1) or by transfection of a synthetic double-stranded viral RNA substitute have shown that cells respond to viral RNA and generate an immune response, generating IFN response [110, 115 - 118].

Viruses are still capable of encoding proteins that weaken the innate antiviral response. Although they are dispensable at the time of viral replication, it plays a fundamental role in viral adaptation in the host's cellular environment and its pathogenesis. A study carried out with cell lines of the bat *R. aegyptiacus and* with the Marburg virus (MARV) indicated that these proteins inhibit the antiviral response, dependent on the viral protein VP35. Other studies, carried out with PEDV, SARS- and MERS-CoVs identified the inhibitory proteins, being able to inhibit the IFN response in mammalian cells; however, to date, there are no studies in bats that observe the role of accessory proteins and their role in the antiviral response [123 - 127].

Studies with an *in vivo* model occur due to the need to understand the infection in live bats, however, they are scarce since they require adequate facilities and reagents, specialized professionals, and selection of suitable species for CoVs.

The study by Watanabe *et al.* [128] isolated CoV in 57.1% of insectivorous bats and 55.6% of frugivores, but they were unable to grow the virus *in vitro*. To isolate the Cov detected in *Cynopterus brachyotis*, intestinal samples were

administered orally to the bats *Rousettus leschenaulti*. After 2 to 5 days of infection, the virus was detected using quantitative real-time PCR (qPCR), and the animals did not show any clinical signs of the disease. The authors noted that although the Cov replicated in *Rousettus leschenaulti*, they were unable to isolate the virus, stressing the importance of choosing the appropriate species.

Munster *et al.* [129], aiming to assess whether bats could be infected with MERS-CoV/EMC 2012 and their possible immune response, used 10 *Artibeus jamaicensis* in their study. It was observed that after nine days, there was the presence of the innate immune response, with no signs of inflammation and viral elimination from the intestinal and respiratory tract, although the animals did not show any clinical signs of the disease. It can be concluded that *Artibeus jamaicensis* may be ancestral hosts of MERS-CoV since they support viral replication [130 - 133].

To study species-specific tropism, Widagdo *et al.* [134] analyzed the tissue receptor distribution of dipeptidyl peptidase 4 (DPP4) in several bat species for the MERS-CoV virus. It was observed that in insectivorous bats, DPP4 is detected mainly in the gastrointestinal tract and in the kidneys, while in fruit bats, they are detected in the respiratory tract and the gastrointestinal tract. The study suggests that the course of the disease and its severity is linked to the tropism of tissues in bats, which occurs in different locations, depending on the species.

The immune response of bats to coronavirus infection requires further studies to assess the virus-host interaction, and it is necessary to better understand how animals serve as reservoirs for CoVs and how to control infections from serious pathologies [64].

CONCLUSION

Bats represent one of the most ancient groups with remarkable peculiarities and belong to the second bigger order within the mammals. Those animals exist almost in all terrestrial environments, thus they are found practically in all continents except cold regions or extremely high altitudes.

Due to their biological and ecological aspects, bats are important for virus maintenance and spread with zoonotic potential in the environment.

The coronavirus, alphaCoV and betaCoV, were detected in many bat species, but the first has a higher occurrence. The virus detection in bat populations all over the world shows these animals' importance as hosts, reservoirs or virus sources, including emerging viruses.

Bats' resistance to viruses and their immune system has been studied in the last years due to their survival capacity despite being host to diverse viruses.

Many studies, as shown in the review, contribute to the vigilant actions of trying to detect and prevent diseases with zoonotic and pandemic potential.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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CHAPTER 2

Hospital Challenges During the COVID-19 Pandemic

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Abstract: COVID-19, was detected in Israel in February 2020. The local government made decisions regarding the different aspects of crisis management, including hospitalization capacity, and each hospital was required to arrange its facilities accordingly to deal with the new threat and its regional impact. While hospitals in Israel are prepared for emergencies, especially trauma events, they still have to adapt according to the newly emerging threats. Ziv Medical Center (ZMC), located in Northern Israel, has long-standing experience with mass-casualty events, especially war situations. This gained experience had to be adapted to the pandemic needs. In turn, new management processes were developed to support the routine work of the hospital and to address the new needs of COVID-19 patients. This chapter presents the three major challenges posed by the new situation, *i.e.*, protecting staff and patients from infection, tending to the medical needs of COVID-19 patients, and preserving routine hospital activities. The technical (new facilities) and managerial (decision-making process) adaptations required to manage the crisis, are detailed. Lessons learned include the need to define and optimally manage the "3 **S**": **S**taff, **S**pace, and **S**upply, to overcome the challenges of the COVID-19 pandemic, with the staff being the most critical resource for success.

Keywords: Administration, Board meetings, COVID-19, Emergencies, Hospital management, Manpower, Morale, New demands, Pandemic management, Specialty, Vaccination.

INTRODUCTION TO CORONAVIRUS DISEASE (COVID-19)

COVID-19 is an infectious viral disease caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2) first observed in China in December 2019 and whose structure and complete genomic sequence were characterized within

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several weeks of the initial case descriptions [1 - 3]. The virus is transmitted from infected individuals *via* saliva droplets, and upper respiratory system secretions [3]. Asymptomatic transmission can occur *via* individuals who are either in the incubation period before the onset of symptoms, or who do not develop significant symptoms. This has been a major contributing factor to its rapid and widespread spread and regional outbreaks [4].

Children infected with the virus may suffer from mild to moderate flu-like symptoms [5] and only rarely require medical intervention [6], although sporadic cases of a Kawasaki-like vascular illness have recently been reported among SARS-CoV-2-positive children [7]. Unlike some flu strains, young children seem to be at particularly low risk for the common adult respiratory complications associated with COVID-19 [8] and thus less frequently require hospitalization. Individuals aged >60 years, and those with underlying chronic medical conditions, are more likely to develop serious COVID-19-related complications. Such patients may require mechanical ventilation and suffer cardiorespiratory collapse, often attributed to an unbalanced immune response, or microcirculatory perturbation related to the renin-angiotensin-aldosterone axis or other mechanisms, possibly augmented by concomitant medical treatment and comorbidities [9 - 11].

The first COVID-19 patients in Israel contracted the virus abroad and were first identified at the beginning of 2020. The government initially attempted to contain the virus by reducing case importation, first by canceling flights from regions of the world with high reported infection rates, prohibiting entry of tourists from these countries, and isolating returning Israeli residents in their homes for 14 days following their return. The same protocol of isolation was mandatory for anyone who had been in contact with infected individuals.

Initially, all patients with COVID-19, regardless of its severity, were mandated to be hospitalized at a level of isolation sufficient to prevent transmission. However, as the number of cases rose, the guidelines were updated to allow infected individuals with mild or no symptoms to self-isolate at home. Standard hospital treatment primarily focused on providing the most severely affected patients with the highest locally available standards of cardiopulmonary, renal, and blood coagulation balance support until sufficient inherent recovery or death.

NEW CHALLENGES POSED BY THE COVID-19 PANDEMIC

During emergency situations, hospitals aim to maintain routine hospital activities while simultaneously addressing the new demands and taking the measures necessary to protect staff and the patients from the presented threat. In war situations, for example, sheltered hospitalization departments are needed to

protect the wounded and the staff, as was the case at our center during the Second Lebanon War. In an ongoing emergency situation, patients and the staff must be protected against infection. The challenges posed by the COVID-19 pandemic are detailed below.

Providing Medical Services During COVID-19 Pandemic

The Israeli Ministry of Health (MOH) directed preparatory measures to be implemented in all public medical centers. At the first stage, most of the preparatory efforts were focused on the emergency department (ED) and the infection prevention and control unit (IPC) and, later on, involved arrangements for new hospitalization wards for COVID-19 patients.

In order to rise to these new challenges, the hospital must address the three "s" components: staff, space, and supplies [12]. The preparatory activities conducted at our hospital to adequately address these three elements included:

New Facilities for the New Medical Needs

While demand for some non-urgent services inevitably drops during emergency situations, many others, *e.g.*, labor and delivery, dialysis and other acute services, must be continuously available. In order to ensure medical service continuity, alongside the developing needs, the hospital made the following arrangements:

Protocols for Handling COVID-19 Case

To provide medical services to the suspected and confirmed COVID-19 patients, a new and separated emergency department (ED) dedicated to COVID-19 patients, was established at our center. New protocols were written to manage suspected and confirmed cases identified in the different departments at the hospital and those admitted from prehospital ambulance services. Pathways for direct registration of confirmed cases for hospitalization and for handling suspected cases first requiring evaluation and diagnostic confirmation at the ED were clearly defined. When the pandemic first began, staff was assigned, and part of the ED space was dedicated to this new challenge. Later, in anticipation of the oncoming flu season, we allocated a new and a separate ED for respiratory diseases to enable the "regular" ED to continue functioning at full capacity.

Establishment of COVID-19 Inpatient Departments

Some COVID-19 patients require hospitalization, including intensive care setup. To address these new demands, the hospital had to arrange for dedicated staff,

space, and other infrastructure and equipment. The new departments were equipped to treat patients while also preventing infection of other patients and staff. Our reinforced 150-bed area was available to house the new, isolated hospitalization departments for COVID-19. When such spaces are unavailable, the hospital administration has to consider new temporary alternatives, such as tents or mobile buildings. In urban areas, with limited available space, park areas or converted departments could serve the new facilities. For example, the surgery departments could be jointly dedicated for this purpose.

Establishing a New COVID-19 Laboratory

Diagnosis and follow-up of COVID-19 cases require dedicated lab infrastructure, equipment, and staff. In Israel, the MOH supplied the necessary equipment and reagents and the staff underwent relevant training for the new mission.

Other Needs of Confirmed and Suspected COVID-19 Patients

In addition to hospitalization departments, other medical services have to be duplicated and adapted for COVID-19 patients. For example, at Ziv Medical Center (ZMC), delivery room and dialysis services were duplicated in the wards dedicated to COVID-19 patients. For services that could not be replicated in the COVID-19 ward, new protocols were established for the delivery of such services to COVID-19 patients while minimizing the risk to others. For example, in our center, the protocol for performing CT scans on COVID-19 patients requires evacuation of the pathway between the COVID-19 department, the respiratory ED, and the CT scan area. All these considerations aim to provide the services needed by the COVID-19 patients while protecting other patients and the staff from potential infection.

New Outpatient Clinics

COVID-19 patients suffer from consequences of the disease and their related treatments, especially intubations [13], impaired pulmonary functioning [14] and other physical and mental effects [15]. Our understanding of COVID-19 and its detrimental effects on the different body systems is growing with time, especially the long-term outcomes [13].

ZMC decided to open an outpatient clinic for COVID-19 patients who suffering from different outcomes of the disease and need medical services. As a commu-

nity hospital, we believe that it is our responsibility to continue monitoring these patients and assist them in coping with the different aspects of the disease.

Regular Services for Non-COVID-19 Patients

Hospitals, especially regional ones, have to fulfill their mission and their commitment toward their community or to assist in finding alternative solutions for local patients. In addition to providing the routine and urgent medical needs, the hospital must protect their non-COVID-19 patients and staff from getting infected on the hospital premises.

Several factors directly related to the pandemic, impaired the hospital's ability to continue providing these services.

a. The main bottleneck was manpower. In order to meet the new demands relating to COVID-19 patients, part of the staff was designated for COVID-19 needs. For example, in order to open new departments, some services had to be reduced due to movement of physicians and nurses to the new services. Highly specialized staff, such as intensive care unit (ICU) staff, is needed in the new ICU for COVID-19 patients. One way to enable reassignment of ICU staff to corona wards was by reducing the number of operations, especially those that require intensive care attention post-surgery.
b. The hospital's capacity to provide services is significantly reduced in cases of staff with COVID-19 symptoms or in quarantine following exposure to COVID-19 patients.
c. Departments closed or down-sized in order to allocate space for COVID-19 patients, do not function at full capacity.
d. Patients refer to hospital services less frequently during the pandemic, for various reasons, *e.g.*, concern of contracting the virus at the hospital or less frequent outings during lockdown.

Hospital Manpower Challenges During COVID-19 Pandemic

The main challenge in any emergency situation of a scale similar to that of the COVID-19 pandemic, is the limited staff. In hospitals, there is no reserve staff that can be recruited in an emergency situation. To provide the new demands, the administration must set new priorities and decide which services will be reduced or closed and which must be provided, *e.g.*, life-saving services in acute medical situations. Shifts can be extended, within the guidelines of labor laws and after negotiations with worker unions. When possible, new staff can be hired.

The following institutional and national measures were taken:

1. ***"Specialized" Staff Designation*:** To deal with COVID-19 patients, staff who regularly provide critical respiratory system care, were designated as "Specialized". This staff included the personnel in the ICU, internal medicine departments, ED, and the anesthesia department. Qualification of such specialized staff requires years and no hospitals have depots of specialized physician or nurses that can be recruited during emergency situation. It this "short blanket" situation, such valuable resources are stretched and pulled from one side to another and priorities are reshaped by the hospital administration, as needed.

2. ***New Employee Recruitment*:** Planning for a potential large-scale outbreak highlighted the degree to which hospitals, especially peripheral ones in Northern Israel, are critically understaffed. While the new staff is generally less skilled and specialized for the newly evolving needs of the hospital, they can contribute in different areas.

3. ***Medical Students*:** During the pandemic, schools and universities in Israel were shut down as part of social distancing measures. On March 13, 2020, the Forum of Deans of Israel's six faculties of medicine, decided to halt clinical rotations; the decision was implemented until May 10, 2020. This decision aligned with the subsequent position paper of the American Association of Medical Colleges (AAMC) [16]. At the same time, medical students at the clinical stage of their studies were encouraged to engage in voluntary activity in the community, *e.g.*, as physician assistants in non-COVID-19 clinical departments, and in COVID-19 testing laboratories. A high engagement rate of approximately 60% was recorded nationwide. Later, students took part in COVID-19 department activities as well. Medical and nursing students were assigned to different departments, such as ED, lab and others.

4. ***Healthcare Staff Training and Working Model*:** As mentioned above, highly professional systems, such as a hospital, are challenged by both the number of available staff members, and by their level of qualification. For example, pediatric physician support is less urgent for new trauma needs presented in the operation theatre at war time and urology expertise is less advantageous in the COVID-19 ICU setting. Thus, precise mapping was necessary to enable judicious reallocation of human resources in accordance with the different needs. To improve staff capabilities, all medical and nursing staff were required to undergo a 4-hour course to learn the basics of treating severe COVID-19 patients. A recommended course syllabus was provided by the MOH and was delivered by ICU staff to small groups of relevant hospital personnel. The syllabus included medical scenarios and the staff has to decide about the different steps of evaluation and

treatment. One scenario was about using the ventilator with patient after intubation aiming to teach and to guide about the using of ventilators, different types, dealing with problems and alerts. The course had been held using simulation devices and real machines such like ventilators and infusion pumps, to make the staff familiar with these devices and to care of ventilated patient. Another scenario dealt with COVID-19 patients at the internal ward that got in worse situation and need mechanical ventilation. The aim of this scenario is to guide the staff evaluate the situation and provide the needed treatments. This approach proved highly effective in increasing staff confidence and ability to address the new demands [9, 17].

The concept of 3 S (Staff, Space and Supply) is related to all potential emergency situations the hospital can face (not only pandemic). In the management of all these situations the most important compound is the staff, especially when your ability to recruit experienced staff in short time is limited, especially in the peripheral regions. The proposed solution is to manage the staff in the routine based on the understanding of the potential emergency. The potential tool for the staff crisis management is to prepare the list of the retired specialists in the area to be able to recruit them in the emergency cases.

COVID-19 Infection Control and Personal Protective Equipment (PPE)

As in any emergency situation, the administration is committed to protecting staff (and patients) against the new threats, *i.e.*, the COVID-19 virus in the current situation. The MOH published guidelines to mitigate the risk of viral transmission to health professionals and the public. The IPC unit, together with the ZMC administration, was responsible for implementing these guidelines. As these guidelines were published and distributed throughout the hospital, employees were able to develop a sense of their personal risk level and express their expectations regarding the appropriate standards of protection. Implementation of these guidelines can be challenging, as the staff is acutely aware of the different standards of protection around the world and justifiably prefer to have access to the best personal protective equipment (PPE) available [18], while the administration must consider rationing available stocks for future needs.

Due to the worldwide PPE shortage, every hospital made its own efforts to maintain a minimal amount of PPE (an “iron” minimal storage to fulfil 1 month of independent functioning). Due to the increasing number of severely ill COVID-19 patients, we had to update the “iron” storage and gradually increase it. At the beginning of the pandemic, the MOH delivered instructions to collect all the PPE for future recycling. Under special precautions, we did so for 4 months until the worldwide and local manufacturing exceeded the increasing demands of PPE.

In addition, each department's staff (physicians and nurses) was organized into three pods, with all pod members working together in the same department for 12 hours and then resting at home for 24 hours. The pods never met face-to-face but only communicated by phone, even during shift transfer, so that if a worker became infected, it would only affect his pod, while the other two pods could continue functioning. This approach preserved teams of physicians and nurses who were already used to working together while also reducing the chance of having to close down an entire department or facility in case of contact with a confirmed COVID-19-infected colleague or patient. As knowledge relating to the transmission route and rate of COVID-19 expanded, it was determined that if the staff was exposed to a COVID-19 patient and was protected according to the standards, he could continue working without the need for isolation at home.

Each staff member was asked to check his/her body temperature at home before leaving for the hospital, and febrile employees were instructed to stay home and call the IPC staff to arrange for COVID-19 testing. Each employee reported his/her temperature to the immediate supervisor and was instructed on effective social distancing and hygiene (forbidden crowding, especially when eating and smoking).

During the first COVID-19 wave in Israel (March 2020), the citizens were not instructed to wear masks; only medical staff wore masks, too, only at work. Later, policies were updated and now (February 2020), all residents are obligated by law to wear a mask outside their homes.

The IPC unit plays an important role in monitoring staff exposure and conducting epidemiology investigations to provide guidance regarding staff isolations. In addition, the IPC must report to the regional health office for epidemiology investigations outside the hospital regarding hospitalized patients or other contacts of the staff.

The IPC unit continues to invest efforts to prevent cross infections. We kept the same amount of hand hygiene observations as during the pre-pandemic time (more than 300 monthly observations). In every department, a hand hygiene nurse manages the local SOP for the infection prevention. These nurses are also used for the local audit of hand hygiene and infection prevention in other departments.

Logistics

There are many logistical issues to be considered in order to protect staff and patients from getting infected at least in the hospital setup. Masks and other PPE are not sufficient; additional measures must be taken to minimize the risk of infection. For example, when selecting the location of the makeshift COVID-19

departments, it was important to consider the logistics, especially the necessity to adapt the space to its new purpose, while taking budget and the time to operation into account. Besides these primary requirements, other issues, such as technology, and access to ventilators and other supplies, *e.g.*, oxygen tanks and pharmaceuticals, were also considered. A scheduled supply purchase plan was prepared.

In addition, a separate air conditioning system was needed and low-pressure rooms were needed for some patients. To ensure this, an air pressure gradient was created by increasing air flow in the clean area and increasing air suction from the COVID-19 department, leading to continuous air circulation from the clean to the infected area. Furthermore, to minimize crowding, a new setup was needed for staff dining, as cafeterias were closed.

Due to closures in the country, especially the education system and kindergartens, many workers had no child-care arrangements, which the hospital administration must consider providing.

Communication

Although communication is part of the MOH's responsibilities, regional and community hospitals, such as ZMC, also played a role in communicating the situation to the public, with explanations tailored to the diverse mosaic of religions and traditions of the local communities. These included dissemination of information and instructions relevant to people requiring hospital services.

At the hospital level, as soon as a working plan was formulated, it was distributed and explained at the level of each hospital unit manager. Most of the communication with the staff was conducted *via* video conference calls. The designated COVID-19 units created a closed group for communication by emails or WhatsApp (administration, IPC, COVID-19 department and ED managers, PCR lab manager and spokespeople). In addition, a weekly report was sent to the unit managers, summarizing the situation at the hospital, regional and the country levels.

Morale

In emergency situations, hospital workers, especially those assigned to COVID-19 departments, face new challenges which impact their morale. Challenges in the working environment include the complexities of performing with PPE, *e.g.*, gowns, face shields, surgical masks, eye protector and gloves, which are hot and physically uncomfortable, alongside increased staff concerns regarding introduction of the virus to their families at home [18]. For this reason, shifts in

COVID-19 units were 3 hours long. In addition, morale was impacted by the disconnect between staff and patients arising from PPE use, which impairs communication and increases psychological stress. Patients hospitalized in COVID-19 departments are generally in severe condition and require long-term hospitalization. Working in such wards is emotionally challenging [16], especially in light of the high mortality rate and regular exposure to patient and family pain and sufferings. We encourage families to visit COVID-19 patients, as we found it important to the patients, the families and the staff.

Our community served as an enormous and gratifying source of support, as expressed by donations of meals, refreshments, flowers, and funds, which were distributed according to needs.

Psychological support teams were established to enable employees feeling distress and burn-out to reach out anonymously for support and relief [17].

The Hospital's Operating Mode

During crises, much attention has to be paid to hospital management, to ensure continuity of its different missions, particularly the non-COVID-19-related medical services, with minimal disruption. At the same time, management must ensure efficient and effective treatment of COVID-19 patients in the dedicated facility, handle suspected COVID-19 patients, mostly at the ED and withstand the surge in COVID-19 cases. Its third mission is to protect its staff from contracting the virus, while also tending to their morale and fatigue.

ZMC has an established Hospital Multi-Component Emergency Center (HMCEC) [19, 20] to handle emergency situations. This center comprises five units: the board round table, the operational unit, logistical support unit, the healthcare professions advisory unit, and a specialized expert advisory unit. Yet, the current COVID-19 pandemic demanded more comprehensive management [12], with more flexibility than our HMCEC, which is more suitable for large crises such like war. The board round table, which includes the hospital board chaired by the hospital director, was responsible for formulating hospital COVID-19 policy and strategy, while the IPC unit tended to all of the other components of hospital management.

The COVID-19 pandemic brought a series of challenges to hospitals. Initially, ZMC continued its routine operation, alongside preparations for a potential surge in demand for clinical care during the pandemic. Departments dedicated to the treatment of severe COVID-19 cases were arranged and employees were trained to treat critically ill and mechanically ventilated patients [21].

Unlike mass casualty events, the COVID-19 pandemic did not place an immediate burden on the local healthcare system and gave the hospital time to prepare ("rising tide" as opposed to "big bang" of a sudden-onset disaster [20]).

In order to deal with the new situation, we decided to tailor the structure of the HMCEC to meet the demands of the pandemic. The hospital administration (chief executive officer, deputy director, logistics manager, emergency director, human resources manager, nurse manager, spokesperson, ambulatory and quality services manager), the head of the relevant department (ED, COVID-19 hospitalization wards, lab manager) and the IPC unit manager, sat at the board round table and acted as the modified HMCEC. When the pandemic began, meetings were held daily (up to 10 people, seated 2 meters apart, with surgical masks, while others joined by remote connection) to evaluate the situation and make decisions for the following days. Later, these daily meetings were held *via* audio or video conference calls only. The HMCEC structure enabled seamless and effective operation of the hospital and adaptation of its structure in accordance with evolving threats. In situations with a rapidly changing environment, uncertainty, and the potential for large medical care demands, the entire HMCEC might be necessary to cope with the challenges, but it hasn't been the situation with COVID-19 pandemic, to date.

At these meetings, we monitored decision implementation and performance, then the IPC staff presented COVID-19 status at the local and the national levels and new policies that were set forth by the MOH. In addition, statistics regarding COVID-19 department hospitalizations, the flow at the ED and the number of COVID-19 samples from hospital and community collection sites being processed in the hospital laboratory, were all presented, as were summaries of bed occupations at the different wards, especially intensive care beds and the ED load. The manpower representative reported on new staff recruitment and on the number of isolated and sick workers. The logistics representative reported on stock levels of relevant resources, such as ventilators, PPE, oxygen and others. At the end of each meeting, the hospital manager made decisions for the next days, especially with regards to restriction of specific hospital activities, such as operation theatres, when the number of severe COVID-19 patients increased and there was higher demand for staff, especially specialty intensive care personnel.

