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Review

Antiviral effects of azithromycin: A narrative review

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ABSTRACT

Viral infections have a great impact on human health. The urgent need to find a cure against different viruses led us to investigations in a vast range of drugs. Azithromycin (AZT), classified as a macrolide, showed various effects on different known viruses such as severe acute respiratory syndrome coronavirus (SARS-CoV), Zika, Ebola, Enterovirus (EVs) and Rhinoviruses (RVs), and Influenza A previously; namely, these viruses, which caused global concerns, are considered as targets for AZT different actions. Due to AZT background in the treatment of known viral infections mentioned above (which is described in this study), in the early stages of COVID-19 (a new zoonotic disease caused by a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) development, AZT drew attention to itself due to its antiviral and immunomodulatory effects as a valuable candidate for COVID-19 treatment. AZT usage instructions for treating different viral infections have always been under observation, and COVID-19 is no exception. There are still debates about the use of AZT in COVID-19 treatment. However, eventually, novel researches convinced WHO to announce the discontinuation of AZT use (alone or in combination with hydroxychloroquine) in treating SARS-CoV-2 infection. This research aims to study the structure of all of the viruses mentioned above and the molecular and clinical effects of AZT against the virus.

1. Introduction

Viruses are generally DNA or RNA particles wrapped in a protein shell. The controversy about considering viruses as "living" organisms continue to this day; however, the importance of these microorganisms in human health cannot be denied regardless of the argument [1]. Coronavirus disease-2019 (COVID-19) pandemic, Ebola and SARS outbreaks, influenza, Zika fever, etc., are examples of difficulties with which humans were involved. As of November 6, 2021, There are 249,896,797 confirmed cases and 5,054,842 deaths from the coronavirus COVID-19 outbreak [2]. Ebola virus also caused a long-lasting, large, and fatal outbreak from December 2013 to March 2016. This outbreak eventually caused 28,652 infections resulting in 11,325 deaths in 10 countries [3]. Severe acute respiratory syndrome coronavirus

(SARS-CoV) is the agent that gave rise to a mini pandemic from November 2002 to July 2003 that led to a total of 8096 probable cases and 774 deaths [4]. These data confirm the significant effect of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other viruses on human health and life.

Different therapies are utilized to treat various viruses worldwide. Azithromycin (AZT), used since the early 1980s [5,6], is a second-generation synthetic macrolide antibiotic used to treat a wide range of bacterial infections. However, its effects aren't limited to bacteria. AZT also showed satisfying effects on viral infections due to its anti-viral and anti-inflammatory activities. Consequently, it is now being investigated as a treatment for different viral diseases such as COVID-19 [7].

The main goal of this to review the structure of the mentioned RNA viruses and evaluate the effects of AZT against them. To achieve this purpose, almost all the related manuscripts published in PubMed, ScienceDirect, Google Scholar, and Scopus databases were studied. For the collection of these articles the following keywords were used in title or abstract with the help of Boolean operators ("and", "or"): "Azithromycin", "Zithromax", "*SARS-CoV-2*", "*SARS-CoV*", "*Zika virus*", "*Ebola virus*", "*Enterovirus*" "*Rhinoviruses*", "*Influenza A virus*", "COVID-19″, and "HAV".

2. Antiviral effects of AZT against enveloped negative-sense RNA viruses

2.1. Influenza A virus

2.1.1. Structure

The influenza viruses (Orthomyxoviridae family members) are enveloped negative-sense RNA viruses which formed up by eight different RNA parts. Each part codes for a special protein and is responsible for the function and construction of the viruses. Influenza viruses are categorized into three types, A, B, and C. The first type is the main reason for pandemic illness, but the two other types, B and C, have been the root of epidemic proportion.

The presence of glycoproteins, hemagglutinin (HA), and neuraminidase (NA) on the surface of influenza assist us in classifying this virus in a different manner.

HA has 16 distinct molecules that allow the virion sticking to the surface of a cell, while NA, consisting of nine molecules, contributes to the absorption of host discharges and release of viral particles from the host cell. Influenza virus is called with this name because there are various types of HA and NA particles. Influenza A Type has a variety of subtypes with a consecutive mixture of 17 H and 10 N antigens (i.e., H1N1, H3N2, and H5N1), but there are no subtypes for types B and C influenza [8].