CONCLUSION

This article described the challenges faced by ZMC during the COVID-19 pandemic and detailed the emerging challenges posed by the new and unique threat. In every emergency, hospitals must cope with the emerging medical demands and best protect staff and patients from the threat. At the same time, the

hospital must strive to continue providing its routine services. Despite the uniqueness of the COVID-19 pandemic, our experience at ZMC with different kinds of health disasters positioned us to translate prior structures and protocols to this novel threat.

Lessons learned from the pandemic included the management structure necessary for dealing with emergencies and the adaptations necessary for the current threat. In our case, the main challenge was not setting up new facilities and adequate equipment, but rather ensuring sufficient resources. These included coping with the increased demand for staff and prioritizing and pulling the "short blanket" to adequately address the dynamic needs throughout the hospital. In the absence of reserve facilities, more attention to training during the new situation is needed. In such circumstances, medical and nursing students presented ideal reserve manpower. At the national level, lessons must be learned about the size of the "blanket" needed for the medical system in the different regions of the country and the impact of the size of the "blanket" during emergencies on national security. The chapter focuses on the unique demands and needs in the emergency situations and proposes practical and useful ways to fulfil those needs by the hospital management, even in the case of required adjustment, as a part of the uniqueness of the medical system in different countries. The chapter and recommendations will keep their relevance in different emergency situations as a toolbox for hospital management.

CONSENT FOR PUBLICATION

Declared none.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 3

Proinflammatory and Thrombotic Manifestations and the Therapeutic Options of COVID-19

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Abstract: COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS CoV 2), has put the global health system into crisis. The complications of the disease include respiratory failure, proinflammatory, and thromboembolic presentations. It is being increasingly recognised that host response with the inflammatory and thrombotic state is responsible for the severity of the disease. Numerous studies are now showing that increased inflammatory markers like interleukin (IL) 6 portray a poor prognosis. Thrombo-embolic complications like pulmonary embolism also produce clinical deterioration in COVID 19. The management of the disease presently includes antiviral, anti-inflammatory, and anticoagulant therapy along with supportive care for respiratory complications. The therapeutic challenge is augmented due to the varied clinical presentations, rapid worsening, and lack of a clear understanding of the pathophysiology. The initial data regarding the treatment options are of low quality and are mostly from cohort analysis. Many randomised controlled trials (RCT) are ongoing, and the results from the RCTs will help in developing better treatment options. We discuss in this review the pathophysiology and mechanism behind the increased inflammation and thrombosis. We will also discuss the available therapeutics options and the recommendations of various guidelines regarding the management of the proinflammatory and thrombotic state.

Keywords: Complications, Coronavirus, COVID-19, Guidelines, Inflammation, Pathophysiology, SARS CoV 2 , Thromboembolism, Thrombosis, Treatment.

INTRODUCTION

The emergence of SARS CoV 2 and its associated COVID-19 disease has put the global health system into crisis. As of the end of September 2020, more than 7

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million people have been affected and more than 100,000 deaths in India have occurred [1]. The understanding of the pathophysiology of the disease process is rapidly evolving.

Coronaviruses (CoVs) (family *Coronaviridae*) are positive-sense single-stranded RNA enveloped viruses with a genome of approximately 26–32 kilobases in size [2]. Prior to the emergence of SARS CoV 2, six other CoVs species were known to infect humans [2]. Four of the CoVs, 229E, OC43, NL63, and HKU1 usually cause mild upper respiratory tract infections in individuals [3]. However, in the year 2002, severe acute respiratory disease coronavirus (SARS-CoV) emerged, showing that CoVs are also capable of causing outbreaks of severe infections in humans. Another severe CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), surfaced in 2012 in Saudi Arabia [3].

The first case of COVID-19 emerged in China in December 2019. It was likely a case of zoonotic transmission. Since then, rapid human-to-human transmission has led to the spread of the disease to almost all parts of the world [4]. On 11th March, 2020, WHO declared it a pandemic [5].

SARS CoV 2 is predominantly transmitted between people by respiratory droplets, aerosols and contact [4, 6 - 9]. It has also been shown to be detected in non-respiratory specimens [10]. The faeco-oral route has also been proposed as a route of transmission [11]. The incubation period is roughly 4–5 days before symptom onset, and the majority (97.5%) of patients become symptomatic within 11.5 days [12].

The respiratory system is primarily affected by the SARS-CoV-2 virus. Symptoms include upper respiratory tract manifestations, myalgias, and smell or taste disorders [13, 14]. Gastrointestinal symptoms have also been reported in COVID 19 [11]. The Chinese Centre for Disease Control and Prevention reported a study that included approximately 44,500 confirmed infections [15]. Their study found that 81 percent had mild (no or mild pneumonia), 14 percent showed severe disease with respiratory distress or > 50 percent lung involvement on imaging within 24 to 48 hours, and 5 percent developed the critical disease (*e.g.*, with respiratory failure, shock, or multiorgan dysfunction). The overall mortality was 2.3 percent. In the hospitals, with a proportion of critical cases being higher, the case fatality rate is also higher, with one study reporting mortality of 39 percent in hospitalised patients [16, 17]. Patients with initial non-severe symptoms can also develop severe symptoms [18]. In an analysis of hospitalized patients in Wuhan for pneumonia due to SARS CoV 2, which studied 138 patients, it was reported that shortness of breath was developed five days after the onset of symptoms, and hospitalisation occurred after a median of seven days of symptoms [19]. The

pathophysiology of infection wit SARS CoV 2 is similar to that of SARS CoV and MERS CoV, and the disease severity in patients is due to both the viral infection and the host response. Complications of COVID-19 that have been reported are:

- Respiratory complications – The major complication which leads to a maximum number of admissions due to severe disease is acute respiratory distress syndrome (ARDS) which develops 8-9 days after the onset of dyspnoea [13]. One study from the USA showed that about 24 percent of hospitalised patients required mechanical ventilation [20].
- Thromboembolic complications.
- Inflammatory complications – Patients have been shown to have increased inflammatory responses.
- Secondary infections – Secondary infections appear to be an uncommon complication. In one review which analysed 9 studies, co-infection was present in only 8 percent of cases [21].

PATHOPHYSIOLOGY OF PRO-INFLAMMATORY STATE IN COVID-19

The steps followed by the virus inside the host include attachment, penetration, replication of the RNA to make full-length copies to be integrated into newly produced viral particles and release [22].

SARS CoV 2 mainly affects cells that express the angiotensin-converting enzyme 2 (ACE2), namely airway epithelial cells, alveolar epithelial cells, vascular endothelial cells and macrophages in the lung [23, 24]. CoVs consist of four structural proteins, Spike (S), membrane (M), envelop (E) and nucleocapsid (N) [25]. The S glycoprotein, a transmembrane protein found in the outer portion of the virus, forms homotrimers protruding in the viral surface and facilitates binding of the viruses to host cells by attachment with ACE2 expressed in the cells in the lower respiratory tract [3, 23]. ACE2 receptors are produced more abundantly on the apical than the basolateral surface respiratory epithelial cells [26]. The virus is more likely to infect and kill these cells. SARS-CoV infection was found to reduce ACE2 receptors in respiratory tract cells. Virus-induced ACE2 downregulation was thought to be important for disease pathology, as this was associated with acute lung injury [27]. Similar pathophysiology also has been proposed for SARS CoV 2.

Innate Immune Response to SARS CoV 2

Epithelial cells, alveolar macrophages, and dendritic cells (DCs) act against the viruses as part of the innate immunity till adaptive immunity gets involved. Antigen presentation *via* DCs and macrophages triggers the T cell responses. Virus-infected apoptotic epithelial cells are phagocytized by DCs and macrophages, which leads to antigen presentation to T cells [28].

Viral infection and replication in epithelial cells lead to pyroptosis and vascular leakage [29]. Pyroptosis is an inflammatory form of programmed cell death that is frequently seen with cytopathic viruses [30]. This probably sets off the subsequent inflammatory response.

Local inflammation, causes increased secretion of the pro-inflammatory cytokines and chemokines like IL-6, IFNγ, MCP1, and IP-10. Secretions of these cytokines draw monocytes and T lymphocytes, but not neutrophils, from the blood into the infected site. Pulmonary infiltration of these immune cells from the blood may account for the lymphopenia and increased neutrophil-lymphocyte ratio seen in around 80% of patients with SARS-CoV-2 infection [13, 31, 32]. However, direct virus killing of lymphocytes has also been thought to contribute to lymphopenia in patients [27].

The lymphocytes and monocytes remove the infection within the lung in most people, and the immune response subsides. However, in some patients, a dysfunctional immune response occurs, which leads to a cytokine storm, resulting in widespread lung inflammation. It has been observed that the patients, who require intensive care in hospitals demonstrated higher levels of IL-1, IL-2, IL-7, granulocyte colony-stimulating factor (G-CSF), IP-10, MCP1, macrophage inflammatory protein 1α (MIP1α), and tumour necrosis factor (TNF) in the blood [13]. IL-6 levels in these patients tend to rise with time and are relatively more elevated in non-survivors than survivors [32]. Also, patients with severe disease exhibit an increased proportion of $CD14^{+}CD16^{+}$ inflammatory monocytes, which contribute to the cytokine storm in peripheral blood than patients with mild disease. It has also been shown that the rise of proinflammatory cytokines like IL-1, IL-6 does not relate with the concomitant rise of anti-inflammatory cytokines [33]. McElvaney *et al.* in their study, showed that although the level of IL-10 was more in stable COVID-19 patients as compared to healthy control, there was no difference in the level between stable and COVID-19 patients in ICU. This contrasted with patients of community-acquired pneumonia in ICU, who exhibited markedly increased IL-10. A similar observation was made with alpha 1 antitrypsin (AAT), which is also a key anti-inflammatory modulator. Raised IL-6:AAT ratio was predictive of prolonged ICU stay and mortality in serious

patients, whereas improvement in the ratio was associated with clinical resolution. In the same study, the authors tried to understand the immune-metabolic pathway behind the inflammatory state. They showed that the neutrophils in COVID-19 patients have higher cytosolic levels of the pro-inflammatory metabolic regulator and glycolytic markers with increased cytosolic PKM2 (pyruvate kinase M2), phosphorylated PKM2, HIF-1a (hypoxia-inducible factor1a), and lactate.

The understanding of how SARS CoV 2 impairs the body's innate antiviral cytokine responses is still lacking, however, studies on SARS CoV show that multiple viral structural and non-structural proteins negate the interferon responses. This occurs at various levels, including the prevention of pattern recognition receptor (PRR) recognition of viral RNA [34, 35]. It is possible that some of these pathways are similar in SARS CoV 2. This results in increased viral replication, with increased release of pyroptosis products that can further induce aberrant inflammatory responses.

The unrestrained inflammatory cell infiltration and cytokine storm can mediate damage in the lung through excessive secretion of proteases and reactive oxygen species, in addition to the direct damage resulting from the virus. Together, these damage the alveolar epithelium and cause pulmonary oedema [31]. This decreases the efficiency of gas exchange in the lung, causing respiratory distress, decreased oxygen saturation, and hypoxia. The lung also becomes predisposed to secondary infections.

Aged people and people with co-morbidities are more likely to develop a dysimmune response, which is an improper immune response that fails to eradicate the virus and also damages the lung parenchyma. The reason for this is unclear; however, the ageing lung microenvironment causes altered dendritic cell maturation and migration to the lymphoid organs. Therefore, defective T cell activation maybe one cause. Children, however, tend not to develop severe disease despite being capable of experiencing high viral titres. In patients younger than 18 years, more than 50% were either asymptomatic or had mild symptoms, and less than 6% of children developed severe symptoms [36].

It is postulated that viral persistence and replication may be necessary to drive the ongoingdamage, although the exact picture remains to be elucidated. The viral titres probably peak in the respiratory tract even before the symptoms of pneumonia occur in SARS-CoV and SARS-CoV-2 infections [37]. However, a study showed that viral RNA was detectable in patients until the point of death, suggesting a correlation between virus persistence, replication and worse outcome [38].

Adaptive Immune Response by the Body

T cell primarily consists of two types of cells, CD4+ and CD8+. CD8+ are involved in killing the virus infected cells, while CD4+ are involved in priming the CD8+ and B cells. The T-cell response to SARS-CoV-2 peaks about one to two weeks after infection and is detectable for several months even after recovery [39].

Autopsy of patients with SARS CoV 2 have revealed increased mononuclear infiltrates in the lung along with bilateral diffuse alveolar damage because of inflammatory exudates. Peripheral CD4 and CD8 T cells were substantially reduced. The results indicate that increased activation of T cells, as shown by an increase of Th17 and high cytotoxicity of CD8 T cells, accounts for lung injury in the severe category of patients [31]. One study of a cohort of 68 patients from China showed that there is functional exhaustion of antiviral lymphocytes. T cell and $CD8^+$ T cell counts were decreased significantly in moderate and severe patients compared with those in healthy controls [40]. It is postulated that T cells are attracted away from the blood and into the area of inflammation. Also, it is speculated that an inverted CD4:CD8 ratio occurs due to the greater reduction of the helper $CD4^+$ T cell population. The inversion of the CD4:CD8 ratio and the activation of the cytotoxic T cells is thought to happen simulataneously [41].

SARS-CoV-2-specific IgA, IgG, and IgM antibodies have been shown to be detected early in the disease [42]. There is a preponderance of IgG1-expression among IgG subclasses. In one study, IgM antibody levels peaked on day 13 and began to fall on day 21, while IgG antibody levels peaked on day 17 and maintained the level until tracking ended (till 38 days) [43].

PROTHROMBOTIC STATE

Previously, SARS CoV and MERS CoV had been associated with an increased risk of thrombosis [44]. Infection with SARS CoV 2 also seems to be associated with a prothrombotic state. A retrospective analysis from China has shown that increased D-dimer levels lead to increased mortality [38]. Both venous and arterial thromboembolism have been documented in cases with SARS CoV 2 [45, 46].

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are very common in acutely ill patients with COVID-19. Autopsy reports of patients who died from SARS CoV 2 have reported DVT, PE, thrombotic microangiopathy in glomeruli [47, 48]. Autopsies of patients who died from COVID-19 have been compared with the autopsies of patients expiring from influenza caused by H1N1. It is reported that the incidence of severe endothelial injury, thrombosis with

microangiopathy, and angiogenesis is higher with COVID-19, as compared to influenza caused by H1N1 [49].

Pathophysiology of Coagulopathy in COVID-19

Laboratory findings from admitted COVID-19 patients have reported increased D-dimer, fibrinogen, factor VIII, von Willebrand factor (vWF), and decreased antithrombin [50]. Virchow's triad states that hypercoagulability is due to endothelial injury, stasis, and hypercoagulable state.

Endothelial injury – It is believed that SARS CoV 2 directly affects the pulmonary endothelial cells and leads to endotheliitis [51].

Stasis – Immobilization leads to the stasis of blood flow in all hospitalized and critically ill patients, regardless of whether they have COVID-19 or not [52].

Hypercoagulable state – COVID-19 has been reported to be associated with increased procoagulant factors, namely elevated factor VIII, elevated fibrinogen, circulating prothrombotic microparticles, neutrophil extracellular traps (NETs), hyper viscosity [50, 53, 54].

Unlike disseminated intravascular coagulation, where the major finding is bleeding, COVID-19 leads to increased thrombosis. In COVID-19, the findings consist of high fibrinogen and high factor VIII activity, suggesting that major consumption of coagulation factors is not occurring.

COVID-19 appears to cause a hypercoagulable state through pathophysiology that is unique to SARS-CoV-2. Thrombosis and inflammation are interrelated, and there is a bidirectional relationship between them [55].

Ranucci *et al.* in their prospective observational study, found inflammatory markers to be elevated and correlated with increased prothrombotic markers [53]. Abou-Ismail MY *et al.* have proposed the following mechanism for the inflammation and thrombosis seen in COVID-19 patients [52]. Infection of the endothelial cells through the ACE2 receptor in COVID-19 leads to a severe inflammatory response that originates in the pulmonary alveolar cells. This triggers endothelial activation and dysfunction, production of tissue factor, and platelet activation, and increased levels of VWF and factor VIII, all of which contribute to thrombin generation and fibrin clot formation. Thrombin, in turn, causes inflammation through its effect on platelets, which promote NET formation in neutrophils and activation of endothelium, and further activation of monocytes.

Some of the studies regarding the procoagulant state are (Table **1**):

- In a study of 81 patients in the ICU of a hospital in Wuhan, China, the incidence of lower extremity venous thromboembolism (VTE) in these patients was 25% (20/81), of which 8 patients with VTE events died [56].
- Lodigiani *et al.* studied 388 patients admitted to a hospital in Milan. Thromboprophylaxis was used in 100% of ICU patients and 75% of those on the general ward. VTE was confirmed in 16 cases, and PE was confirmed in 10 cases. The rate of ischemic stroke and ACS/MI was 2.5% and 1.1%, respectively [57].
- In a study of 198 patients in the Netherlands, the cumulative incidences of VTE at 7, 14 and 21 days were 16%, 33% and 42%, respectively. VTE appeared to be associated with death (adjusted HR, 2.4; 95% CI, 1.02-5.5). The cumulative incidence of VTE was higher in the ICU (26%, 47%, and 59% at 7, 14 and 21 days) than on the wards [46].
- Study of 3334 patients in New York reported thrombotic events in 16 percent patients. 207 (6.2%) were venous (3.2% PE and 3.9% DVT), and 365 (11.1%) were arterial; 1.6% had an ischemic stroke, 8.9% had MI, and 1.0% had systemic thromboembolism. Among 829 ICU patients, 29.4% had a thrombotic event (13.6% venous and 18.6% arterial). All-cause mortality was 24.5% and was higher in those with thrombotic events (43.2% *vs*. 21.0%, $p < .001$) [58].
- A series of 184 sequential patients with severe COVID-19 in the ICU reported PE in 25 (14 percent), DVT in 1, and catheter-associated thrombosis in 2. The cumulative incidence of VTE (based on different durations of follow-up) was calculated at 27 percent. All received at least standard dose thromboprophylaxis [45].
- In a series of 150 patients in French hospitals, sixty-four clinically relevant thrombotic complications were diagnosed in 150 patients, mostly PE (16.7%). Increased D-dimer and fibrinogen were noted in more than 95 percent of patients. No patient developed disseminated intravascular coagulation. Increased levels of Von Willebrand (vWF) antigen, vWF activity, and FVIII were found, and positive lupus anticoagulant was found in 50/57 tested patients (87.7%) [59].
- A single hospital reported five cases of acute ischemic stroke in patients less than 50 years of age in association with COVID-19 over a two-week period, with symptoms suggesting large-vessel occlusion [60].
- Meta-analysis from 102 studies with 64503 patients reported VTE in 14.7% cases (95% CI 12.1% to 17.6%, I^2=94%; 56 studies; 16,507 patients). The overall prevalence rates of pulmonary embolism (PE) and leg deep vein thrombosis were 7.8% (95% CI 6.2% to 9.4%, I^2 = 94%; 66 studies; 23,117 patients) and 11.2% (95% CI 8.4% to 14.3%, I^2 = 95%; 48 studies; 13,824 patients), respectively [61].

Table 1. Studies regarding the incidence of the prothrombotic state in COVID-19.

Place of Study	Type of Study	Sample Size	Use of Thromboprophylaxis	Venous Thromboembolism	Arterial Thrombosis
Wuhan, China (Cui *et al.*)	Retrospective; single centre; hospitalized patients	81; mean age - 59.9 years Range of age – 32-91 years	No	VTE 25%; all lower extremity thrombi	None
Italy (Lodigiani *et al.*)	Retrospective; single centre; hospitalized patients	388; median age - 66 years Range of age – 55-77 years	LMWH Ward: 75% used (41% prophylactic dose, 21% intermediate dose; 23% therapeutic dose) ICU: 100% used (3.2% Therapeutic, 28% weight adjusted thromboprophlxis)	VTE 21% (cumulative rate) ICU 27.6% and general ward 6.6%	Ischemic stroke 2.5% and ACS/MI 1.1%
Netherlands (Middeldorp *et al.*)	Retrospective; single centre; hospitalized patients	198; Mean age - 61 years	Yes (nadroparin 2850 units daily for <100 kg and 5700 units daily for >100 kg)	7-day incidence of VTE (15%) and 14-day incidence of VTE (34%)	None
New York, USA (Bilaloglu *et al.*)	Retrospective; single centre; hospitalized patients	3334; median age - 64	Low dose LMWH	3.2% PE and 3.9% DVT	(1.6% ischemic stroke, 8.9% MI, and 1.0% systemic thromboembolism
Netherlands (Klok *et al.*)	Retrospective; multicentre; hospitalized patients	184; mean age – 64 years	Yes (nadroparin at different doses)	VTE (n = 28), PE (n = 25)	Ischemic strokes (n = 3)
France (CRICS TRIGGERSEP Group)	Prospective; multicentre; hospitalized patients	150 ICU patients; median age – 63 years	Yes (80% prophylactic dosing; 20% therapeutic)	16% PE and 2% DVT	2 cases of cerebral ischemia, 1 case each of limb and mesenteric ischemia

VTE – Venous thromboembolism, LMWH – Low molecular weight heparin, PE – Pulmonary embolism, DVT – Deep venous thrombosis, ICU – Intensive care unit, the percentage in the table is with respect to incidence per sample size.

To summarise, the prothrombotic state can involve many organs [52]:

- Lungs – PE, microthrombi leading to hypoxia
- Heart – Myocardial infarction
- Cerebral circulation – Stroke
- Kidneys – Thrombotic microangiopathy
- Peripheral circulation – Chilblains
- Skin – Livido reticularis
- Bowel – Ischemia

THERAPIES AGAINST THE PROINFLAMMATORY STATE

In this section, we highlight some of the treatment strategies to mitigate the proinflammatory state. Sun *et al.,* in their review, noted that MODS occurred after a window period of about 5-7 days after the diagnosis. After this period, about 80% of the patients tend to improve, whereas 20% of the patients progress to severe pneumonia, with approximately 2% mortality. To improve the prognosis, they have suggested that patients should be given immunotherapy treatment at the time of diagnosis in order to block the possibility of a subsequent dysimmune response and cytokine storm [62]. However, it is still unclear if anti-inflammatory therapies should be administered in mild cases and the dose of such therapies in moderate/severe cases.

Convalescent Plasma (COPLA) from Patients Recovered from COVID-19

COPLA is a form of passive immunization in which plasma from patients recovered from SARS CoV 2 is transfused to active patients for transfer of antibodies that act directly to neutralize viral infectivity. COPLA has previously been used in patients with SARS CoV and MERS [63]. Sun *et al.* analysed 40 studies on COPLA in SARS CoV, SARS CoV 2, MERS, Ebola, and influenzas and reported that COPLA could reduce the risk of mortality, with a low incidence of adverse events, promote the production of antibodies, with reduction of viral load and course of illness [64]. Very few randomised controlled trials regarding plasma therapy in COVID-19 patients have been published to date. Two RCTS (one from China and one from the Netherlands) were halted prematurely, with no mortality benefit being reported by them [65, 66]. The Dutch study also showed that 53 of 66 patients tested had anti-SARS-CoV-2 antibodies at baseline, with SARS-CoV-2 plaque reduction neutralization test showing neutralizing antibodies in 44 of the 56 (79%) patients tested with median titres comparable to the 115 donors (1:160 *vs*. 1:160, p = 0.40) [66].

PLACID study was an open-label, parallel-arm, phase II, multicentre, randomized controlled trial, sponsored by ICMR, and was conducted in thirty-nine government and private hospitals across India. Their findings reported that COPLA was not associated with a reduction in mortality or progression to severe category. They also reported that about 83.2% of plasma recipients had detectable neutralising antibodies at enrolment [67].

The present evidence suggests that plasma recipients already have neutralising antibodies when they are prescribed COPLA, and the use of this therapy in the treatment of COVID-19 does not have any favourable outcome *vis-à-vis* disease progression and mortality.

Steroids

Corticosteroids are commonly used to decrease various hyperinflammatory conditions. The immunosuppressive effects of glucocorticoids are based on the trans-repression of proinflammatory genes. Glucocorticoids inhibit NF-κB signaling and induce inhibition of synthesis of downstream proteins, such as IL-1, IL-6, granulocyte-macrophage colony-stimulating factor, and inducible cyclooxygenase-2. This results in reduced proliferation, activation, differentiation, and survival of T cells and macrophages.

Yang *et al.* in their meta-analysis of 15 studies regarding the use of corticosteroids in SARS CoV, MERS, and SARS CoV 2, reported that corticosteroid use was associated with increased mortality in patients with coronavirus pneumonia [68]. The initial findings of the RECOVERY trial in the UK published in the month of July, 2020 reported that the use of dexamethasone led to lower 28-day mortality in patients who required respiratory support (either invasive mechanical ventilation or oxygen alone) at randomization but not among those receiving no respiratory support [69].

Chen *et al*., in their retrospective study of 99 patients in Wuhan, China, in January 2020, reported that 19 (19%) patients were treated with glucocorticoids for 3–15 days. The subgroup analysis of mortality for various treatment modalities was not provided; however, they recommended that methylprednisolone (1–2 mg/kg per day) should be used for patients with ARDS for a short duration [70]. In another study from Wuhan, China, Wang *et al.* reported that 44.9% of patients of COVID-19 were given glucocorticoid therapy, with no effective outcomes observed [19]. A retrospective analysis of 201 patients out of whom 84 developed ARDS showed that treatment with methylprednisolone decreased the risk of death [71]. Wu *et al.* analysed 1514 severe and 249 critical hospitalized COVID-19 patients from two medical centers in Wuhan, China. Corticosteroids were administered in 531 (35.1%) severe and 159 (63.9%) critical patients. Compared

to the non-corticosteroid group, systemic corticosteroid use was not associated with a beneficial effect in reducing in-hospital mortality in both severe cases and critical cases [72].

The RECOVERY Collaborative Group in the UK conducted a controlled, open-label trial and randomly assigned patients to receive oral or intravenous dexamethasone (at a dose of 6 mg once daily) for up to 10 days or to receive usual care alone [73]. 2104 patients received dexamethasone and 4321 received usual care. Overall, 482 patients (22.9%) in the dexamethasone group and 1110 patients (25.7%) in the usual care group died within 28 days after randomization (age-adjusted rate ratio, 0.83; 95% confidence interval [CI], 0.75 to 0.93; P<0.001). The use of dexamethasone in patients receiving invasive mechanical ventilation and among those receiving oxygen without invasive mechanical ventilation led to lower mortality. However, this was not the case in patients who required respiratory support at randomization.

WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group pooled data from seven RCTs from 12 countries and performed a meta-analysis that evaluated the efficacy of corticosteroids in 1703 critically ill patients with COVID-19 (Table **2**). 222 deaths were reported among the 678 patients who received corticosteroids and 425 deaths among the 1025 patients randomized to usual care or placebo (summary OR, 0.66 [95% CI, 0.53-0.82]; p < .001 based on a fixed-effect meta-analysis) [74]. Three trials each in the meta-analysis involved hydrocortisone and dexamethasone, and one focused on methylprednisolone. The summary OR for death was 0.64 (95% confidence interval [CI], 0.50 to 0.82; p < .001) among patients receiving dexamethasone *versus* standard care or placebo. The OR for hydrocortisone was 0.69 (95% CI, 0.43 to 1.12; P = .13), while it was 0.91 (95% CI, 0.29 to 2.87; P = .87) for methylprednisolone.

Table 2. Association Between Corticosteroids and 28-Day All-Cause Mortality in Each Trial, Overall, and According to Corticosteroid Drug [74] [Modified from a table published by WHO REACT group].

Drug & Trial	Trial	Dose	No. of Deaths/Total no. of Patients		Odds Ratio
			Steroid	No Steroid	
Dexamethasone					
DEXA-COVID 19	NCT04325061	High: 20 mg/d Intravenous	2/7	2/12	2.00 (0.21-18.69)
CoDEX	NCT04327401	High: 20 mg/d Intravenous	69/128	76/128	0.80 (0.49-1.31)

(Table 2) cont.....

Drug & Trial	Trial	Dose	No. of Deaths/Total no. of Patients		Odds Ratio
			Steroid	**No Steroid**	
RECOVERY	NCT04381936	Low: 6 mg/d orally or Intravenous	95/324	283/683	0.59 (0.44-0.78)
Subgroup fixed effect			166/459	361/823	0.64
Hydrocortisone					
CAPE COVID	NCT02517489	Low: 200 mg/d Intravenous	11/75	20/73	0.46 (0.20-1.04)
COVID STEROID	NCT04348305	Low: 200 mg/d Intravenous	6/15	2/14	4.00 (0.65-24.66)
REMAP-CAP	NCT02735707	Low: 50 mg Intravenous 6 hourly	26/105	29/92	0.71 (0.38-1.33)
Subgroup fixed effect			43/195	51/179	0.69 (0.43-1.12)
Methylprednisolone					
Steroids-SARI	NCT04244591	High: 40 mg Intravenous 12 hourly	13/24	13/23	0.91 (0.29-2.87)
Overall (fixed effect)			222/678	425/1025	0.66 (0.53-0.82)
Overall (random effect)			222/678	425/1025	0.70 (0.48-1.01)

CAPE COVID - Community-Acquired Pneumonia: Evaluation of Corticosteroids in Coronavirus Disease, CoDEX - COVID-19 Dexamethasone, COVID STEROID - Hydrocortisone for COVID-19 and Severe Hypoxia, DEXA-COVID 19 - Efficacy of Dexamethasone Treatment for Patients With ARDS Caused by COVID-19, RECOVERY - The Randomized Evaluation of COVID-19 Therapy, REMAP-CAP - Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia, Steroids-SARI, Glucocorticoid Therapy for COVID-19 Critically Ill patients With Severe Acute Respiratory Failure, mg – milligram, mg/d – milligram per day.

The dose and duration of steroids to be prescribed are still not clear. Conflicting results have been obtained with regards to the use of high-dose steroids. Recent study with high dose methylprednisolone in non-intubated severe patients showed reduced progression of the disease and need for mechanical ventilation without serious side effects [75]. In an observational study in Spain, the use of 1.5 mg/kg/24 h of methylprednisolone or dexamethasone equivalent led to reduced mortality (HR = 0.087 [95% CI 0.021-0.36]; P < 0.001). The laboratory markers proposed for the usage of high dose steroid to negate the hyperinflammatory state are IL-6 > = 40 pg/ml, and/or two of the following: C-reactive protein > = 100 mg/L, D-dimer > = 1000 ng/ml, ferritin > = 500 ng/ml and lactate dehydrogenase > = 300 U/L [76]. A retrospective controlled trial in Turkey showed that pulse-steroid treatment (250 mg/day methyl prednisolone) had a shorter ICU stay, but it

did not lead to reduced mortality compared to the standard dose steroid [77]. Retrospective cohort analysis by Monreal *et al.* showed high dose steroid (≥ 250 mg/day of methylprednisolone) led to increased mortality than standard dose methylprednisolone (≤ 1.5 mg/kg/day of methylprednisolone) (adjusted OR 2.46, 95% CI 1.59–3.81, $p < 0.001$). It was also associated with an increased risk of the need for mechanical ventilation or death (adjusted OR 2.35, $p = 0.001$) [78].

For mild cases, the use of steroids seems to be counterproductive. Li *et al.* retrospectively analysed 475 patients with non-severe COVID-19 pneumonia. 55 patients had received early, low-dose, and short-term corticosteroids therapy, whereas 420 patients had not received corticosteroids therapy. Compared to the non-corticosteroids group, use of corticosteroid resulted in prolonged duration of fever (median 5 *vs*. 3 days, $p < 0.001$), virus clearance time (median 18 *vs*. 11 days, $p < 0.001$), and length of hospital stay (median 23 *vs*. 15 days, $p < 0.001$). It was also associated with increased antibiotics therapy (89.1% *vs*. 23.6%, $p < 0.001$) and antifungal therapy (7.3% *vs* 0, $p = 0.042$), and more patients developed severe disease (12.7% *vs*. 1.8%, $p = 0.028$). There was no significant difference in mortality between the two groups (1.8% *vs*. 0, $p = 0.315$) [79].

The clinical interpretation study of the RECOVERY study by Calzetta *et al.* found that dexamethasone increased mortality compared with usual care in patients not requiring oxygen supplementation, leading to a number needed to harm value of 26.7 (95% CI 18.1-50.9, $p < 0.05$). They recommended avoiding steroids in patients not requiring respiratory support [80].

An open-label, parallel-group, phase 2, randomised controlled trial of inhaled budesonide, compared with usual care, was performed in 146 adults within 7 days of the onset of mild COVID-19 symptoms in the UK [81]. 73 received usual care, and 73 received 2 inhalations of 400 μg of budesonide delivered *via* a turbohaler twice a day. The number needed to treat with inhaled budesonide to reduce clinical deterioration was 8. Patients who received budesonide recovered 1 day earlier as compared to the usual care group.

Tocilizumab

Tocilizumab, a humanized anti-human IL-6 receptor antibody, is used for several inflammatory and autoimmune diseases, such as rheumatoid arthritis, giant cell arteritis, polyarticular juvenile idiopathic arthritis, and systematic juvenile idiopathic arthritis [82].

Initial case reports reported success with the use of tocilizumab [83 - 86]. Luo *et al.* retrospectively analysed 15 patients who had received tocilizumab therapy. Tocilizumab treatment ameliorated the increased CRP in all patients rapidly.

Three patients, who were critically ill, expired. The expired patients showed an increasing trend of IL-6 even after the administration of tocilizumab and methylprednisolone, supporting the indication of repeat doses of tocilizumab patients who are critically ill [87]. A case-control study of 45 patients in France suggested that tocilizumab may reduce the number of patients requiring intensive care and mortality [88]. Rodríguez-Baño *et al* studied a cohort of 778 patients from 60 hospitals in Spain. They compared treatment with tocilizumab with steroids and no treatment. They reported that tocilizumab was also associated with a lower hazard of death and concluded that it should be prioritized for randomized trials [89].

The timing of administration of tocilizumab is a matter of debate. 58 patients were analysed in a study in China; 39 of which received tocilizumab. This was compared with 19 patients who declined tocilizumab treatment as the control cohort [90]. They reported that patients who received tocilizumab treatment performed better than those without treatment (P = 0.0273). They also noted that the patients with a baseline IL-6 level of ≥ 100 pg/ml in the -treatment group had poorer clinical outcomes than those with an IL-6 level of < 100 pg/ml (P = 0.0051). They concluded that the administration of tocilizumab in an early stage of cytokine storm (IL-6 level < 100 pg/ml) may improve the outcome. Similar results were reported by a retrospective study of 146 patients in Spain [91]. The study reported that IL-6 levels greater than 30 pg/mL predicted the need for invasive mechanical ventilation. Also, the early administration of the drug led to an improvement in arterial oxygen tension/fraction of inspired oxygen (P/F) ratio in patients with high IL-6 (P = .048).

The indications for the use of tocilizumab include:

- Patients who have increased oxygen requirements and mechanically ventilated patients not responding to steroids.
- Extensive and bilateral lung disease on X-ray and CT.
- In the case of cytokine storm, if IL-6 levels are increased > 10 times (normal 7 pg/mL) along with any one of the following:

  * Raised ferritin > 1000 (normal up to 200 to 300 μg/mL)
  * LDH > 500 (normal 110-250 U/L)
  * High CRP levels > 100mg/mL (normal 8 mg/L)
  * Increased fibrinogen levels

Cautions:

- After the administration of tocilizumab, patients should be monitored for secondary infections and neutropenia (< 500/mm^3).
- AST/ALT > 5 times the upper limit of normal value
- Platelets < 50,000 cells/mm^3
- Complicated diverticulitis and intestinal perforations
- Active infections and tuberculosis should be excluded before use.

Dose: 8 mg/kg (maximum 800 mg at one time) given slowly in 100 ml NS over 1 hour; dose can be repeated once after 12 to 24 hours, if needed.

Itolizumab

Itolizumab, an anti-CD6 humanized IgG1 monoclonal antibody, binds to domain-1 of CD-6 (responsible for activation and differentiation of T-cells). It decreases the T-cell proliferation along with the reduction of the production of cytokines/chemokines. It has been used for moderate to severe chronic plaque psoriasis since 2013 [92].

A phase 2 multi-centric, open-label, randomized, controlled trial to study the efficacy and safety of Itolizumab in COVID-19 complications with 36 patients was conducted in India. 1.6 mg/kg was administered as the first dose. An additional dose of 0.8 mg/kg was administered after 1 week in some patients. The trial reported improved oxygenation, with a greater reduction in IL-6 and TNF α, with fewer deaths in the patients treated with itolizumab [93].

Anakinra

It has been suggested that anakinra, which is a recombinant IL-1 receptor antagonist, might negate the hyperinflammatory state. Two cohort studies with either 5 mg/kg twice a day intravenously [high dose] or 100 mg twice a day subcutaneously [low dose] have shown favourable results with improvement in clinical status [94, 95]. Cohort studies have also shown that anakinra is a potential alternative to tocilizumab for patients with elevated aminotransferases and may be useful in non-responders to tocilizumab [96].

Other Anti-inflammatory Drugs

Other drugs that are being studied in response to the increased inflammatory markers include – siltuximab, sarilumab, baricitinib etc [97 - 99]. Another novel adjunctive therapy that has been proposed is cytosorb, which acts by absorbing cytokines, DAMPs, and PAMPs in order to reduce their circulating levels and

ameliorate immunopathology [100]. The use of gimsilumab, lenzilumab and namilumab has also been tested in clinical trials [27]. The different drugs that can be used for the proinflammatory state in COVID 19 are summarized in Table **3**.

Table 3. Different drugs that can be used for the proinflammatory state in COVID-19.

S. No.	Drug	Pathway	Dose
1.	Steroid	Trans-repression of proinflammatory genes	Moderate – For 3 days, then the clinical response Methylprednisolone - 0.5 to 1 mg/kg Dexamethasone - 0.1 to 0.2 mg/kg Severe – For 5-7 Days Methylprednisolone - 1 to 2 mg/kg Dexamethasone - 0.2 to 0.4 mg/kg
2.	Tocilizumab	Antibody against IL-6 receptor	8 mg/kg (maximum 800 mg at one time) given slowly in 100 ml NS over 1 hour; dose can be repeated once after 12 to 24 hours
3.	Itolizumab	Antibody against CD6	1st dose - 1.6 mg/kg, 0.8 mg/kg may be repeated after 1 week
4.	Anakinra	IL 1 antagonist	High dose - 5 mg/kg IV BD or Low dose - 100 mg SC BD 7 days
5.	Sarilumab	Antibody against IL-6 receptor	400 mg IV single dose
6.	Other drugs		
i)	Siltuximab	IL-6 blocker	Used in Castleman's disease Proposed dose – 11 mg/kg IV once
ii)	Baricitinib	Janus Kinase inhibitor	Used in rheumatoid arthritis Proposed dose – 4 mg PO for 2 weeks

BD – twice daily, IL – interleukin, IV – intravenous, mg – milligram, kg – kilogram, ml – millilitre, NS – normal saline, PO – per oral, SC - subcutaneous

Based on the evidence available till now, it is clear the proinflammatory state is responsible for the complication and mortality in COVID-19. Early recognition is essential for the effective management of the patients. In mild cases, inhaled budesonide can be prescribed. However, systemic steroids should not be prescribed as they prolong the disease duration, with delayed viral clearance, and may increase mortality.

For moderate and severe cases, inflammatory markers, such as IL-6 can be considered as a marker of inflammatory state and severity. Systemic steroids should be considered as a standard of care and immunotherapy should be considered early to halt the inflammation-mediated lung injury.

MANAGEMENT OF THROMBOSIS

Numerous studies have reported the role of increased D-dimer in prognostication of the severity of the disease [13, 101]. The D-dimer is suggestive of the pathological activation of the haemostatic pathway, as it reflects the fibrin formation and degradation. Zhang *et al.,* in their study of 343 patients, reported a cut-off value of D-dimer of 2000ng/mL with a sensitivity of 92.3% and a specificity of 83.3% to predict in-hospital mortality [102]. Lodigiani *et al.* in their study reported that the median D-dimer of survivors at the time of admission was 353 ng/mL and increased to 529 ng/mL a week later, in comparison to 869 ng/mL and 1494 ng/mL in patients who died [57]. These studies show the importance of monitoring the thrombotic markers in assessing the outcome in the COVID-19 patients.

According to the recommendations of the American Society of Hematology (ASH), platelet count, PT/aPTT, D-dimer, and fibrinogen should be monitored. Progressive elevation in the level of D-dimer is suggestive of worsening severity of COVID-19 infection and predicts that more aggressive critical care will be needed [103]. Monitoring of anti-Xa level is recommended over aPTT in patients who are on anticoagulation with heparin products since the latter may be elevated in COVID-19 patients [55].

Diagnosis of DVT or PE - Given the high frequency of these occurrences, the possibility of DVT or PE should always be considered:

DVT – Individuals with suspected DVT should undergo ultrasonography.

PE – ASH guidelines suggest that Elevated D-dimer (> 500 ng/mL) level may result from many causes and does not confirm a diagnosis of PE/DVT in a patient with COVID-19.

Tachycardia, unexplained hypotension, worsening of respiratory status, or other risk factors for thrombosis should raise the suspicion of PE, and computed tomography with pulmonary angiography (CTPA) should be performed to confirm or exclude the diagnosis. A ventilation/perfusion (V/Q) scan is an alternative if CTPA cannot be performed or is inconclusive, although a V/Q scan may be unhelpful in individuals with significant pulmonary involvement from COVID-19.

ASH Guidelines [104]

ASH guidelines suggest that:

- Prophylaxis with LMWH is preferred as compared to unfractionated heparin in all hospitalized adults with COVID-19.
- Prophylaxis is given unless the risk of bleeding outweighs the risk of thrombosis.
- Fondaparinux should be given in the case of heparin-induced thrombocytopenia.
- Mechanical thromboprophylaxis (*e.g.*, pneumatic compression devices) can be used in patients if anticoagulants are contraindicated or unavailable. Combined pharmacologic and mechanical prophylaxis is not recommended.
- Pharmacological thromboprophylaxis can be given even with abnormal PT or aPTT.
- Therapeutic anticoagulation is to be given in the presence of specific indications like VTE, atrial fibrillation, or mechanical valve.
- If a particular patient is already on anticoagulation for VTE or atrial fibrillation, therapeutic anticoagulation should continue unless the platelet count is less than 30-50 x 10^9/L or if the fibrinogen is less than 1.0 g/L.
- The dose should be increased in patients, who experience recurrent clotting of access devices (*e.g.*, central venous catheters, arterial lines) or extracorporeal circuits (*e.g.*, CRRT, ECMO) despite prophylactic anticoagulation. It should be increased from standard-intensity prophylaxis to intermediate-intensity prophylaxis or from intermediate-intensity prophylaxis to therapeutic-intensity or anticoagulants should be switched in these settings.

Treatment of the underlying condition is essential if a patient develops coagulopathy/DIC. The decision to initiate blood product transfusion needs to be individualized.

Blood component therapy should be done with patients with active bleeding, requiring an invasive procedure, or who are otherwise at high risk for bleeding complications and should not be instituted on the basis of laboratory results alone. Transfusion of blood products just to correct laboratory parameters in patients who are not bleeding has not been shown to improve outcomes and is not recommended. Disseminated thrombosis might worsen with replacement and further deplete scarce blood products.

In a patient who is actively bleeding, the following transfusion doses are recommended:

- If the platelet count is less than 50 x 10^9/L – transfuse platelets (one adult dose).
- If the INR is above 1.8 - transfuse plasma (4 units).
- If the fibrinogen level is less than 1.5 g/L – transfuse fibrinogen concentrate (4 grams) or cryoprecipitate (10 units).
- If the patient has severe coagulopathy and bleeding, transfusion of 4F-PCC (*e.g.*, 25 Units/kg) should be considered.
- The role and effectiveness of tranexamic acidare not known.

If imaging studies to diagnose PE or DVT cannot be performed, and if there are no contraindications for therapeutic anticoagulation, empiric anticoagulation should be initiated in the following scenarios [105]:

- If intubated patients develop findings suggestive of PE, such as a decrease in oxygen saturation, tachycardia, increased CVP or PA wedge pressure, or evidence of right heart strain on echocardiogram, when chest X-ray and/or inflammatory markers are stable or improving.
- If patients develop physical findings suggestive of thromboses, such as superficial thrombophlebitis, peripheral ischemia or cyanosis, thrombosis of dialysis filters, tubing or catheters, or retiform purpura (branching lesions caused by thrombosis in the dermal and subcutaneous vasculature).
- If in patients with respiratory failure, PE or microvascular thrombosis is highly suspected, and other causes are not identified (*e.g.*, ARDS, fluid overload), especially when D-dimer and/or fibrinogen levels are increased.

International Society on Thrombosis and Haemostasis (ISTH) Guidelines [106]

ISTH guidelines like the ASH guidelines recommend measurement of D-dimers, PT, and platelet count (in decreasing order of importance) along with fibrinogen in all patients who presented COVID-19 infection.

Prophylactic dose LMWH should be given in all patients (including non-critically ill), who require hospitalisation for COVID-19 infection, provided there are no contraindications (active bleeding and platelet count less than 25 × 10^9 /L; monitoring advised in severe renal impairment). Deranged PT or APTT is not a contraindication for prophylactic dose.

Anticoagulation in Post-discharge Patients

Robert *et al.,* in their study of 1877 hospital discharges associated with COVID-19, reported a VTE of 4.8 per 1000 discharges within 42 days of the discharge as compared to 3.1 VTE per 1000 discharges in 2019 due to other medical illnesses

[107]. The authors recommended against using anticoagulation in the post-discharge setting.

According to the ASH guidelines, all patients with COVID-19 who are on empiric therapeutic anticoagulation for presumed or documented PE should be continued on a therapeutic dose for a minimum of 3 months. It should be taken care of that the patient does not suffer from serious bleeding. Therapeutic anticoagulation can be stopped after 3 months if the patient has recovered from COVID-19 and has no other ongoing risk factors for thrombosis or other indications for anticoagulation (*e.g.*, atrial fibrillation) [108].

Clinical trials in non-COVID patients [109] have shown that post-discharge thromboprophylaxis provided up to 7 weeks in patients with combinations of high-risk factors such as old age, active cancer, and elevated D-dimer > 2 times the upper normal limit can be beneficial. An individualised decision has to be taken regarding the use of post-discharge thromboprophylaxis, which should be considered based on the patient's VTE risk factors at the time of discharge.

Fibrinolytics

Hardaway *et al.* in 2001, reported in their study that administration of either streptokinase or urokinase to patients with terminal ARDS reduced the expected mortality from 100% to 70% with no adverse bleeding events [110]. Wang *et al.* described a case series of three patients with COVID-19 suffering from ARDS and respiratory failure. They were treated with off-label intravenous administration of tPA (Alteplase). All three cases demonstrated an initial improvement in their P/F ratio ranging from 38% ~100% improvement [111]. However, this improvement was maintained only in one patient, and it deteriorated in the other two patients after the completion of their tPA infusion.

Medcalf *et al.* in their review, have suggested that a "consumptive fibrinolysis," as a result of increased levels of fibrin and misfolded proteins/necrotic tissue in the lung, develops in patients with severe COVID-19 disease. This can be decreased by enhancing plasmin formation either *via* the administration of t-PA or plasminogen [112]. In 2019, a study on 60 ARDS patients reported that oxygenation improved more rapidly with nebulized streptokinase than with nebulized heparin [113].

On-going Research

Asakura and Ogawa explored the use of nafamostat, a serine protease inhibitor, as a potential anticoagulant treatment for patients with COVID-19. Nafamostat has weak anticoagulation properties. They advised combining it with heparin to

augment its positive effects [114]. Recently, evidence from three COVID-19 pneumonia cases has shown improvement in clinical status following the administration of nafamostat [115].

As inflammation and cytokine storm have been suggested in the pathogenesis of thrombosis, anti-inflammatory therapies may prove to be effective in the management of thrombosis also.

CONCLUSION

COVID-19 is an ongoing global health crisis. Comprehension of the proinflammatory and prothrombotic cascades is crucial in developing therapies for appropriate management. Early recognition of patients going into cytokine storm and thrombotic state is crucial in the early initiation of treatment. All patients requiring respiratory support should be prescribed systemic steroids and prophylactic anticoagulation. Monitoring of inflammatory markers like IL-6, CRP should be done. Patients deteriorating despite steroids need to be given tocilizumab in the presence of raised inflammatory markers. Patients who develop a thrombotic state should have the anticoagulation upgraded to a therapeutic dose and should be continued post-discharge.

CONSENT FOR PUBLICATION

Not Applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 4

Common and Rare Dermatologic Manifestations Registered in COVID-19 Patients

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Abstract: The novel coronavirus (COVID-19) causes a severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that has become a pandemic. In spite of several studies, the more time passes, the more symptoms are reported among COVID-19 patients. Surprisingly, numerous dermatological manifestations are also reported. This chapter focuses on the dermatological manifestations caused by COVID-19 infection. We overviewed and classified common and rare dermatological symptoms among COVID-19 patients and their pathophysiological mechanisms. We also discuss appropriate therapeutic management and attitudes, which may provide insights for dealing with similar cases in medical centers.

Keywords: COVID-19, Dermatological manifestations, Pathophysiology, Therapeutic management.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which caused Coronavirus disease 2019 (COVID-19), has become a pandemic pulmonary disease since early 2020 with common symptoms such as cough, fever, sore throat and abdominal pain. Global organizations such as the World health organization (WHO) have focused on studying the structure, pathogenesis mechanisms, treat-

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ment protocols and vaccines. In addition to typical symptoms, as the number of patients increases (more than 267 million confirmed cases of COVID-19, including more than 5 million deaths up to December 2021), more symptoms and complications have been reported for COVID-19 and also pre- and post-COVI--19 syndrome, such as neurological and cerebral, cardiovascular, musculoskeletal and even cutaneous complications (Fig. **1**) [1].

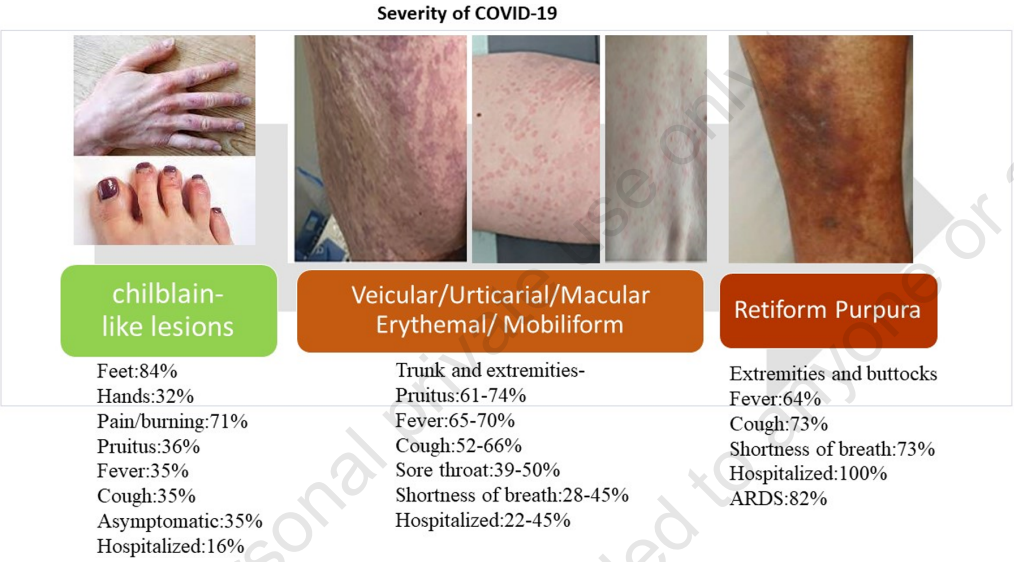

Fig. (1). Spectrum of skin symptoms in patients with COVID-19. Disease severity is calculated based on the proportion of patients who were hospitalized due to ARDS, Acute Respiratory Distress Syndrome (Designed by authors) (All figures are free to access reports).

Evidence-based reports for dermatologic manifestations of COVID-19 are rising, which could be helpful in the early detection and treatment of COVID-19 [2 - 7]. Despite the uncertainty of the pathogenesis mechanisms, many efforts are being made to explore them.

Besides COVID-19 and its medications, lifestyle changes and side effects of medicines, such as drug reactions, could be other possible mechanisms that could cause skin manifestations [8].

Vesicular eruptions, urticarial lesions, maculopapular eruptions and livedo or necrosis are the most commonly reported skin complications in different ages with wide ranges of severity from mild to severe cases [4].

However, there are some challenges that question if these complications are directly contributed to COVID-19, or if they occur because of altered immunity, weakness of the skin barrier or frequent use of immunosuppressant medications in the treatment of SARS-CoV-2 or vaccines complications [9, 10].

Receptors of angiotensin-converting enzyme-2 (ACE2) protein help SARS-CoV-2 to bind and enter into cells and endocytosis in epithelial cells. SARS-CoV-2 disrupts the normal activity of ACE2 by binding it. ACE2 could deactivate Angiotensin (Ang) I and II, which are responsible for fibrosis, oxidative stress and inflammation, and also cause a systemic response in most tissues, such as vasculature, brain and kidney. Reactive oxygen species formed by increased activity of Ang II could disrupt physiological processes by interrupting antioxidant and vasodilator molecules [11].

There are also some evidences that the increased risk of SARS-CoV-2 infection and its complications is associated with owing some diseases, such as blistering skin diseases, including pemphigus Vulgaris (PV) and bullous pemphigoid which could increase skin rashes due to skin barrier disruption, immune dysfunction and use of systemic immunosuppressive therapy [12]. Cytokine storm of COVID-19, which increases TNF-α and IL-17, could explain these findings. The same results were reported for psoriasis [13], peripheral arterial disease in diabetic patients [14] and also autoimmune bullous skin diseases (AIBD) [15].

Rapid viral infection of COVID-19 passage in the dermal vascular system stimulates the immune system to activate the Langerhans cells in all parts of the body that trigger circulating immune complexes to cause urticarial vasculitis or reactions. Subsequently, these immunocomplexes stimulate CD4+ T helper lymphocytes to produce cytokines, including IL-1, interferon (IFN-γ), or TNF-α. They also may recruit B cells, eosinophils, CD8+ cytotoxic T cells and natural killer (NK) cells which could cause lymphocytic thrombophilic arteritis (LTA) and livedo vasculopathy [16, 17].

In this chapter, we tried to review and classify common and rare dermatological symptoms among COVID-19 patients and their pathophysiological mechanisms. We also discussed impressive therapeutic management and attitudes, which may provide insights for dealing with similar cases in medical settings.

COMMON AND RARE DERMATOLOGICAL SYMPTOMS

Although certain pathological pathways are not introduced for SARS-COV-2 associated skin manifestation, three pathological mechanisms are suggested to explain them: (1) Immune responses to viral infections, which resulted in clinical features such as viral exanthem, (2) COVID-19-associated vasculitis and

vasculopathy on which cause secondary cutaneous lesions and (3) adverse drug and vaccination reactions. The vaccination-induced skin lesions are rare in reports [8].

A strong immune response due to SARS-CoV-2 is derived from unique and effective nonstructural protein (NSP) functions. NSP3 could block the host's innate immune system response and amplified cytokine expression, NSP5 may inhibit IFN signaling and prevent melanoma differentiation-associated gene 5 (MAD5) recognition, and NSP16 impedes innate immunity. Direct T cell viral infection by SARS-CoV-2 is also confirmed by the detection of RNA of SARS-CoV and Virus-like particles (VLP) in T lymphocytes which could trigger a severe immune reaction and may cause dermal lesions.

COVID-19 could trigger cytokine storm (IL-6) by initiating hyperactive immune responses in patients who may impact the skin by activation of resident cells of the skin immune system (dermal dendritic cells (DDCs)), mast cells, macrophages, lymphocytes, and neutrophils, and can promote the development of skin lesions which are similar to hyperimmune response and excessive cytokine releases in diseases, such as antiphospholipid syndrome and systemic lupus erythematosus (SLE) [18].

Vesicles or Pustules (Pseudo-Chilblain)

Cold weather could trigger maladaptive vascular response and inflammatory skin reaction, which may cause chilblains. Late symptoms, including erythematous and edematous macules, nodules, violaceous or purpuric patches and swellings and sometimes ulcerous plaques over the dorsal surface of fingers and toes on the feet (74%–100%) with similar appearance, were also reported in COVID-19 patients called pseudo-chilblain lesions which often cause pain and itching [19]. Pseudo-chilblain, also known as “COVID toes”, are usually round and have multiple lesions with a wide range of sizes. They could impact the whole toe with a clear distinction at the metatarsophalangeal (MTP) joints. In rare cases, pseudo-chilblain lesions appeared in the legs, ankles, arms, elbows, thighs, forearms and ears. The common features in all reported cases are red, purple, brown or grey background areas with red to purple globules. In one of three cases, a grey-brown reticular network is reported in the peripheral background area [20, 21].

France, Italy, Spain and the Middle East reported the first cases with SARS-CoV-2- associated chilblain-like lesions. As one of the most common COVID-19 skin lesions, pseudo-chilblain lesions are more frequent in young patients (children and adolescents) with mild symptoms and particularly white patients (89% in the United States and Europe) and rare in black or African Americans (0.7%) and Chinese (no cases) from November 11, 2019, to September 30, 2020 [22].

Reported cases of these lesions during the COVID-19 pandemic have increased (28.6 per 100,000 person-years (95% CI 26.8-30.4)) in comparison with the pre-pandemic period from three years before December 2019 (5.2 per 100,000 person-(95% CI 4.8-5.6)).

Viral-induced intravascular diffuse coagulopathy may cause these lesions that could form the induction of diffuse thrombosis with the presence of eosinophils and also activation of the mannose-binding-lectin (MBL) pathway through the SARS-CoV-2 spike glycoprotein [23].

In the presence of an antigen, innate immunity defense is nonspecific. Cell-associated receptors (PRRs), or pathogen-recognizing molecules (PRMs) as pathogen-detecting receptors, identify foreign agents by pathogen-associated molecular patterns (PAMPs). Endogenous serum of MBL (sMBL), as one of the PRPs/PRMs, could bind microorganisms and activate the complement system. However, infection of SARS-CoV-2 disrupted immune reaction by its spike protein [24]. sMBL interferes with the entry of SARS-CoV-2 and binding of S protein to host receptors, and the triggering of a downstream anti-viral innate immune response. N-linked glycosylation plays a critical role in the specific interaction between sMBL and SARS-CoV S1 protein. Some reports investigated sMBL deficiency correlation and SARS-CoV-2 infection.

There are some reported cases of nail splinter hemorrhages (13%) [25] and dilated nail fold capillaries with loss of polarity and sub-corneal hemorrhagic dots [26]. Pericapillary oedema and micro-hemorrhages were reported on the toes frequently [27], but the rate of capillary anomalies, including dilated capillaries, was equal on the fingers and toes (Fig. **2**).

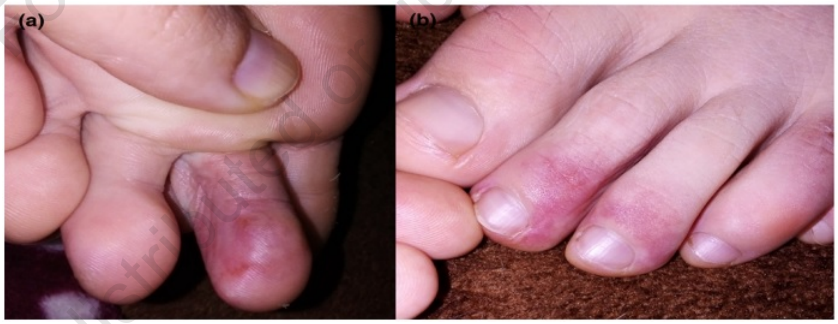

Fig. (2). Pseudo-chilblain lesions. **(a)** Erythema and swelling on the third left toe; **(b)** purpuric macules on the second and third right toes [28]. (Copyright © 2021 *the International Society of Dermatology*).

The pseudo-chilblains are often reported in the last stage of the disease and are associated with the progression of the disease. Pathophysiologic mechanisms are

suggested for pseudo-chilblains in COVID-19, such as immune-mediated inflammation or micro-thrombosis. Pseudo-chilblains and chilblain lupus have some histopathologic resemblances. IFN 1-induced immune stimulation in both of them could produce chilblain-like lesions by creating microangiopathic changes. An immune receptor induced by IFN 1 and IFN-induced helicase C domain-containing protein 1 (IFIH1) could trigger a cascade of anti-viral immune responses by inducing proinflammatory cytokines. The distinct frequency of pseudo-chilblains in white patients may be attributed to the presence of specific IFIH1 polymorphism (Allele T in chr2:162267541 (GRCh38.p13) allele C>T) [29] in them, which enhanced interferon production. In contrast, the allele C is more common in African and Chinese [30]. This could explain the effect of skin color in appearing these pseudo-chilblain lesions due to significantly higher IFN repone [28]. The other suggested causing mechanism is prothrombotic coagulopathy with venous thromboembolism, microvascular thrombosis, elevated D-dimer and high fibrinogen. The association of antiphospholipid antibodies with pseudo-chilblain lesions was also found in some studies [31]. Most patients recover spontaneously without any particular complication from 10 to > 8 weeks. Oral gabapentin is suggested for pain control. Rarely oral analgesics and anti-histamines were prescribed to the patients (Fig. **3**) [32].

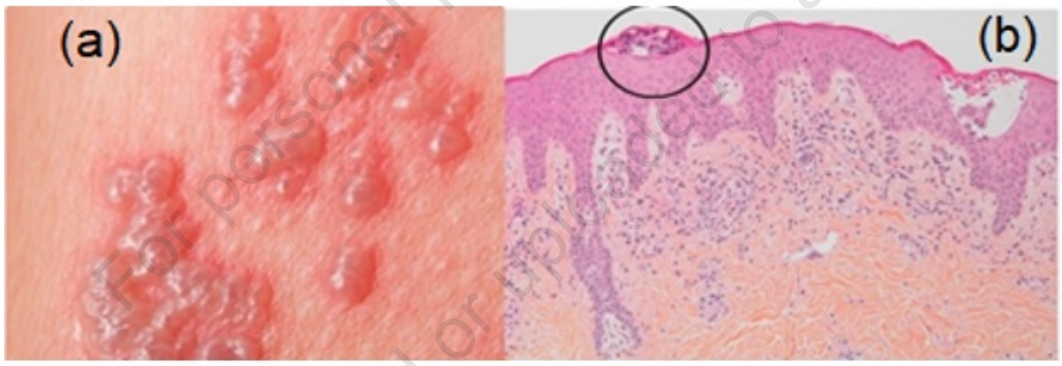

Fig. (3). Vesicular lesions associated with COVID-19. **(a)** Vesicular eruptions associated with COVID-19; **(b)** skin biopsy with spongiotic vesicular eruption [33] (open access license).

The dys- and hyperkeratotic cells, epidermal atrophy and vacuolar degeneration are histopathological findings of this viral infection. The other features of these lesions include unilocular vesicles formed by suprabasal non-ballooning acantholysis, the absence of vasculitis and “Pomegranate-like” eosinophilic dyskeratosis. Although there is no evidence that confirms the existence of SARS-

CoV-2 viral particles within vesicles by RT-PCR, false-negative results must be considered due to low viral loads [34].

The underlying mechanisms of forming such vesicular eruptions are unknown yet. It seems that this virus could stimulate dermis cells, such as secretory and vascular endothelial cells, by expressing spike protein which leads to certain changes in the surface of the epidermis [35]. Some reports suggested that COVID-19 infection can lead to lymphocytic vasculitis, similar to that seen in thrombophilic arthritis, activating cytokines by the formation of immune complexes in the blood, which causes the following immune response that is responsible for vesicular eruptions [36]. Such manifestations could create by aggregation of micro-thromboses in organs which decreases blood flow in the cutaneous microvasculature system and leads to low-grade disseminated intravascular coagulation (DIC) and hypoxia-related deoxygenated blood pooling in the venous plexuses [33].

Urticarial Lesions

COVID-19 infection could induce urticaria with trunk or generalized distribution as circumscribed erythematous wheals by histamine-mediated angioedema and degranulation of mast cells. Urticaria could occur in deeper dermal edema. These rashes may develop or arise before or simultaneously with systemic symptoms of COVID-19 [37]. The reported cases belonged to all ages and more in women. Anti-histamines, such as systemic and topical steroids, are good candidates for the management of these rashes [38]. Due to previous studies, urticarial lesions are recognized as dermal lesions of adverse drug reactions. However, this doubt was removed by reporting urticarial lesions in COVID-19 cases with no changes in their prescription or presenting prior to the commencement of therapy,

Systemic inflammatory response, including complement system activation through a triggered-enzyme cascade and alteration of the cytokine-chemokine environment following the response of the human immune system to an acute infection, could cause these skin manifestations in COVID-19 patients. This progress could activate degranulated mast cells, representing subsequent systemic organ damage in COVID-19.

Increasing circulating IL-6 was also reported in similar cases. Other studies also showed that SARS-CoV-2 glycoproteins were colocalized in peripheral cutaneous blood vessels, which led to increased levels of respective complement mediators that may be attributed to urticarial pathogenesis [34].

The association of urticaria was shown with eosinophilia in some COVID-19 cases. This could help to use protective mechanism of eosinophilia for prognosis. In some cases, urticaria appeared as an initial symptom of COVID-19 before the appearance of the typical COVID-19 symptoms. The appearance of these lesions before the positive result of the PCR test is a very interesting issue for clinical

trials as a prognosis symptom of COVID-19. Eosinophilia may also occur as a result of drug hypersensitivity in cases of urticaria [39]. Urticarial rashes also frequently coincided with the onset of non-cutaneous symptoms (63.2%) compared to before (2.6%) or after (34.2%).

Maculopapular Eruptions

Maculopapular eruptions with general characterizations, including erythematic, purpuric rash and morbilliform, usually appear in COVID-19 patients before any other symptoms. A maculopapular lesion with diverse morphological representations and variation in distribution and appearance showed several features, such as the exhibition of perivascular dermatitis (usually superficial) with infiltration of lymphocytes and dilatation of vessels in the papillary and mid dermis. The epidermis showed sub-corneal eruptions, foci of parakeratosis, hydropic variations, basal cell vacuolation, and minor spongiosis. Biopsy of skin lesions also showed a lichenoid esophagitis pattern (LEP) [40].

These SARS-CoV-2 linked maculopapular lesions have various distributions and appearances, including diffuse to distributed erythematous maculopapular lesions and macules coalescing into papules to plaques. These lesions could be valuable for application in the detection, control, and prediction of the disease's severity and may lead to early identification of this eruption as signs of COVID-19 (Fig. **4**) [40].

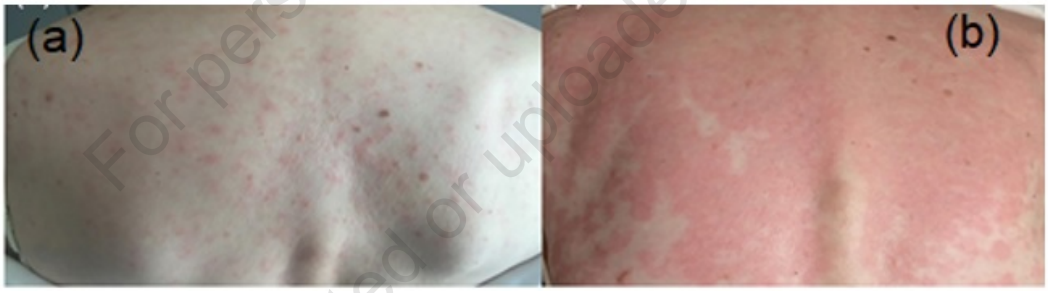

Fig. (4). Appearance of morbilliform eruptions after the start of the COVID-19 symptoms on the back of a 90-year-old woman **(a)** first day **(b)** after 3days [41]. (License Number 5366930898500).

In a novel report, a case showed maculopapular eruption, some chills and generalized fatigue after receiving the COVID-19 vaccine, and classic symptoms of COVID-19, including oppressive chest pain, fever, cough, tachycardia and fatigue [42]. In other reports, it has been shown that Erythematous maculopapular rashes were more likely to present concurrently with other symptoms (58.6%) compared to before (1.7%) or after (39.6%) [43].

Some drug therapies for managing COVID-19 disease, like chloroquine/ hydroxychloroquine and lopinavir/ritonavir, have reported skin complications such as maculopapular eruptions. However, similar rashes were reported in other patients who had not administrated any drugs that could reject the association of these maculopapular rashes with drugs [44].

Livedo or Necrosis

SARS-CoV-2 binds to vascular endothelium and causes inflammation, and massive systemic thromboembolic procedures could induce the formation of spotted, erythematous lace- or net-like vascular pattern and violaceous discoloration, called Livedo [37]. Necrotic acral skin manifestations induced by a hypercoagulability state were also reported in severe COVID-19 [45]. Livedo or necrosis is associated with vascular phenomena. Pathogenic necrosis showed changes in hyper congestion of microscopic venous vasculature by restricted arterial inflow, exaggerated dilation of venous, or impaired venous outflow.

Endotheliitis may have also contributed to the necrosis. SARS-CoV-2 could directly infect endothelial cells in numerous tissues and cells, including the brain, gut, kidney and lungs. The spike protein of this virus is associated with necrosis. Endothelial cell infection could cause endotheliosis changes that may impact coagulation and vascular homeostasis, resulting in necrosis. Increasing fibrinogen and alterations of d-dimer in the prothrombin and partial thromboplastin could show coagulopathy of COVID-19 as an underlying mechanism of necrosis as an early sign of developing the disease [46].

COVID-19-linked livedoid exhibits inflammation of superficial perivascular composed primarily of lymphocytes as well as thrombotic vasculopathy of the deep dermis. These rashes have also shown elevated vascular deposition of C4d and C5b-9 in the deep dermis.

Complement activation occurs in many dermal diseases through three pathways: classical, lectin and alternate [47].

C5b-9 is a component of complement activation in several tissues, such as choriocapillaris, kidney and endoneurium, and the incused by nephropathy, neuropathy and retinopathy. Microvascular deposition of C5b-9 is assumed to be a pathogenetic factor for microangiopathy. By activation of classical and lectin pathways, the C4d is produced as a sign of complement activation. C4d could bind covalently to erythrocytes, B lymphocytes and reticulocytes to create a stable, long-lived cell-bound marker that could be quantified by flow cytometry (Fig. **5**) [47].

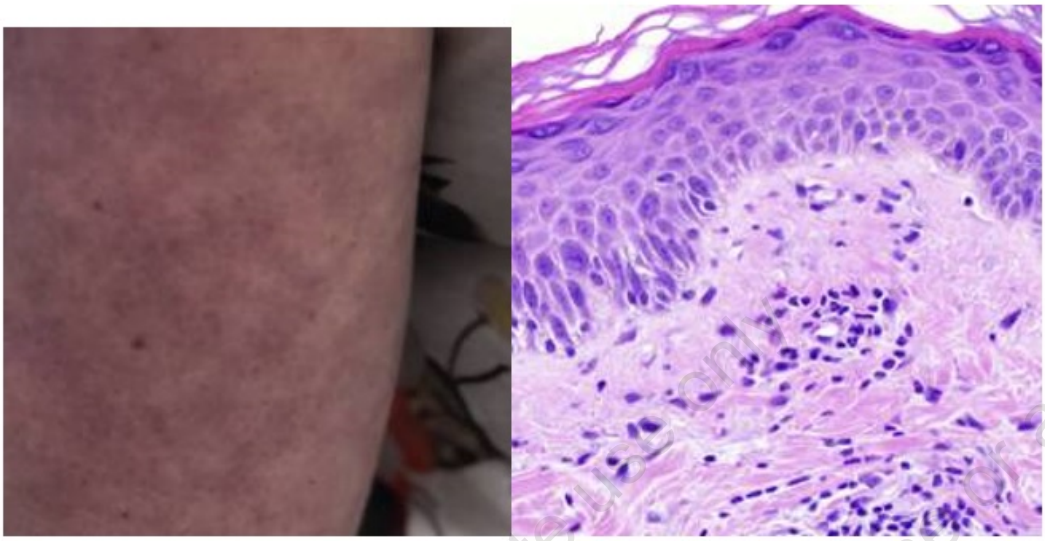

Fig. (5). Morphologic and histologic features of SARS-CoV-2–associated LR **(a)** Morphologic features (Photographs of the arm.) **(b)** Histologic features (Hematoxylin-eosin stain. Original magnifications: ×200.) Four-millimeter skin punch biopsies of the wrist and thighs found perivascular lymphocytic inflammation, increased superficial dermal mucin, and necrotic keratinocytes consistent with viral exanthem (open access article under the CC BY-NC-ND license).

RARE MANIFESTATIONS

Enanthem or Purpuric Flexural Lesions

Larger variants of petechiae called purpura are less common lesions of COVID-19. Fever and significantly thrombocytopenic as a rare complication were described in these few patients with extensive purpuric skin rash and coalescing macules on the right periaxillary region [48]. The other reported symptoms were intermittent respiratory distress, debilitating neuropathic pain and sensorimotor peripheral neuropathy, which explain these rashes as a result of the multisystem inflammatory syndrome [49].

These findings introduced purpuric vasculitic pattern as one of the classified COVID-19 dermatological manifestations. In some cases, the purpuric features are shown in a large morbilliform lesion that preserves mucosa in acute symptomatic stages of COVID-19 illness, disproving the post-viral syndrome. Although some cases showed purpuric rash with a milder form of SARS-CoV-2-associated disease, the other cases represented severe inflammatory reactions linked with COVID-19 (Fig. **6**) [50, 51].

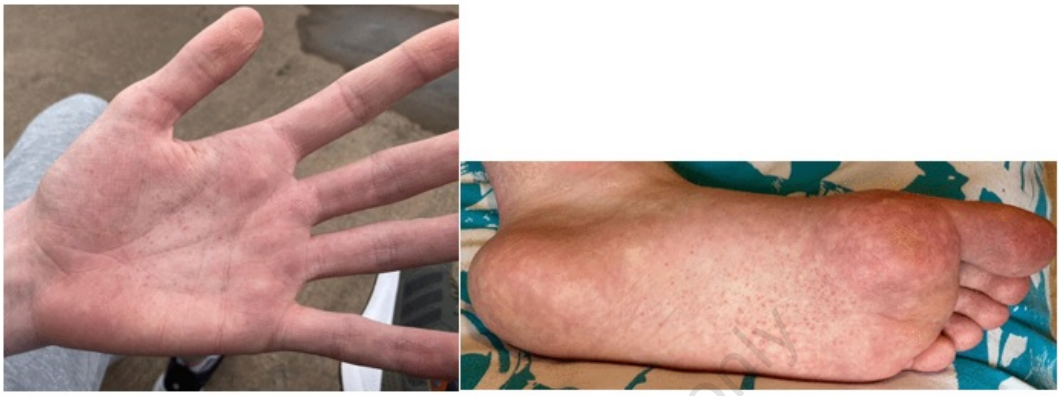

Fig. (6). Palmar (left) and Plantar (right) petechial rash in SARS-CoV-2 multisystem inflammatory syndrome [50]. (License Number 5366960822343).

Thrombotic microangiopathy in the superficial dermis with blistering and vesicle formation is suggested as an underlying histopathological finding within livedoid and purpuric lesions and vesicular lesions [52]. Other reported dermal rashes in association with COVID-19 are symmetrical drug-related intertriginous and flexural exanthema (SDRIFE)-like. The patient had bilateral pneumonia and severe hypoxaemia. Thus, she receives hydroxychloroquine and azithromycin. Axillae and antecubital fossae also showed developed erythematous with subcorneal pustules and superficial infiltrates of lymphocytes and eosinophils, representing histopathological features in acute generalised exanthematous pustulosis (AGEP). Considering possible drug reactions, it did not determine if the lesion was an atypical SDRIFE-like rash secondary to COVID-19 [53].

In other cases, the flexural accentuation of AGEP is a rare drug reaction that showed widespread erythema with several small pustules. Histopathological findings represented spongiform subcorneal and intracorneal neutrophilic pustule, acanthosis, neutrophilic exocytosis, and rare necrotic keratinocytes as side effects of Hydroxychloroquine. These features were reported in at least 35 cases [54].

Multisystem Inflammatory Syndrome in Children (MIS-C)

The other rare reported in COVID-19 patients is MIS-C, characterized by fever, inflammation evidences, multisystemic and severe disease and dermatologic features including polymorphic, maculopapular, morbilliform, erythrodermic, urticarial, reticular, petechial, purpuric in variable anatomic distribution [55].

There is also a similar report about three pediatric cases with skin rashes in their faces and on the trunk induced by Hydroxychloroquine toxicity [56].

MEDICINAL PLANTS FOR COVID-19 SKIN INFECTIONS

COVID-19 skin manifestations are challenging conditions for patients and medicines. We overview these dermatological manifestations. Fortunately, most of these rashes eliminate gradually and spontaneously without the requirement of drugs. However, some of them may remain as scars that could affect the appearance of the patients. Medicinal plants play a vital role in hindering viral infections. Their active constituents with anti-septic properties, including flavones, alkaloids, and polyphenols, have shown favorable effects in treatment. They showed positive effects on treating various forms of dermatitis. They also could moisturize and smoothen the skin, possess antimicrobial and high antioxidant activity, and inhibit lipid peroxidation. Some well-known herbals are oils of sunflowers, flaxseeds, castor, lavender, rosemary, thyme, sandalwood, olives, eucalyptus, cloves, and fennels. In the following section, we described important plants with healing properties of skin manifestations [3].

Leaves of Devil's horsewhip or *Achyranthes aspera*, a perennial tropical plant (India, Sri Lanka, tropical Asia, Africa, Australia, and America), are applied as traditional medicine with many health benefits. Triterpenoid saponins, as the main chemical ingredients, contain oleanolic acid as an aglycone and are rich in alkaloids, flavonoids, saponins, and steroids, such as sitosterol, ecdysone, and ecdysterone that provide anti-allergic activity. In India, they mix leaves and roots with water or milk and use them as a paste on the skin to treat rashes and irritation.

Acorus calamus or sweet flag, a perennial plant in India, central Asia, southern Russia and Siberia, and Europe, has alpha and beta asarone as oils in their leaves which treat all types of skin lesions like rashes, while acorenone and isocalamendiol such as saponins, lectins, sesquiterpenoids, steroids, and lignans in rhizomes. Antimicrobial, anti-fungal, and anti-viral features of this plant are also considerable.

The other perennial plant *Alpinia galangal*, belongs to the ginger family in all Southeast Asian countries like India, Bangladesh, China, and Surinam, showed antibacterial and anti-fungal activities by reducing the formation of acne which is because of the presence of flavonol. It could reduce skin inflammation and remove scars or calluses from the skin. The people applied it as an anti-septic cleanser to smoothen the skin and give its antibacterial activity.

Bulb onion or *Allium cepa* contains polyphenols, anthocyanins, phytosterols, and saponins, which exhibit antioxidant properties and could treat acne and reduce skin inflammation. Anti-histamine property of quercetin in its onion declines nasal congestion.

Garlic or Allium sativum contains sulfur compounds, such as allicin, ajoene, diallylpolysulfides, vinyldithiins, and S-allylcysteine with enzymes, saponins, and flavonoids. Its anti-fungal, antibacterial and anti-viral activity is well-known. Its anti-viral properties make it the most applied in diets as a food ingredient or plant medicine for the treatment of COVID-19. It could enhance natural killer cell activity to eliminate the infected cells. Its antioxidant properties help to protect DNA against free radicals and UV-induced skin cell damage.

Aloe vera contains aloe emodin, aloetic acid, anthranol, steroids such as campesterol, beta-sitosterol, and various enzymes that help to decline scars by enhancing collagen content. It is also applied for the treatment of radiation dermatitis, seborrheic dermatitis, and acne in the skin.

Shatavari or *Asparagus racemosus* contains asparagamine A, a polycyclic alkaloid, steroidal saponins, shatavaroside A, shatavaroside B, filiasparoside C, shatavarins, and isoflavone and also flavonoids such as quercetin and rutin which showed antibacterial and anti-fungal properties to protect the skin.

Neem or *Azadirachta indica* contains various important constituents such as nimbin, nimbidin, nimbolide, limonoids, quercetin, beta-sitosterol (polyphenolic flavonoids), azadirachtin, azadirachtin A, and betasitosterol, which could modulate various genetic pathways. The plant has strong antioxidant and antimicrobial properties, including anti-viral characteristics. Recently, this plant extract has been used in the treatment of COVID-19 as it contains polyphenols. It could positively affect the treatment of dermatitis eczema, skin rashes, skin redness, itching, acne, and skin damage from ultraviolet.

Acorus calamus or sweet flag, a perennial plant in India, central Asia, southern Russia and Siberia, and Europe, has alpha and beta asarone as oils in its leaves which treat all types of skin lesions like rashes, acorenone, and isocalamendiol such as saponins, lectins, sesquiterpenoids, steroids, and lignans in rhizomes. The antimicrobial, anti-fungal, and anti-viral features of this plant are also considerable.

Calendula officinalis or pot marigold contains lutein, zeaxanthin, and beta carotene, while the flowers contain flavonol glycosides, triterpene, saponins, and sesquiterpene could be useful in treating skin rashes, dermatitis, skin irritation due to radiation, and skin inflammation. Camellia sinensis, or green tea, is well-known for antioxidant activity owed to carotene, riboflavin, nicotinic acid, pantothenic acid, and ascorbic acid. Cannabis or Cannabis sativus: contain cannabinoids such as cannabidiol, cannabichromene, cannabigerol, terpenes, and phenolic compounds, which help in the treatment of contact dermatitis, pruritus, skin inflammation, and even skin infections given the presence of polyphenols and

flavonoids. The papaya plant, or Carica papaya, contains carotenoids, vitamin C, and many other vitamins, their leaves have alkaloids, carpaine, and pseudocarpine, and their fruits owe various nutrients. Protease enzymes or papain could treat various skin rashes such as acne, dermatitis, and eczema [57].

DISCUSSION

COVID-19 as a pandemic directly or indirectly had adverse impacts on all aspects of people and society, which almost hindered all non-vital activities. Some complications disrupt the person's daily activities, and some of them affect the person's health for a long time. Several medical centers faced numerous challenges in dealing with SARS-CoV-2: lost medical staff, lack of facilities, lack of sufficient knowledge for dealing with large numbers of suspected COVID-19 cases, lack of accurate and rapid diagnosis, and confusion in prescribing medication. Several case reports have been published over these two years. In early cases, the researchers reported symptoms such as cough, fever, sore throat and abdominal pain. However, the number of reporting other complications like dermatological manifestations is also rising [58]. Initially, the skin lesions in COVID-19 patients were suggested as post-viral or drug (such as immune suppressive medications) complications. However, evidences confirmed them as the main symptoms of SARS-CoV-2 infection [59].

For instance, skin manifestation associated with COVID-19 may cause disfigurement and cutaneous symptoms, which cause considerable chronic and recurrent discomfort and disability. These complications could result in psychological disorders which impair the quality of life [60].

Although the actual frequency of skin findings varies widely in the literature, recent studies indicate a frequency close to 6% [61]. The most frequent dermatological manifestations based on large national and international series on skin manifestations were maculopapular eruptions. Urticarial and vesicular eruptions, as well as purpura and necrosis, are described less frequently. In terms of onset, skin lesions usually occur simultaneously as general symptoms and may rarely precede them, as in urticaria and vesicular lesions. Vascular disorders such as livedo, purpura and necrosis are also late manifestations but are associated with greater severity and late prognosis [62]. Some cutaneous findings were also reported in COVID-19 patients as a consequence of vaccination, especially in mRNA vaccines [10, 63 - 68]. Investigation of various cutaneous manifestations also showed that they coincide with the onset of other COVID-19 symptoms (45.05% of all cases rather than before or after them) [43].

Decreased reporting of skin complications can be attributed to insufficient knowledge of non-dermatology frontline workers in recognizing and diagnosing

cutaneous complications and diseases for evaluating patients with COVID-19 [69], particularly in color people [70]. Failure to perform the PCR test at the optimal time could also affect the number of reported skin manifestations associated with COVID-19 [71].

CONCLUSION

Clinical presentations of dermatological findings in SARS-CoV-2 infection are extremely varied from early appearance before classic symptoms to presenting after becoming symptomatic. The diversity and variability of reported morphological appearances observed could resemble various underlying mechanisms of SARS-CoV-2 [58].

Genomic variations in SARS-CoV-2, which affected its antigenicity, may induce a different immunologic reaction. The most reported mutations are contributed to the spike protein. However, this important issue has not been considered in SARS-CoV-2-associated skin manifestations studies [59].

Despite increased knowledge of SARS-CoV-2 virus pathogenesis and its complications, further studies are necessary to improve our information to understand the underlying mechanisms and suggest medical management for the consequences.

In spite of increasing COVID-19-associated skin manifestations in reported cases worldwide, one of the considerable limitations is the low number of cases reported due to a lack of knowledge in recognition of these features. Thus, improving the knowledge of clinical staff and employing an expert physician to reveal clinical characteristics and pathological mechanisms of these manifestations.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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CHAPTER 5

Circulating Biomarkers of Cardiopulmonary Disturbances in COVID-19

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Abstract: Significant findings have been obtained on the relationship between underlying cardiovascular disease and the severity of COVID-19 infection. Using plasma profiles of patients with COVID-19, biomarkers in circulation were also identified that varied depending on the patient's characteristics and disease. The purpose of this study is to review the sources that focus on circulating biomarkers of cardiopulmonary disorders. In addition to conventional biomarkers such as troponin, we consider data from new emerging biomarkers about their roles in the prognosis of severity, mortality in the hospital and effectiveness of treatment. Consideration of mechanisms associated with circulating biomarkers in various conditions associated with COVID-19 can provide broader tools for the diagnosis, treatment, and prognosis of at-risk patients.

Keywords: Circulating Biomarkers, Prognosis, Severity, Cardiopulmonary Disorders, COVID-19.

INTRODUCTION

Due to the complex nature of COVID-19, there exist increased severity of SARS-COV-2 disease and the high mortality rate of such patients, especially due to cardiovascular diseases, decreased viral clearance, increased ACE2 expression,

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and metabolic disorders. Circulating biomarkers are indicators that can detect and monitor the status of biological processes, pathological features, or responses to a therapeutic intervention.

HEMATOLOGICAL BIOMARKERS

In severe COVID-19, there may be a significant increase in leukocytes, neutrophils, infection-related biomarkers (CRP, PCT), tumor necrosis factor (TNF), ferritin, and various cytokine levels (IL-2R, IL-6, IL). -8, observed IL-10) [1].

The coagulation profile of COVID-19 is characterized by released intravascular coagulation. However, with increased fibrinogen, prothrombin time (normal or slightly prolonged) and activated partial thromboplastin time (aPTT) as well as platelet counts vary by more than per milliliter (without significant bleeding) [2]. Increased levels of D-dimer can often be seen in patients with COVID-1.9. This feature is effective in determining the prognosis and is associated with the severity of the disease and mortality in the hospital. At admission, D-dimer levels above 2.0μg / ml may even determine mortality in COVID-19 patients [3, 4].

CARDIAC BIOMARKERS

The role of cardiac biomarkers in the diagnosis, triage, treatment, and prognosis of COVID-19 is also well known. An increase in cardiac biomarkers such as Lactate Dehydrogenase (LDH), Creatine Kinase (CK), Cardiac Troponin I (cTnI), Creatinine kinase-muscle/-brain activity (CK-MB), Myoglobin (Mb), Alpha Hydroxybutyrate Dehydrogenase (α-HBDH), Aspartate Aminotransferase (A) N-terminal (NT) -pro hormone BNP (NT-proBNP) has been observed in patients with COVID-19 [5]. Elevated CK-MB, cTnI, Mb, and NT-proBNP have been associated mainly with myocardial injury and especially with higher mortality, in severe and critical conditions [6 - 8].

Galectin-3

Galectin-3 (Gal-3) is found as a member of the galectin family that specifically binds to beta-galactosides, especially in a variety of immune cells, sensory neurons, epithelial and endothelial cells [9]. In cardiac cases, Gal-3 has been introduced as a useful tool in the diagnosis of acute heart failure (AHF) and is increased in cases of heart failure (HF). The increase in Gal-3 is also recognized as an effective diagnostic tool in the diagnosis of AHF. Galectin-3 expression levels increase over time in heart failure associated with fibrosis, atherosclerosis, adverse cardiac remodeling, and inflammation [10]. Increased activity of fibroblasts and macrophages in cardiac tissues during HF is associated with the

overexpression of Gal-3 [11]. Clinical value of effective predictors such as the novel biomarkers like MR-ProADM [12] and GDF-15 [13] has been shown in various studies (Fig. **1**) [14].

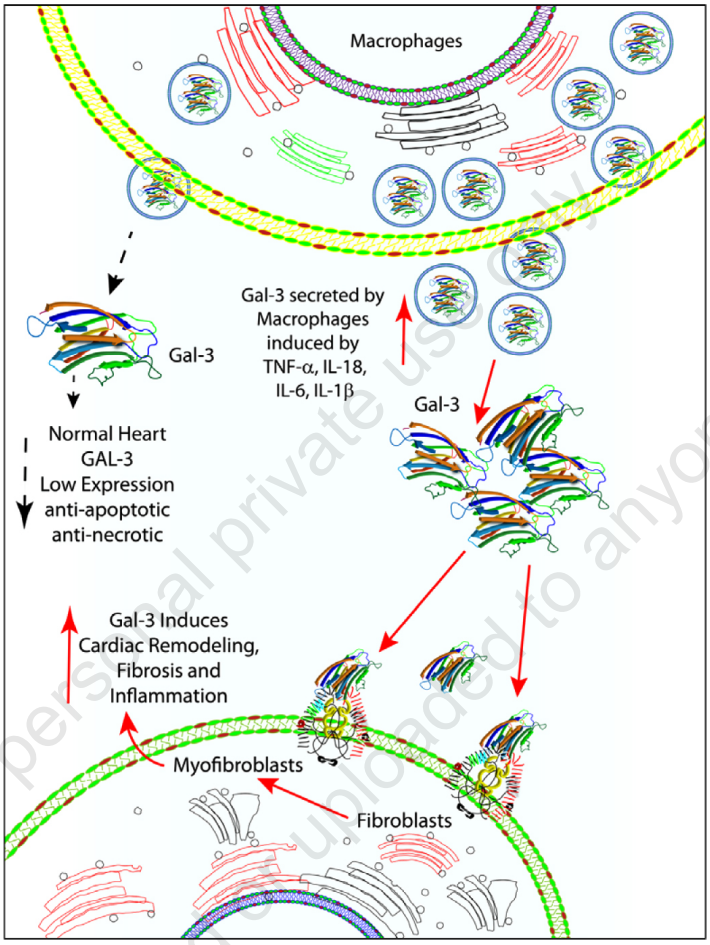

Fig. (1). Gal-3 and cardiac remodeling in COVID-19 [8].

COVID-19 AND CARDIAC BIOMARKERS

Cardiac Troponins (TNS)

Cardiac troponins include three types of regulatory and functional proteins: troponin C (TnC), troponin T (TnT), and troponin I (TnI), which play a major role in myocardial contractility. TnC binds intracellular calcium and subsequently TnI, initiating cardiac muscle contraction. During COVID-19 infection, cardiovascular disorders such as microangiopathy, viral myocarditis, renin angiotensin (RAS)

system problems, hypoxia, elevated serum TnC due to inflammatory storms, and cytokine-induced myocardial damage have been observed [15].

Recent international guidelines advise measuring TnT and TnI levels as a prognostic factor for severity of illness and a metric for virus-related cardiac injury in COVID-19 patients, respectively.

Cardiac troponin I (cTnI)

In severe cases of COVID-19, cTnI levels are increased significantly [16]. There is a linear correlation between cTnI> 0.09 ng/dL, underlying cardiovascular disease, severity, poor prognosis and of course the highest mortality. There are also some evidences of an association between high cTnI levels (due to cardiac dysfunction) and mortality in COVID-19 [17]. Elevated cardiac TnI level reflects myocardial injury in COVID-19 infection which is associated with significantly higher in-hospital mortality when compared to patients with a normal TnI level. Mechanisms include myocarditis, microangiopathy, myocardial infarction, and cytokine storm that are the possible causes of this injury [18].

Cardiac troponin T (cTnT)

There is some evidence that indicate correlations between cardiac functional and biochemical changes and high-sensitivity cardiac troponin (hs-cTnT) in COVID-19. Coordinated increases in cTnT with inflammatory biomarkers also indicate myocardial damage (associated with underlying inflammatory responses) [19].

There is some evidence that hs-cTnT correlates with other cardiac functional and biochemical changes in COVID-19. Coordinated increases in cTnT with inflammatory biomarkers also indicate myocardial damage (associated with underlying inflammatory responses) [19].

Endotheliopathy

The incidence of endotheliopathy is an important factor in determining the severity, predicting clinical manifestations, and even mortality of patients during COVID-19 and is associated with increased circulatory factors due to endothelial stress, complement activation, and dysfunction of fibrinolytic processes [20]. Severe acute respiratory syndrome (SARS) caused by COVID-19, along with cellular infection, requires the presence of the angiotensin-converting enzyme (ACE2) [21]. Usually type II pneumocytes, enterocytes as well as endothelial cells are the site of ACE2 expression [21, 22].

Possible pathobiological aspects of endothelial involvement include inflammation, immune system overactivity, lack of protective barrier, and

angiogenesis, leading to decreased fibrinolysis, extensive thrombosis, and, ultimately, end-organ damage [23]. The rapid reaction to COVID-19 first activates innate immunity, and in severe cases, tissue damage (proportional to microvascular damage due to complement activation) is seen (Fig. **2**) [24].

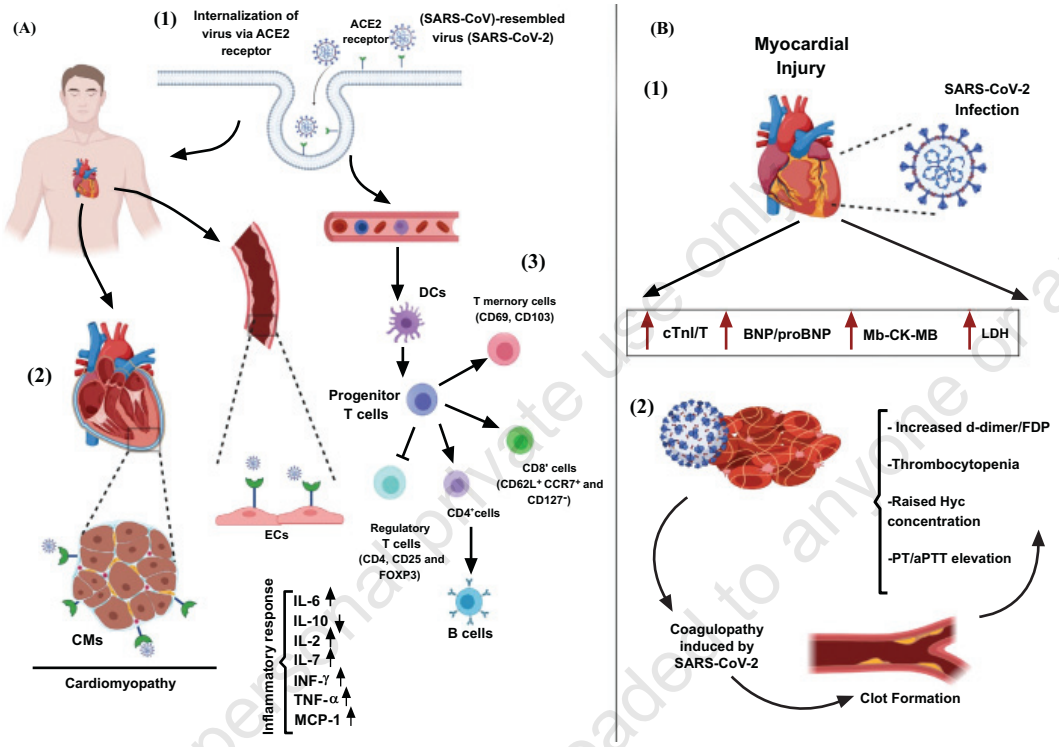

Fig. (2). Mechanism of cardiovascular effects of SARS-CoV-2 virus: part A- 1. Transmission of the virus through binding to ACE2 receptors and expression in pneumocytes, cardiomyocytes and endothelial cells. 2. Cardiovascular damage caused by the virus 3. Hyperactivity of inflammatory responses (cytokine storms) in the late severe phase of the disease. Coronavirus suppresses dendritic (DC) cells and releases interferon-gamma (INF-γ), interferon-gamma (INF-γ), IL-6, IL-10, IL-2, and IL-7, tumor necrosis factor is alpha (TNF-α) and monocyte chemoattractant protein 1 (MCP-1/CCL2) through inflammatory responses. Part B- The effect of SARS-CoV-2 virus on the cardiovascular system leads to cardiomyopathy, which can be detected by measuring specific biomarkers (including cTn I / T, LDH, Mb, CK-MB and BNP / NT-proBNP). Coagulation events often cause clot formation and are associated with high levels of d-dimer / FDP, PTT / aPTT, Hyc, and fibrinogen [25].

Vascular contraction with endothelium (along with increased production and release of cytokines) is caused by endothelial dysfunction and leads to extensive coagulation, which eventually leads to thrombosis and significantly compromised fibrinolysis [26, 27].

Pathological loss of the endothelial glycocalyx surface layer impairs tissue or limb function. Viruses (such as SARS-CoV-2) use glycans, including

glycosaminoglycans, for primary interaction with host cells. Circulation of heparan sulfate (HS) can also be used as one of the indicators of endothelial barrier breakdown [28].

Inflammatory processes affect acute endothelial cells, causing acute phase reactants (such as von Willebrand factor -VWF). Adhesion receptors then enter the bloodstream, causing pathophysiological phenomena that underlie severe COVID-19 [29, 30].

D-dimer

A fibrin degradation product, called D-dimer, as a biomarker for thrombotic disorders has been identified as a potential indicator for its prognosis in COVID-19 patients. A D-dimer value less than 0.5 μg/mL is usually considered normal, and values increase with increasing age and in pregnancy. Increased severity of community-acquired pneumonia could rise the level of D-dimer. Elevated D-dimer and thrombotic complications have been widely reported in COVID-19 patients. Some studies found that a higher D-dimer value on hospital admission was significantly associated with in-hospital mortality in patients of COVID-19. The optimal cutoff value of D-dimer for predicting mortality in COVID-19 patients is 1.5 μg/ml, as compared to 0.5 mg/L in mild cases [31, 32]. Hepatic damage in subjects with severe form of COVID-19 is related to changes in coagulative and fibrinolytic pathways which cause the evaluation of D-dimer blood levels [33].

Post-treatment Changes in Biomarkers

Corticosteroids (especially low to moderate doses of dexamethasone) in COVID-19 change the levels of some dynamic biomarkers. Although there are still limited findings on their effect on circulating cardiac and non-cardiac biomarkers [34], corticosteroid therapy has been reported to significantly improve serum CRP, IL-6, and D-dimer levels in young patients (≤65 years of age) and females [35].

A recent clinical study in hospitalized patients also showed that after treatment with dexamethasone, plasma IL-6 levels were still higher (>10pg/ml) in 62.5% of cases, possibly due to co-expression of the glucocorticoid receptor expression (GR or NR3C1) and IL-6 [36].

In a clinical trial, it was shown that Tocilizumab could lead to a significant reduction in CRP, whereas changes in LDH and D-dimers were not significant [37]. In a retrospective study, Sarilumab administration rapidly decreased CRP levels, which was consistent with clinical improvement. Lower levels of IL-6 and NLR ratio also occurred in a large proportion of patients [38].

NEW EMERGING BIOMARKERS

New biomarkers such as Hcy (homocysteine), Ang II (Angiotensin II), Angiotensin (1-7) and alamandine have been suggested for further investigation, especially in the prognosis of severe cases with cardiovascular complications. The Ang (1-7), renin– angiotensin system (RAS), and alamandine apply their cardioprotective effects (*e.g.*, vasodilation) by increasing the nitric oxide release from endothelium and decreasing nicotinamide adenine dinucleotide phosphate to produce superoxide. Both *in vitro* and *in vivo* tests have shown the anti-inflammatory effects of Ang-(1-7) and alamandine [15].

Recent evidence suggests that high levels of Hcy are of predictive values for cardiovascular complications following macro and microangiopathy (especially in coronary and peripheral arteries). In order to determine the severity of COVID-19 cases, an increase in Hcy levels is also considered by affecting platelet activity on thromboembolic events that may eventually lead to cell death through ferroptosis [39].

More recently, some evidences of the therapeutic and diagnostic value of exosome nanoparticles have been proposed that have a significant effect on intercellular transport of lipids, proteins, and microRNAs (in pathophysiological conditions such as endocrine disorders, cardiovascular disease, inflammation and malignancies) [40]. In COVID-19, exosomes appear to be of great importance, either as interfering with the pathogenesis of the disease or as new biomarkers (especially in personalized diagnostic and therapeutic criteria) [41].

In both critically ill and non-critical patients, a proteomic profile of exosomes was identified, which is primarily associated with coagulation, inflammation, and the reaction of the immune system. Tenascin-C (TN-C) and fibrinogen-β (FGB) have been detected in patients' circulating exosomes, plentifully [42, 43].

As a member of the interleukin (IL) 1 receptors, ST2 is a ligand for IL-33. Its two isoforms include cellular (ST2L) and soluble (sST2). IL-33 and its receptors act as a component of the cardiac defense system which inhibit cardiomyocyte hypertrophy and apoptosis. By binding sST2 to IL-33, several cardiac protective pathways are activated. In fact, IL-33 prevents fibrosis and cardiac hypertrophy *via* κB NF-κB, MyD88, IRAK and ERK signaling pathways [44 - 48]. Presumably, an increase in circulating sST2 levels may be considered as an indicator to assess neurohormonal activation, inflammation, and hemodynamic stress [49, 50].

Acute heart failure (AHF), hospitalization period and death of patients during one year of follow-up [51] can be predicted by measuring plasma levels of sST2 and other biomarkers such as cardiac troponins and NP [52, 53].

Indeed, sST2, along with NPs, are valuable prognostic biomarkers. A strong association has been found between higher sST2 levels at the time of hospital admission and Hospital-patient One-year Mortality Risk in dyspneic patients with or without heart failure (HF) (Fig. **3**) [54 - 56].

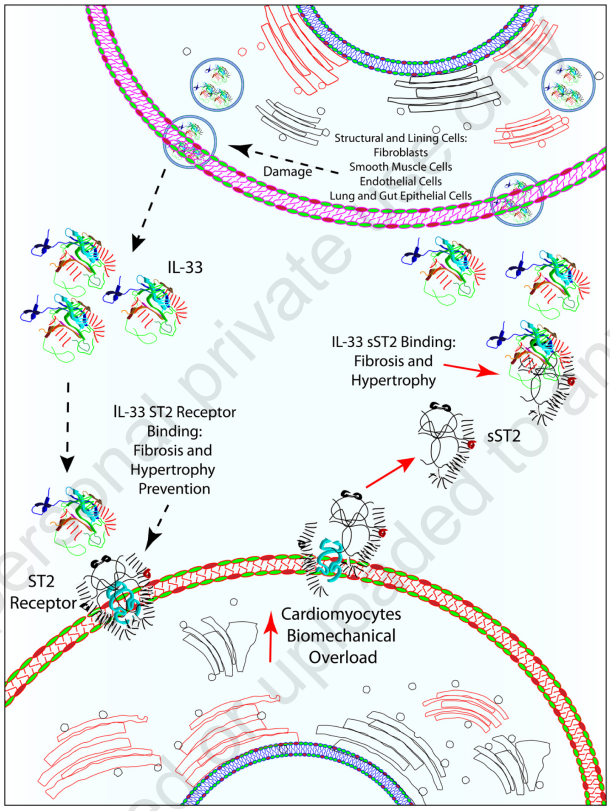

Fig. (3). Schematic effect of ST2 and sST2 on the process of fibrosis and cardiac hypertrophy [8].

In particular, the poorer prognosis was related to the higher sST2 rates at the time of admission to the hospital and significant long-term mortality was predictable through its changes during the first 48 hours [8].

Changes in Cardiovascular Biomarkers During Follow-up

During acute COVID-19, patients with high levels of cardiac troponin may also have an increase in Native myocardial T1 (possibly), extracellular volume (ECV),

late gadolinium enhancement (LGE), and an increase in the number of macrophages on myocardial biopsy [57].

After 6 months, higher levels of cTnT and NT-proBNP were also present on cardiovascular MRI (CMR) along with scarring or reduced left ventricular ejection fraction (LVEF). The highest concentration was detected in patients with major cardiovascular magnetic resonance (CMR) pathology. Fortunately, patients with scarring or decreased LVEF did not experience a greater concentration increase in cardiovascular biomarkers. Therefore, this phenomenon may reflect pre-existing subclinical cardiovascular disease rather than COVID-19-induced cardiac injury [58, 59].

There was restricted significant associations between CMR assessments, edema (*via* T2-weighted CMR), and increased cTnT and NT-proBNP during the hospitalization index, especially as T2 levels increased with age [60]. However, after demographic adaptation and the incidence of cardiovascular disease (CVD), the association between high cTnT and higher T2 levels during hospitalization remained significant. The findings suggest that patients with acute myocardial injury may still be at risk for persistent myocardial edema during recovery in acute coronary infection. However, there is still a limited correlation between cTnT values and other CMR-based pathology criteria [61].

Post-treatment Inflammatory Response and Myocardial Involvement

Higher levels of inflammatory biomarkers and signs of viremia (presence of RNA in plasma) are associated with increased disease severity [62, 63]. However, no association was found between viremia during acute phase or pathological findings on CMR after recovery and inflammatory biomarkers. This indicates a limited relationship between the severity of acute infection and persistent cardiovascular disorders (CVD).

Although COVID-19 causes overactive and inefficient interconnected inflammatory, immunological, and coagulation cascades, observations show that the severity of immune activation is not associated with persistent cardiovascular pathology [64].

CONCLUSION

Cardiovascular diseases are one of the last but most serious complications of COVID-19. Therefore, there is a basic need for prognostic and diagnostic criteria to modify optimal treatment protocols and minimize medical bias. In addition to clinical considerations, initial evaluations focus on continuous monitoring of specific cardiac biomarkers (including cTnI/T, NT-proBNP, Mb, and CK-MB)

and coagulation parameters (such as D-dimer, platelet count, PT, and Hyc) may prevent cardiovascular complications and death from COVID-19.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Declared none.

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CHAPTER 6

Some Aspects of Pathology and Pathogenesis of Coronavirus Infection

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Abstract: This chapter presents an overview of pathology and pathogenesis in coronavirus infections in humans and animals based on literary data and our own experience, illustrated by numerous original images.

Keywords: Coronavirus infection, Coronavirus infection in humans, Coronavirus infection in animals, Pathogenesis, Pathology.

INTRODUCTION

Coronaviruses (Coronaviridae) are a large family of RNA-containing viruses that can infect both humans and animals (their natural hosts) According to the results of serological and phylogenetic analysis, coronaviruses are divided into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. In humans, coronaviruses can cause a range of diseases – from mild forms of acute respiratory infection to severe acute respiratory syndrome (SARS). Four coronaviruses (HCoV 229E, OC43, NL63 and HKU1) have known to be circulating for a long time in the population, they are present year-round in the structure of acute respiratory viral infections and, as a rule, cause damage to the upper respiratory tract of mild to moderate severity [1 - 3]. The histopathology of such lesions remains unknown.

Over the past decades, it has been proven that coronaviruses can cause lesions of varying severity in both wild and domestic animals, causing both chronic and acute life-threatening diseases [4]. In addition to pure veterinary aspects, the questions of comparative pathology in different species are also interesting. Of particular importance, is the possibility of the interaction of various coronaviruses

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with the appearance of strains with new properties [5]. In domestic cats, there are two pathotypes of coronavirus infection. The intestinal form was initially believed to affect only mature enterocytes, but it was shown by improved molecular methods that macrophages are also involved in this process [6]. The generalized infection is called feline infectious peritonitis (FIP). FIPV exists in two serotypes (I and II according to neutralizing antibodies): I has a distinctive S-protein, S-protein of II is recombinant with canine coronavirus. The incidence of FIP caused by type I strains is higher than that of type II [7]. The main feature of feline infectious peritonitis is multiple organ granulomatous perivascular inflammation. Small caliber veins are typically most injured. Activated macrophages and monocytes destroy the basal lamina of affected vessels. B cells are shown to progressively replace macrophages in FIP granulomas [8].

Grossly, granulomatous polyserositis is found. The most affected organs are the brain, kidneys, and eyes, lesions are frequently found in the liver and lungs. Internal organs are often affected by lymphoplasmacytic infiltrates [9]. Sometimes there is pyogranulomatous rhinitis that partially obliterates the ethmoturbinates [10].

The splenic red pulp and lymph node sinuses show follicular hyperplasia, followed by T- and B-cell depletion [6]. Lesions of the central nervous system include signs of hydrocephaly, perivascular pyogranulomas in the leptomeninges, the choroid plexus, the periventricular space, and the parenchyma of the spinal cord and brainstem [11].

A new form of zoonotic coronavirus infection originated in 2002 (reservoir - bats, intermediate hosts-camels), causing acute respiratory syndrome (SARS) with high mortality in humans In 2002-2003 an outbreak of this novel virus originating in Asia resulted in more than 8000 cases and 744 deaths in 29 countries worldwide. No cases were reported since 2004. The virus uses angiotensin-converting enzyme 2 (ACE2) for entry into host cells and infects tracheobronchial and alveolar epithelial and immune cells; an important role is also played by TMPRSS2.

In 2012 a new coronavirus infection – Middle East respiratory syndrome (MERS) has been described in Saudi Arabia, other neighboring countries and Korea. In spite of high mortality, histopathology was reported only in single cases [12]. The primary finding at autopsy was viral-mediated lung damage with features of ARDS. pneumocytes, multinucleated epithelial cells, and bronchial submucosal glands were infected. These infected cells expressed DPP-4 surface antigen, which serves as the host cell receptor for MERS-CoV. Viral inclusions were demonstrated by electron microscopy in respiratory epithelium and in renal proximal tubules.

There are few pathological descriptions in the literature [12], according to which at an early stage (<11 days) the disease was characterized by diffuse alveolar damage with edema, hyaline membranes, alveolar collapse, desquamation of alveolar epithelial cells, the appearance of scattered multinucleated giant cells of uncertain diagnostic significance. The virus antigen was detected in alveolar epithelial cells and macrophages during IHC and viral particles as nucleocapsid inclusions and typical double-membrane vesicles during electron microscopy [12]. After 10-14 days, interstitial/airspace fibrosis and pneumocyte's hyperplasia were described. No extrapulmonary injuries were reported and no specific features were identified at autopsy.

We had no opportunity to self-examine those cases. Since 2020, humanity has faced an infection caused by a new strain of human coronavirus (SARS-CoV-2), characterized by a variety of clinical manifestations of the disease, the lack of etiological therapy, a significant deterioration in the course of concomitant somatic pathology, a fairly high mortality rate, which according to various studies varies widely (from 0.5 to 15%).

SARS-CoV-2 is a single-stranded RNA-containing virus belonging to the family coronaviridae, the genus betacoronavirus. Given its high pathogenicity, it is considered within group 2 of pathogenicity. The genetic sequence of SARS-Co--2 is similar to that of SARS-CoV by at least 79%. A high rate of mutations and successive circulating of different genotypes names after Greek letters (alpha, delta, omicron *et al.*) is typical for the pathogen.

The early symptoms of patients infected with SARS-CoV-2 generally consist of fever, headache, and myalgia. On days 2 to 7, nonproductive cough and dyspnea are typical, followed by radiographically confirmed pneumonia, although an untypical course is possible.

MATERIAL AND METHODS

We present our views on the pathology and pathogenesis of the new coronavirus infection based on our own experience and literary data. A morphological analysis of 1200 lethal observations were carried out. In all cases, clinical and morphological comparisons and complete clinical autopsies were performed. Pieces of all major internal organs were fixed in formalin and embedded in paraffin. Thereafter sections were stained according to generally accepted methods, including in some cases PAS, Romanovsky and Mallory. In several cases, immunohistochemical studies were performed using sera for CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD31, CD34, CD56, CD57, CD68, collagen 1 and 3, spike and nuclear proteins of SARS-Co2, caspase-3 (as a marker of apoptosis), MLCM (as a marker of necroptosis), ACE2 and TMPRSSS as virus receptors in

the tissues of the lungs and a number of internal organs. In several cases, we succeeded to study the colocalisation of antigens due to the Akoya system. As a control, autopsy observations related to the pre-covid period were used.

At the initial stage of the work, the possibility of using a number of sera for IHC studies of SARS-CoV-2 for diagnostic purposes and studying the pathogenesis issues were evaluated. It turned out that along with positive staining of sections from observations with coronavirus infection, pronounced false positive reactions were also noted probably associated with cross-linking with similar sites in other proteins. These false positive reactions were detected during IHC studies using different commercial sera and imaging systems under different reaction conditions.

In order to study the pathological aspects of coronavirus infection, an autopsy and histological study of two cats, which died from a verified coronavirus infection of cats, was conducted. For comparison, a cat that died from the feline immunodeficiency virus was studied.

RESULTS OF OWN STUDIES IN MEN

The entranceway of the pathogen is the epithelium cells of the upper respiratory tract, stomach and intestine. The initial stage of infection is the entry of SARS-CoV-2 into target cells that have angiotensin-converting enzyme type II (ACE2) receptors. According to modern concepts, this receptor is expressed on the surface of various cells of the respiratory system (Fig. **1**), esophagus, intestines, heart, adrenal glands, bladder, brain (hypothalamus), and pituitary, as well as endothelium and macrophages. A certain role is played also by TMPRSSS (Fig. **2**).

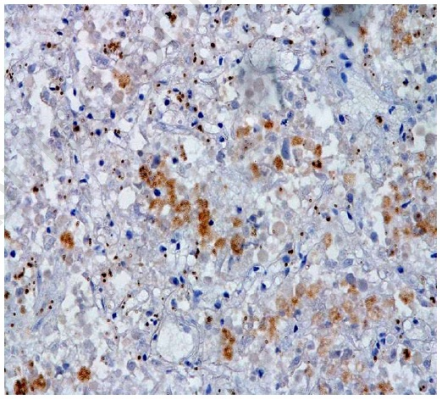

Fig. (1). ACE-2 receptor in lung tissue. IHC x 200.

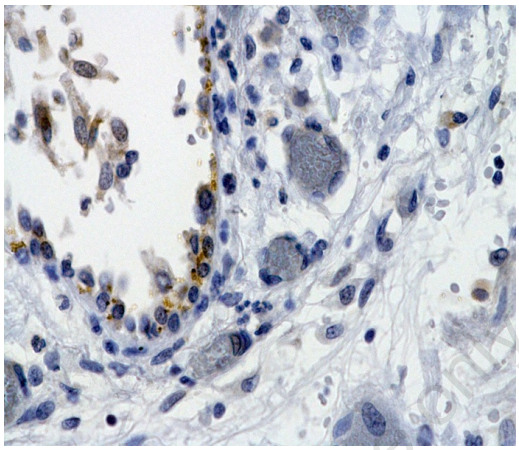

Fig. (2). TMPRSS receptor in bronchial epithelium. IHC x200.

The nature of morphological changes in the mild course of COVID-19 is unknown. Based on the analysis of clinical symptoms, it is possible to assume the tropism of the virus to the laryngeal epithelium, the ciliated epithelium of the respiratory tract throughout, and alveolocytes of types I and II. Apparently, viral lesions in such patients do not lead to the development of pronounced exudative inflammation and, accordingly, catarrhal phenomena. In cases where COVID-19 was considered a concomitant disease and the death was related to other diseases (such as ischemic heart disease, tuberculosis, HIV, chronic hepatitis in cirrhotic stage *etc.*), we also noted moderate overgrowths of the ciliary epithelium (Fig. **3**).

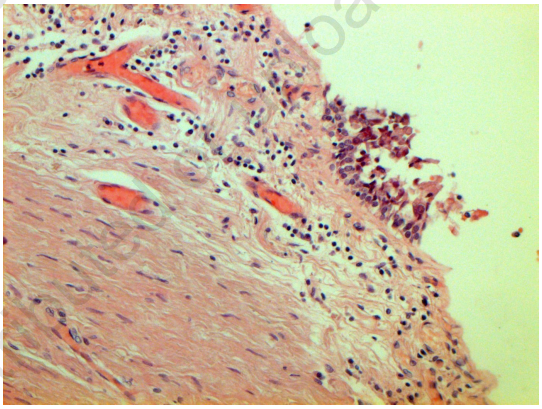

Fig. (3). Overgrowth of the ciliary epithelium of bronchus in a case where COVID-19 was considered a concomitant disease. Stained by H-E, x 200.

During the pathological examination at the autopsy of the deceased due to new coronavirus infections specific macroscopic signs of COVID-19 were not

established, although the morphological picture can be considered characteristic. In the observations, in which signs of severe respiratory failure are sharply predominant, there is a picture of acute respiratory distress syndrome ("shock lung" or diffuse alveolar damage): sharp fullness and diffuse compaction of the lungs, almost indistinguishable from that observed in "swine" influenza A/H1N1pdm (in 2009 and subsequent years), except for the greater severity of the hemorrhagic syndrome. The lungs are enlarged in volume and mass, of a dough-like or dense consistency, low-air or airless; of a lacquer appearance from the surface, dark red (cherry) color, when pressed from the surfaces of the incisions, a dark red liquid flows down. In addition to the different sizes of hemorrhages, there are hemorrhagic infarcts, obstructing blood clots, mainly in the branches of the pulmonary veins. Significant tracheal lesions are not observed, and serous purulent exudate and mucosal hyperemia seen in intubated patients are associated with nosocomial infection.

With a high degree of probability, it can be assumed that the pronounced rise in temperature in many patients is due to the syndrome of a systemic inflammatory reaction (cytokine storm) with severe alteration of lung tissue in the form of diffuse alveolar damage, in which lymphocytes, macrophages, and various cytokines play a leading role. Persistent inflammatory status in patients with severe and critical severity COVID-19 acts as an important trigger for the coagulation cascade, in particular IL-6, which can activate the coagulation system and suppress the fibrinolytic system.

The main and rapidly achievable target of SARS-CoV-2 is alveolar cells of type II (AT2) of the lungs, which determines the development of diffuse alveolar damage. With COVID-19, catarrhal gastroenterocolitis can develop, as the virus affects the epithelial cells of the stomach, small and large intestines that have ACE2 receptors. There is evidence of the possibility of specific damage to blood vessels (endothelium), myocardium and kidneys [13]. There are suggestions about the possible significance of cytokine storm in severe damage to the lungs and other organs and, as a result, damage to the microcirculatory bed with disorders in the blood clotting system. A number of studies postulate the leading pathogenetic role of autoimmune mechanisms based on theoretical assumptions and individual observations [14]. The role of $CD147^{+}$ in SARS CoV-2 cell invasion is also discussed. It has been established that the dissemination of SARS-CoV-2 through the systemic bloodstream or through the plate of the ethmoid bone can lead to brain damage. Changes in the sense of smell (anosmia) and taste (ageusia) in patients at an early stage of the disease may indicate both CNS damage by a virus penetrating the olfactory nerve, and edema of the nasopharyngeal mucosa or viral damage to the cells of the nasal mucosa, the role of vasculitis is not excluded [15].

The main pathogenic mechanism of COVID-19 leading to patient death is severe respiratory failure, which manifested in the form of acute respiratory distress syndrome (ARDS), the main morphological manifestation of which is hyaline membranes. If at the beginning of studying the disease, the emphasis was on artificial lung ventilation in an intensive unit, similar to the severe flu (H1N1), at the moment, many experts recognize a much less effective treatment that has affected the change of tactics of this category of patients [16]. General pathology in COVID-19 was similarly described by several research groups [17 - 24].

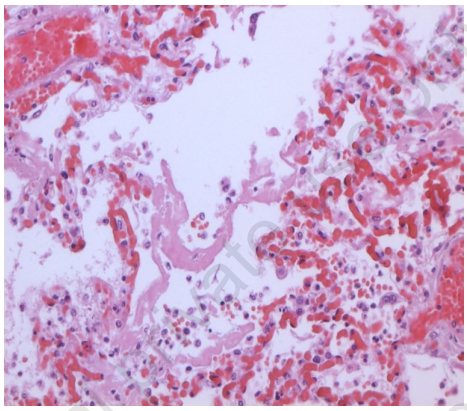

Fig. (4). Moderate number of hyaline membranes in lung alveolus in COVID-19. Stained by H-E, x100.

Severe manifestations of general infectious intoxication in patients with COVID-19 are due to the development of a systemic inflammatory reaction ("cytokine storm" or hyperinflammation) [25]. Previously, it was also found that "cytokine storm" is the main factor of severe course in SARS-CoV and MERS-CoV, and increased serum IL-6 levels correlate with the development of respiratory failure, ARDS and adverse clinical outcomes [26].

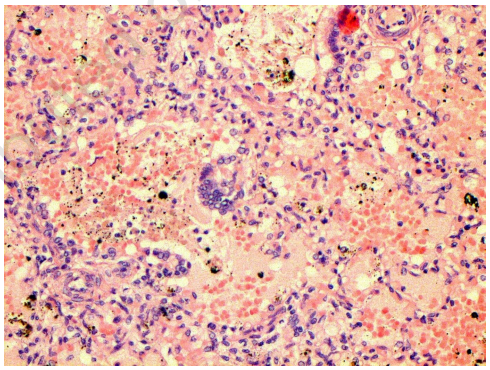

Fig. (5). Squamous metaplasia of the bronchial epithelium in Covid-19. Stained by H-E, x 100.

The dynamics of changes in ARDS associated with COVID-19 can be judged by analogy with SARS and influenza A / H1N1pdm. In the late (productive) stage (after 7-8 days or more from the onset of the disease) of diffuse alveolar damage, macroscopically lungs are enlarged, low-air, dense, fleshy, can resemble the density of the liver, and sometimes with diffused whitish layers and areas of different sizes. Microscopically, siderophages, a relatively (in comparison with swine influenza) small number of hyaline membranes (Fig. **4**), fibrin, squamous metaplasia of the bronchial, bronchiolar and alveolar epithelium can be detected in the lumens of the alveoli, respiratory and terminal bronchioles (Fig. **5**), thickening of the interalveolar septa due to sclerosis, lymphoid (mostly $CD3^+$ and $CD\ 8^+$) (Figs. **6-7**) and macrophage (Fig. **8**) infiltration and proliferation of type II alveolocytes. $CD3^+$ T lymphocytes predominate, among the suppressors ($CD2^+$, $CD5^+$, $CD8^+$). B lymphocytes ($CD20^+$,) are relatively few and natural killers ($CD57^+$) are practically absent.

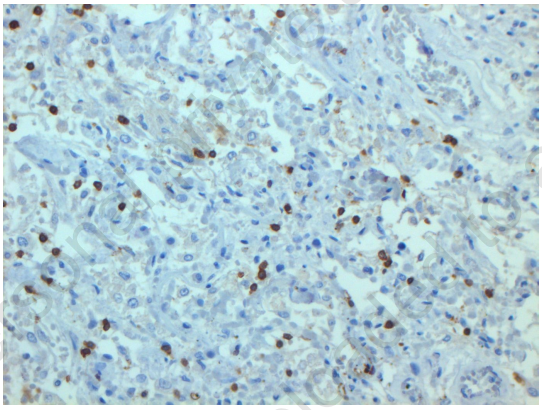

Fig. (6). Lung infiltration by $CD3^+$ lymphocytes in COVID-19. IHC, x100.

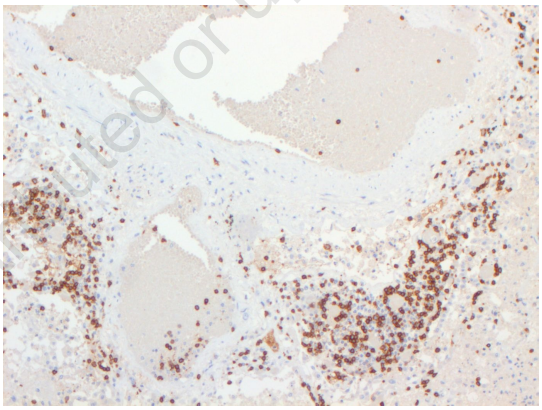

Fig. (7). Lung infiltration by $CD8^+$ lymphocytes in COVID-19. IHC, x 200.

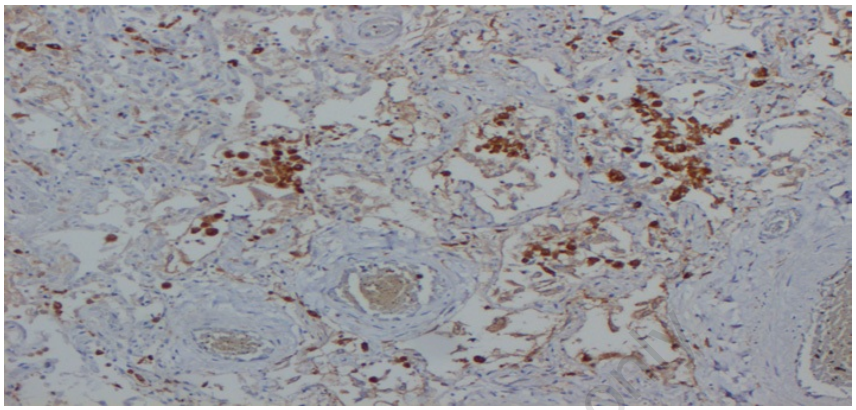

Fig. (8). Lung infiltration by $CD68^+$ macrophages in COVID-19. IHC, x200.

The term viral pneumonia, widely used in the clinic, essentially reflects its development. In turn, severe diffuse alveolar injury is synonymous with the clinical concept of "acute respiratory distress syndrome" (ARDS).

The most important is damage to the vascular bed of different calibers, leading to disorders in the blood clotting system including the lesion of the vascular endothelium and increased thrombosis from disseminated intravascular coagulation to the formation of large blood clots, including the development of thrombembolism of the main trunk and branches of the pulmonary artery with the formation of extracellular traps, the concept of immunothombosis has also been justified [27 - 29],

Damage to the microcirculatory bed, the genesis requires further study, but direct viral damage is most likely. A pronounced alveolar hemorrhagic syndrome is characteristic of most cases, up to the formation, in fact, of hemorrhagic infarcts (although true hemorrhagic infarcts are not uncommon). Pulmonary blood clots are important to distinguish from thromboembolism, as pulmonary embolism (PE) is also characteristic of COVID-19. Thrombosis of the pulmonary arteries sometimes progresses to the right parts of the heart, and thrombosis of the arteries of various organs with the development of their infarcts (myocardium, brain, intestines, kidneys, spleen) is described. This distinguishes changes in the lungs in COVID-19 from those previously observed in influenza A / H1N1. Despite the pronounced hemorrhagic syndrome, significant deposits of hemosiderin are not observed. The described lung lesions can be the cause of death without the addition of bacterial or mycotic superinfection. In many vessels, we observe trombi in different vessels (Figs. **9** and **10**). We succeeded to detect virus spike antigen in the bronchiolar epithelium (Fig. **11**) and macrophages as well in endothelial cells as well as in other layers of the vascular wall (Fig. **12**). The

nucleocapsid protein of the virus was found in the cytoplasm of epithelial cells of the salivary glands, stomach, duodenum and rectum, urinary tract, as well as in lacrimal fluid [13].

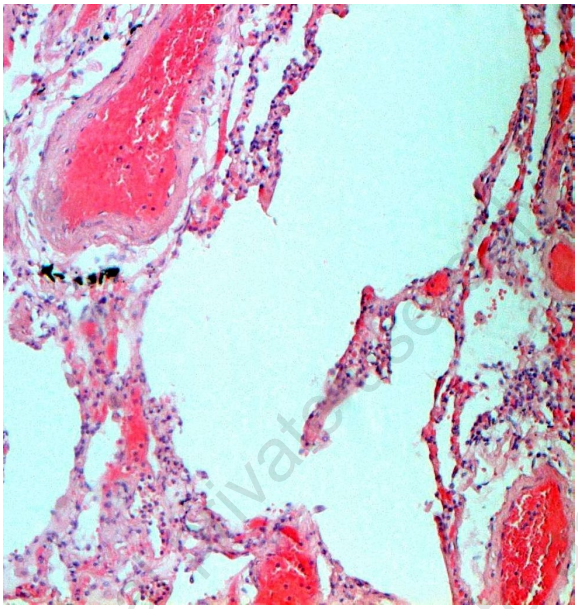

Fig. (9). Thrombi in lung vessels in COVID-19. Stained by H-E, x 100.

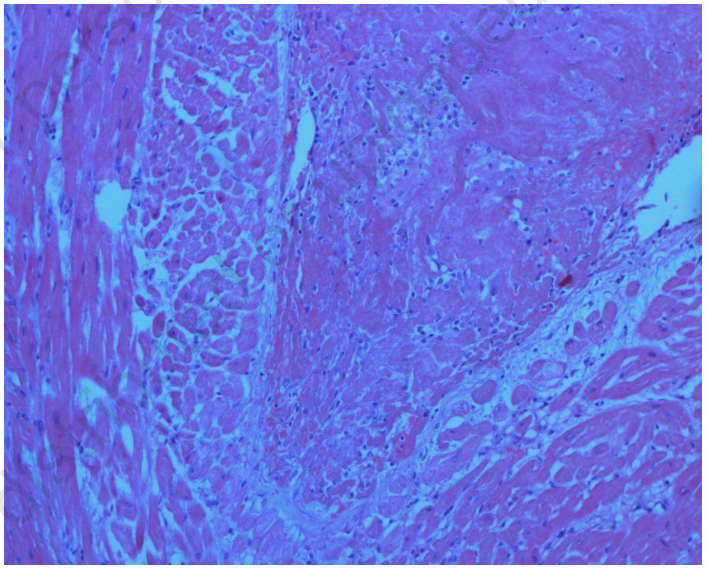

Fig. (10). Thrombus in the coronary artery in a combination of COVID-19 with IHD. Stained by H-E, x 100.

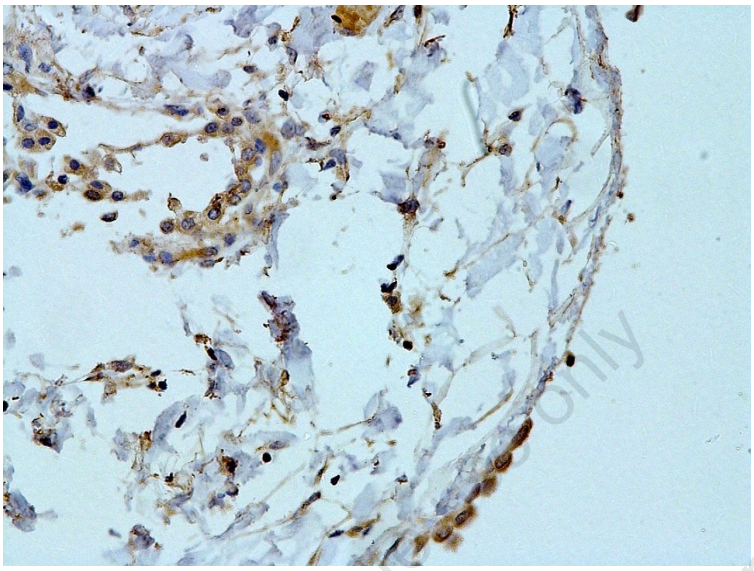

Fig. (11). Spike antigen of SARS- CoV-2 in the bronchiolar epithelium. IHC. x 100.

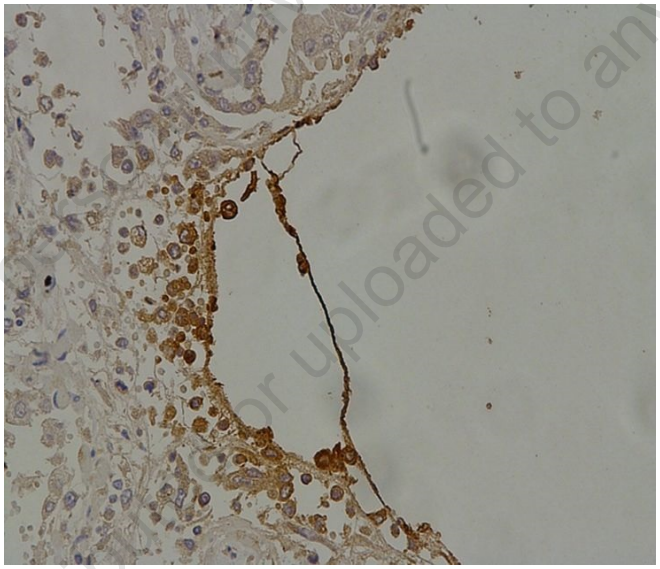

Fig. (12). Spike antigen of SARS- CoV-2 in endothelium in the lung. IHC. x 200.

The nature of cytoproliferative changes of epithelium in the trachea (Fig. **13**) and bronchi (Fig. **14**) remain unclear.

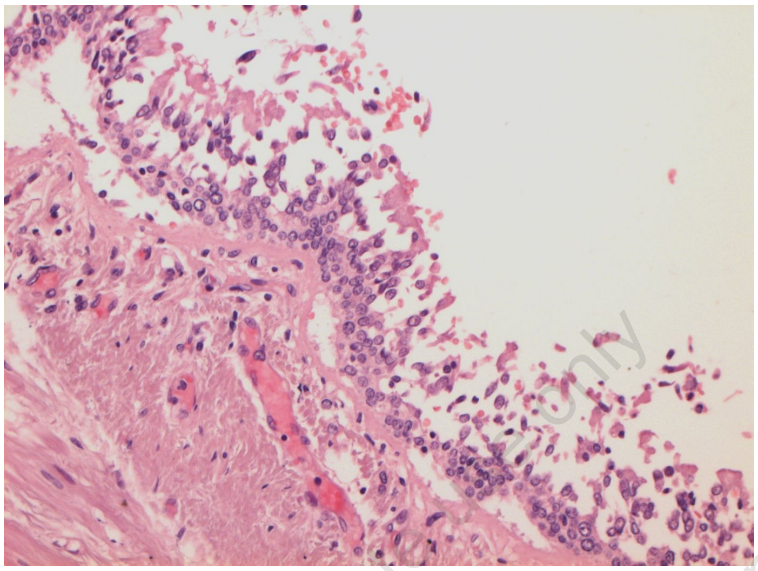

Fig. (13). Cytoproliferative changes of epithelium in the trachea in COVID-19. Stained by H-E, x200.

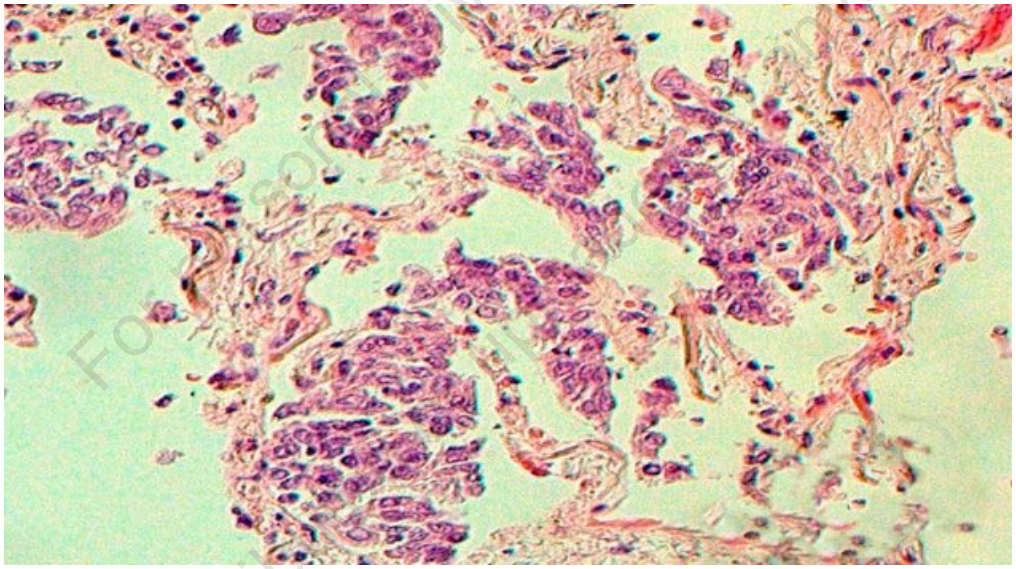

Fig. (14). Cytoproliferative changes of epithelium in bronchus in COVID-19. Stained by HE, x200.

In the final stage of the disease, sections of fibrous tissue may develop in all parts of the lungs (usually in the lower lobes) (Figs. **15** and **16**), which contributes to the development of chronic respiratory failure.

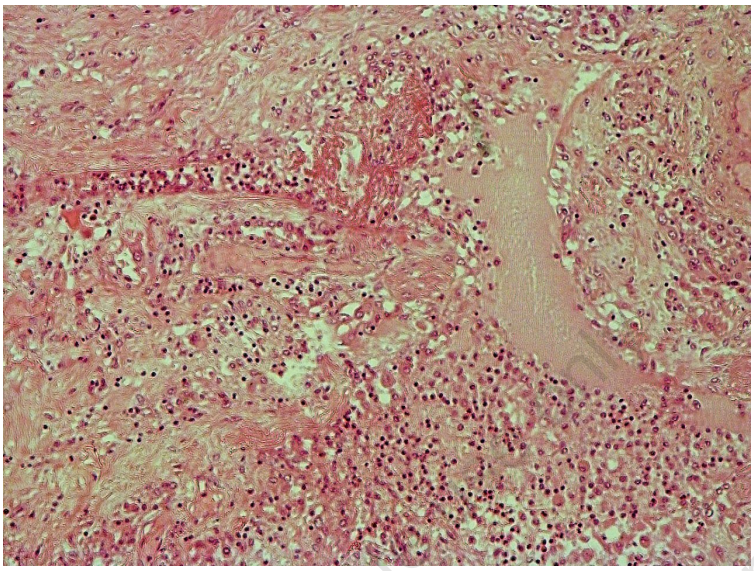

Fig. (15). Expressed lung fibrosis in COVID-19. Stained by H-E, x 100.

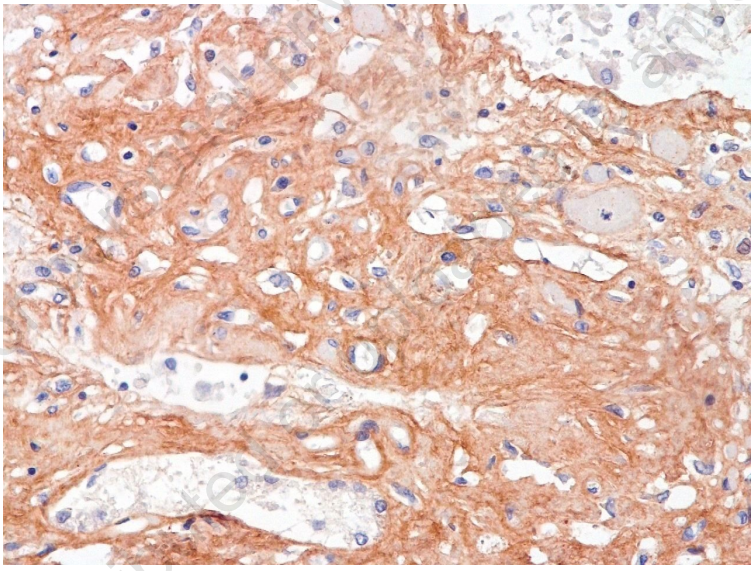

Fig. (16). Collagen 3 in lung in COVID-19. IHC, x100.

The hallmarks of apoptosis (according to an expression of caspasa3) were revealed predominantly in the area with the area of small granular rexis (Fig. **17**), necroptosis also explains additional tissue damage (Fig. **18**).

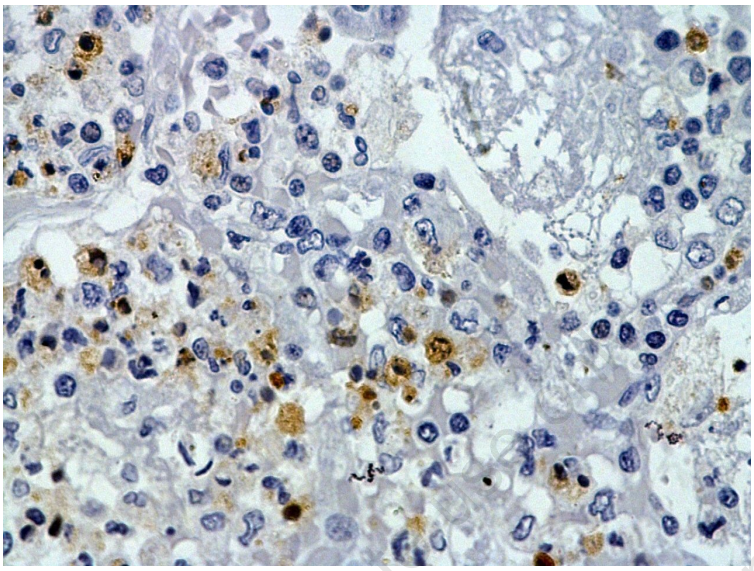

Fig. (17). Caspase 3 in lung in COVID-19. IHC, x100.

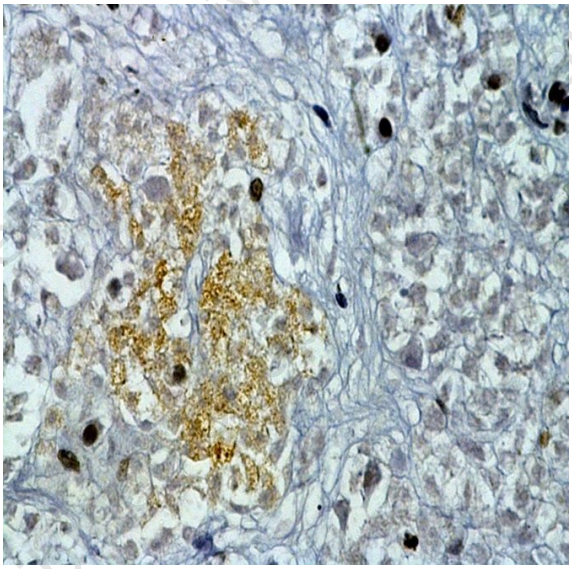

Fig. (18). Necroptosis in the pancreas in COVID-19. IHC, x 100.

Changes in immunocompetent organs have not been sufficiently studied. It can be noted that probably we deal with a combination of direct damage related to the presence of the virus and consequent disturbances of the cooperation of immune cells, leading to the development of a pathologic immune response [30]. There are numerous evidences that the new coronavirus infection evokes autoimmune

reactions contributing to the clinical picture in the acute stage of the disease and in postcovid complications [14]. The accumulation of $CD8^+$ lymphocytes, especially without direct contact with a virus antigen can be considered as evidence for such a mechanism (Fig. **19**). Numerous literary data report about erythrophagocytosis, which can be considered as a sign of an autoimmune lesion as well.

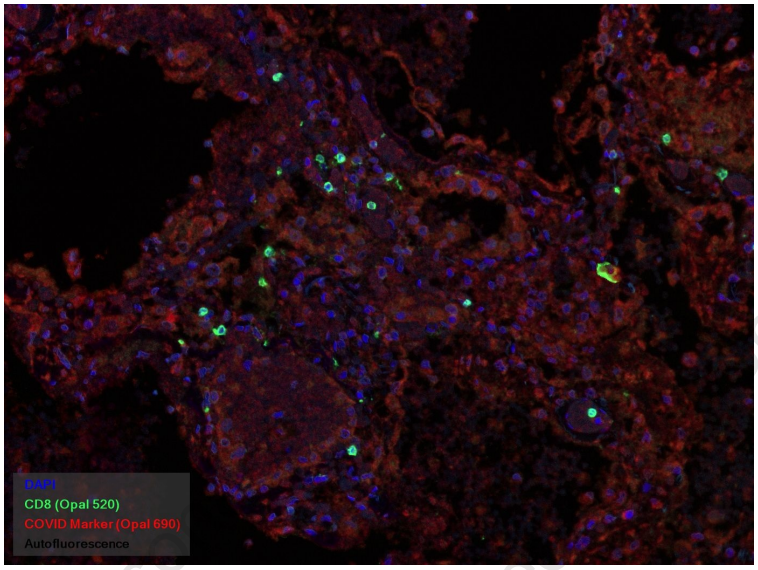

Fig. (19). Colocalization of $CD8^+$ cells (green), spike antigen (red) in lung tissue in COVID-19. Luminescent microscopy with Akoya technique. x 200.

Type I interferons (IFNs) have also been shown to play an important role in the pathogenesis of COVID-19. While the rapid induction of type I IFN from the onset of the disease limits viral replication, a sustained rise in type I IFN levels late phase of infection is associated with aberrant inflammation and poor clinical outcome. It has been recently demonstrated, an alternative pathway for enhancing the production of type I IFN - the cyclic GMP-AMP synthase (cGAS)-Stimulator of interferon genes (STING)-pathway. A STING-dependent type I IFN signature was found, primarily mediated by macrophages adjacent to areas of endothelial cell injury. In addition, cGAS-STING activity was detected in lung samples from patients with COVID-19 with severe tissue destruction and associated type I IFN response. The «lung-on-a-chip» model has shown that, in addition to macrophages, SARS-CoV-2 infection activates cGAS-STING signaling in endothelial cells through the release of mitochondrial DNA, leading to cell death and the production of type I IFN. In mice, pharmacological inhibition of STING reduces SARS-CoV-2-induced severe lung inflammation and improves disease outcomes [31].

Although the role and frequency of joining a bacterial infection 4 to 7 days after the onset remain unclear, this contributes to the development of viral-bacterial pneumonia, which is mainly described in the later stages of the disease. In several cases we observed an accumulation of bacteria in the blood vessel lumen and alveoli during histo bacterioscopic investigation (Fig. **20**). There is evidence that SARS-CoV-2 is able to activate pre-existing chronic infectious processes.

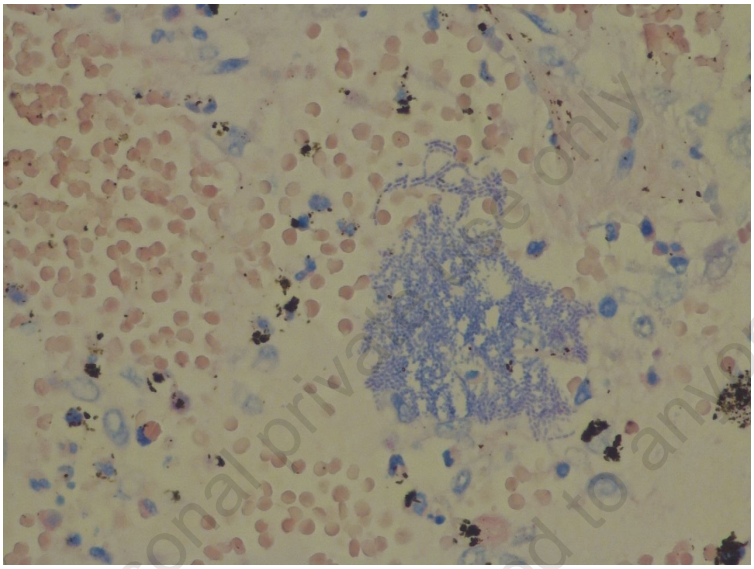

Fig. (20). Accumulation of cocci in lung tissue in COVID-19. Stained by azur-eosin. X400.

The possibility of developing a generalized infection with damage to other organs is evident. In other organs and tissues, alternative and necrotic changes of parenchymal cells, changes in the nuclei (Figs. **21** and **22**), the formation of fibrin thrombi in blood vessels (probably DIC) (Fig. **23**), infiltration by T- lymphocytes, including cytotoxic (Fig. **24**) were observed not only in the parenchyma of the organs but also in surrounding tissues as well, as well as pathological changes associated with comorbid chronic diseases that previously existed in the dead. The nature of such changes can be different and has to be evaluated. We succeeded to detect spike antigens of the virus in lymph nodes (Fig. **25**), pancreas (Fig. **26**) and adrenals. We described certain lesions in the adrenals probably associated with the SARS-CoV-2 virus [32]. Direct viral lesions of the placenta are also very likely, and isolated observations have shown the possibility of intrauterine infection, the clinical significance of which requires further study [33].

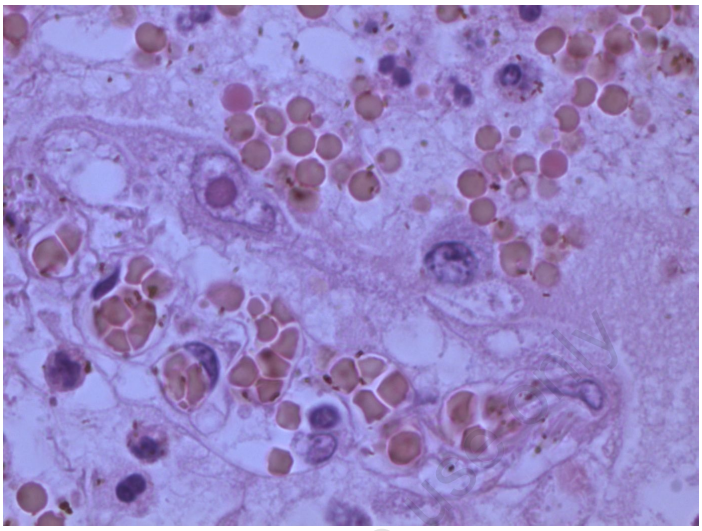

Fig. (21). Considerable changes of nuclei of lung macrophages with the formation of inclusion in COVID-19. Stained by H-E. x400.

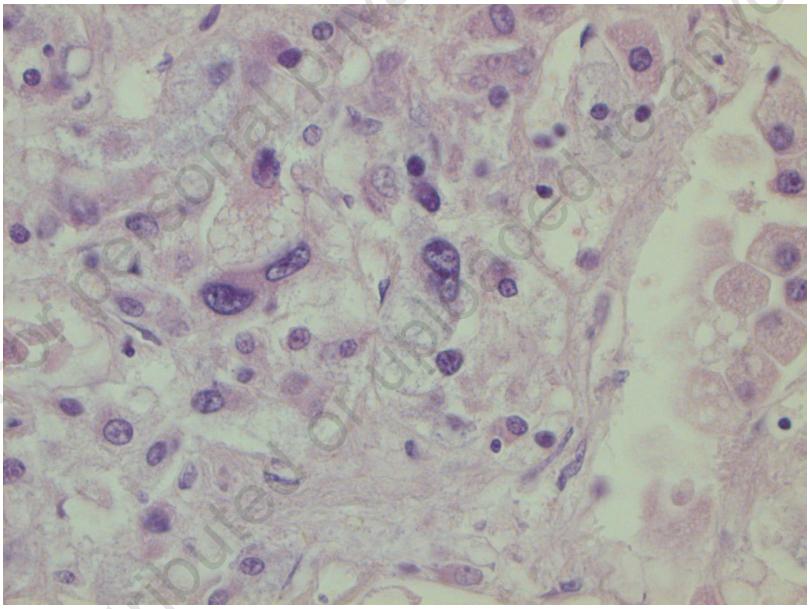

Fig. (22). Considerable changes of nuclei of lung macrophages in COVID-19. Stained by H-E. x400.

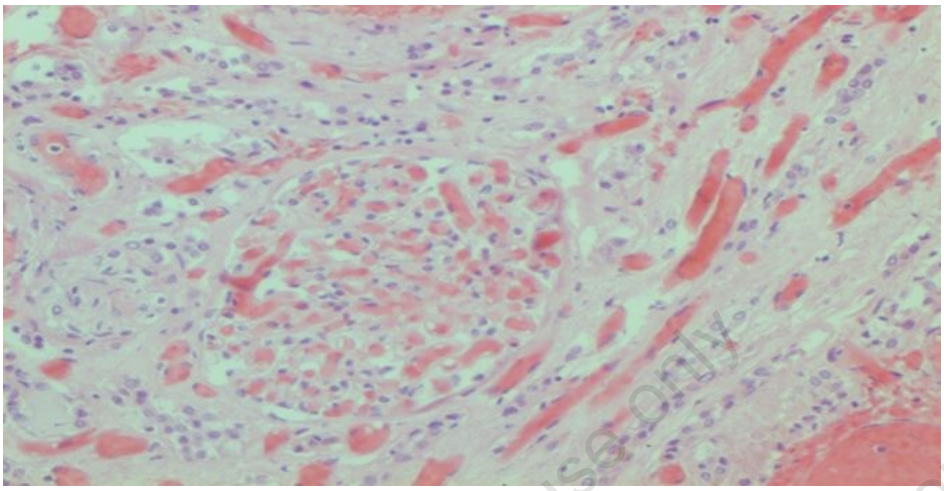

Fig. (23). Disseminated intravascular coagulation in kidney glomerulus in COVID-19. Stained by H-E. x400.

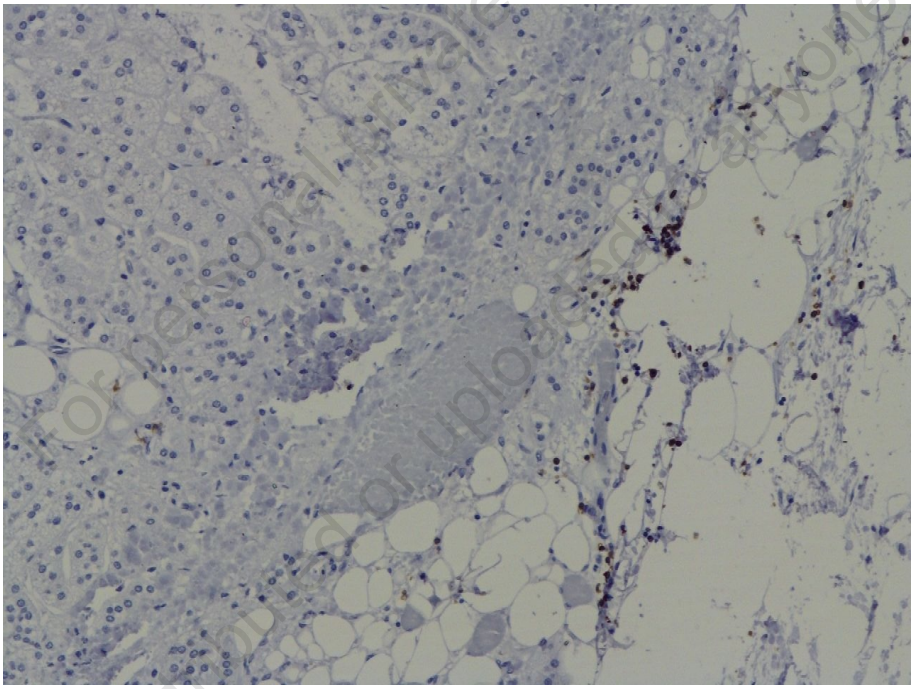

Fig. (24). $CD8^+$ lymphocytes in the connective tissue surrounding the adrenal gland in COVID-19 IHC. x200.

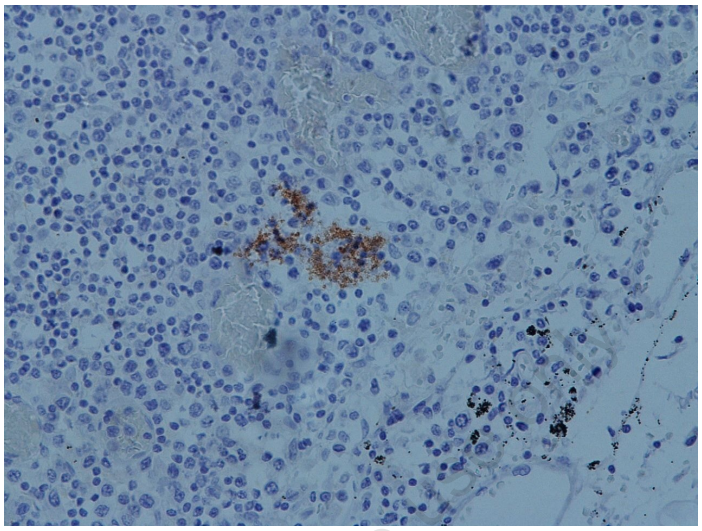

Fig. (25). Spike antigen of SARS COV2 in lymph node in COVID-19. IHC. x200.

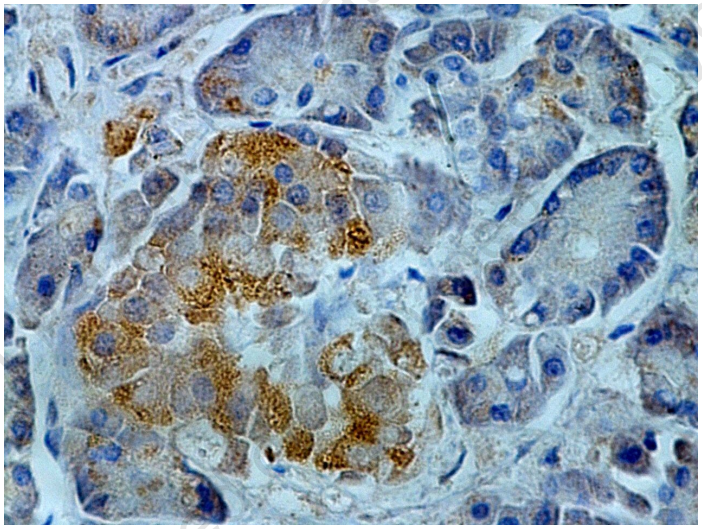

Fig. (26). Spike antigen of SARS-COV2 in the pancreas in COVID-19. IHC. x400.

SOME ASPECTS OF DIAGNOSIS FORMULATION

Clinical autopsies in COVID-19 require certain precautions for the staff. Presently they are elaborated and allowed to work without high risk of personal challenges [34, 35].

Currently, there are no clear ideas about approaches to statistical accounting of deaths due to coronavirus infection in the world. In some cases, a combination of typical COVID-19 clinical symptoms with a positive PCR test is considered

sufficient for its registration. At the same time, it is obvious to practicing pathologists all over the world that despite the presence of pronounced comorbid pathology in the vast majority of the deceased, the ratio of changes associated with various nosological forms can vary significantly, which justifies the need for a differentiated formulation of a post-mortem diagnosis.

When formulating a postmortem diagnosis, it is necessary to differentiate:

1. The occurrence of a fatal outcome from covid-19 when covid-19 is the main disease (the original cause of death);
2. The onset of death from other diseases, in the presence of an infection caused by SARS-CoV-2 (diagnosed due to the detection of the SARS-CoV-2 virus by PCR), but without its clinical and morphological manifestations that could cause death. At the same time, COVID-19 can unfavorably influence the course of diseases of the circulatory system, cancer and other diseases that cause death. In such situations, COVID-19 should not be regarded as the underlying disease (the original cause of death) and is indicated in the diagnosis as a comorbid disease.

It is also necessary to analyze the possibility of developing iatrogenic complications and causes of death, primarily associated with ventilation. Pseudomembranous colitis is associated with long-term, not optimal antibiotic therapy.

The most common complication of COVID-19 is acute respiratory distress syndrome (ARDS). In addition, complications that can also be considered as a direct cause of death have been reported, such as:

- Acute heart failure.
- Acute renal failure.
- Septic shock.
- Intravascular disseminated coagulation syndrome (DIC).
- Multiple organ failure (dysfunction of many organs and systems).
- Secondary bacterial and fungal infections.

On March 25, 2020, World Health Organization (WHO) published an extension of codes for recording covid-19-related deaths. According to the WHO rules:

- The emergency code U07.1 is assigned in the case of covid-19, confirmed by laboratory tests;
- The emergency code U07.2 is assigned in the event of a diagnosis of covid-19

based on clinical or epidemiological data if laboratory confirmation is not final or absent.

WHO notes that COVID-19 is listed on the death certificate as any other cause of death, and the rules for choosing the original cause of death are the same as, for example, influenza. A respiratory infection can turn into pneumonia, which can lead to respiratory failure and other consequences. No special instructions are required. Pathology potentially contributing to death (problems with the immune system, chronic diseases), is registered in part II of the medical death certificate in accordance with the rules for filling in. Clarification of the validity of the data is recommended, if COVID-19 is listed on the Medical Death Certificate, but is not selected as the original cause of death.

It should be noted that in some observations, the order of various pathological processes in the diagnosis has certain subjectivity.

PATHOLOGY OF CORONAVIRUS INFECTION IN CATS

Pathological examination of two feline cases of coronavirus infection in our laboratory showed some typical lesions for FIP diagnosis (Fig. **27**). In one case we observed severe lung injury with changes both typical and atypical for feline lesions. The latter was presented as lung lesions similar to those observed in men (Fig. **28**).

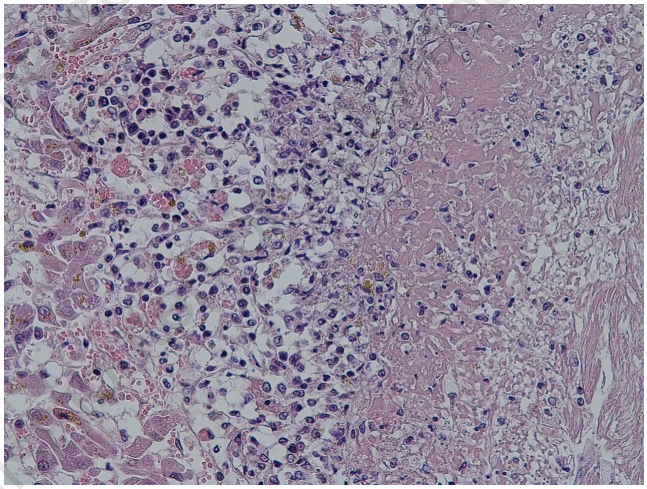

Fig. (27). Typical necrotic granuloma in the liver in a cat that died of feline infectious peritonitis. H-E, x 100.

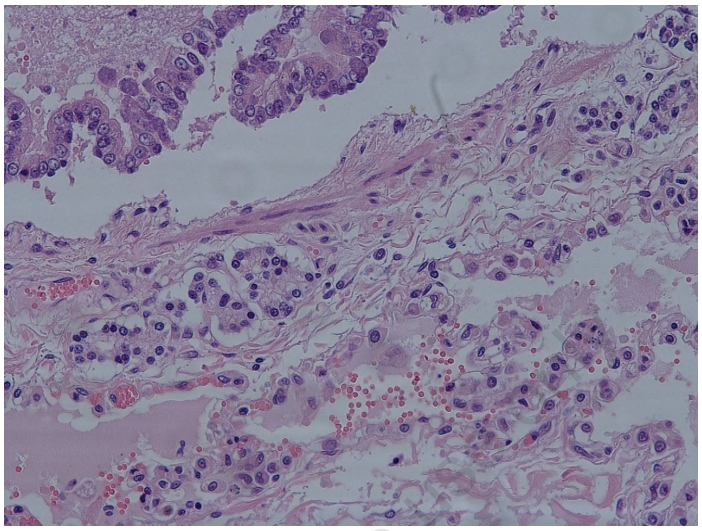

Fig. (28). Proliferation of bronchial epithelium in a cat deceased due to feline coronavirus infection H-E. x 200.

CONCLUSION AND DISCUSSION

The results of histological and immunohistochemical studies of the material of deceased people with varying degrees of severity of coronavirus infection and cats confirm the ability of these pathogens to cause cyto proliferative changes, primarily in the epithelial and endothelial cells. At the same time, lesions of various organs are possible, while the reasons for significant differences in tropism to different organs remain unclear. The substantial differences between clinical course and histopathological features of coronavirus infection in different animals, including men, were never discussed in the literature but obviously, the appropriate investigations are necessary.

There are numerous potentially life-threatening types of pneumonia and other infectious lung lesions (croupose pneumonia, influenza and tuberculosis as the most common); although the immediate death cause is constantly, respiratory failure, significant differences in clinical and pathological features allow us to suppose substantial peculiarities in its pathogenesis [36]. It is notable that the detailed study of patho- and thanatogenesis is absent in many diseases. Severe respiratory failure in COVID-19 in humans is explained by a very peculiar viral pneumonia. It is quite legitimate to use the term respiratory distress syndrome (ARDS). It should be emphasized, however, that the term “non-specific” is hardly applicable to it since many of its clinical and morphological manifestations significantly distinguish it from the one, we observed in swine influenza A H1N1, which did not allow us to transfer the therapeutic approaches formed in those years to a new coronavirus infection without correction.

In the pathogenesis of COVID-19, no doubt, the most important role is played by lesions of the microcirculatory bed, the genesis of which requires further study, but direct viral damage is most likely. Endothelial damage can be associated with both thrombosis in the vessels of various calibers, leading to characteristic complications, and the development of DIC syndrome with maximal kidney damage. Such lesions can be the basis of clinically diagnosed septic shock, while there are no morphological data in favor of classical sepsis caused by bacteria or fungi, although we have found signs of bacteremia with an intravascular accumulation of microorganisms in separate observations. There were no signs of mycotic lesions on our material. It should be noted that in observations, with long-term antibiotic therapy postmortem autopsy revealed pseudomembranous colitis in which a significant role is taken to take *Clostridium dificile* on the background of long-term antibiotic therapy.

Massive infiltration of lung tissue and other organs mainly by T-lymphocytes, including those with suppressor properties, makes it necessary to conduct a differential diagnosis between the morphological manifestation of the protective cellular immune response and direct viral lesions but does not exclude the hypothesis of an immunopathological component of pathogenesis. Currently, the morphological work in which such a diagnosis was carried out is unknown. The presence of false positive reactions when using a number of both poly-and monoclonal sera can be presumably associated with the presence of numerous decussations between SARS-CoV-2 and human antigens [14, 37].

To assess the possible consequences of the identified lesions in surviving patients, catamnesic observation is certainly required, but judging by the literature data, the formation of fibrosis is very likely, the degree of reversibility of which is still impossible to judge. A wide variety of clinical and neurological syndromes are described as the genesis of which remains undeciphered. It is also necessary to take into account the emerging data on the possibility of preserving the virus in the body after clinical recovery [38]. It should also be remembered about the ability of coronaviruses to cause chronic lesions in a number of animals with the development of granulomatous changes in them, which, however, have not yet been described in humans.

In many of the deceased, even in the absence of clear clinical symptoms, a variety of extrapulmonary lesions are also detected. The mechanism of their development probably has a complex nature: direct lesions associated with the generalization of viral infection, vascular disorders associated with endothelial damage and having an autoimmune nature. Many aspects of the pathogenesis of coronavirus infection require further comprehensive study.

The most promising is the search for antiviral drugs that act on the pathogens capable of long-term persistence and pronounced cytoproliferative activity, suppressing autoimmune reactions and normalizing hemostasis.

In the present chapter, we were able to present preferably the most typical structural changes we observed in lungs during our study of autopsy material, trying to correlate them with certain aspects of pathogenesis. Many questions remain unclear and require further complex study, including histopathologic.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Pathologists of SP Botkin Infectious Hospital, Saint-Petersburg, Russia.

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Jean-Marc Sabatier

Jean-Marc Sabatier is a Director of research at the French CNRS, with Ph.D. and HDR degrees in Biochemistry and Microbiology. He headed several academic research teams (CNRS, INSERM, and University), as well as a combined academic-industry research laboratory devoted to the engineering of therapeutic peptides (ERT62, Marseilles, France). He was also a Director of Research for several French private companies as well as a Canadian public company. He acts as a Consultant for top pharmaceutical and cosmetic companies. Dr. Sabatier works in the field of animal toxins and microbes. He contributed to several books in toxinology and virology and more than 170 scientific articles, 180 communications, and 55 patents in both biology and chemistry. He is a member of 61 Editorial Boards of scientific journals, such as 'Peptides', 'Molecules', 'Antibiotics', and the 'Journal of Biological Chemistry'. He also reviewed articles submitted for publication in >100 international journals and acts as an expert for numerous national and international institutions. He won the 'Citizen of the Year Award' from the Nouvel Economiste (1994) for his work on antivirals. He is a member of a dozen scientific societies, such as the 'American Peptide Society' (charter member), 'European Peptide Society', 'American Society for Microbiology', 'Biochemical Society', and 'New-York Academy of Sciences'.