The molecule or virion of the influenza A virus is depicted in Fig. 8 both type A and B influenza are described as negative-sense RNA viruses having genomes with eight single-stranded RNA portions located inside the virus particle. The viral proteins encoded by the viral genome of the two mentioned influenza viruses are similar in terms of function but dissimilar in terms of antigens. PB1, PB2, and PA, the three subunits of the viral RNA-dependent RNA polymerases, are encoded by the three biggest fragments of RNA and are responsible for RNA combination and replication in infected cells. Two parts of RNA encode the viral glycoproteins HA, which has a key role in the formation of receptors containing sialic acid and viral passage. The RNA genome is joined by the viral NP, which is encoded by the RNA section.

The matrix (M1) and membrane (M2) proteins are encoded by RNA portions 6 and 8. The function of M1 protein is to create a platform that contributes to the formation of the virion and, accompanied with NEP, controls the trafficking of the viral RNA fragments in the cell when combined with NEP. As a proton particle channel, the M2 protein is required for the viral passage and, in accompany with the HA and NA glycoproteins, is located on the exterior layer of the virus joined to a lipid layer that comes out from the infected cell [9].

2.1.2. Effects of AZT against influenza A virus

As macrolides possess anti-inflammatory and immunomodulatory effects, it is interesting to consider the conceivable use of these drugs in respiratory viral infection. The severity of an infection is linked with the cytokine dysregulation induced by the virus, which is responsible for the progress of lethal clinical symptoms, e.g., acute bronchopneumonia, pulmonary edema, alveolar hemorrhage, reactive hemophagocytosis, and acute respiratory distress syndrome.

Evidence has revealed that macrolides are able to decline virus-related aggravation, downregulate the inflammatory course, and diminish extra cytokine release in viral infections. Macrolides may also have an effect on phagocyte movement by changing their functions, such as phagocytosis, oxidative burst, cytokine production, bacterial killing, and chemotaxis. Moreover, macrolides could interfere in the replication cycle of the influenza virus, thereby leading to the inhibition of virus production from infected cells, chiefly by preventing intracellular HA0 proteolysis [10].

In Du et al.'s investigation, AZT reuse is a potential alternative for antiviral treatment for highly pathogenic influenza A virus (IAV). They used a designed replication-competent strain of PR8 with luciferase reporter quality (IAV-luc) and various pseudotyped IAV. To evaluate the pH change of vesicles having IAV, they developed an A549 cell line with the endosomal and lysosomal expression of pHluorin2. AZT was recognized as a powerful inhibitor against IAV. In their study, treatment with AZT could extremely suppress the IAV disease in vitro without any damage to the connection and internalization of IAV virions, as well as to the viral replication. However, it suppressed the combination of viral with vacuolar layers.

The results of a lipophilic dye-based fluorescence dequenching assay using two fluorescence dyes, i.e. SP-DiOC18 and R18, indicated the inhibition of the fusion of viral and vacuolar membranes by the treatment with AZT. Moreover, the results of this membrane fusion are in conformity with the pseudovirus assay. Thus, the data from their investigation revealed that for the inhibition of the IAV entry, the AZT antiviral effect is dependent on the alkalinization of acid vesicles. Treatment with AZT could not impair the binding and internalization of IAV virions and also could not prevent the fusion between viral and vacuolar membranes.

Their results verified that treatment with AZT could alkalize the vesicles containing IAV virions by applying an NPC1–pHluorin2 reporter cell line. NPCI-pHIuorin2 reporter cell line is a fused pHluorin2 (pH-sensitive green florescent protein) with N and C terminate of NPC1 (a multipass-transmembrane protein essential for egress of cholesterol from late endosomes/lysosomes), which later prevented pH-dependent membrane fusion. The mechanism by which AZT alkalizes acid vesicles has not yet been defined. Due to the physical and chemical properties of AZT, it belongs to the amphiphilic cationic drugs that can accumulate in acidic and alkaline vesicles. AZT may act as a weak base to prevent endosome acidification [11].

In a randomized test on patients with influenza A, oseltamivir was administered together with 500 mg of AZT for five days daily. The result indicated a faster reduction in the plasma concentrations of interleukin 6, interleukin 17, interleukin 8, C-X-C motif chemokine ligand 9, soluble tumor necrosis factor, and C-receptive protein (CRP). Of note, in that study, the sample size (n = 50) was limited, and the effect was small. Also, no significant alterations were observed in viral freedom or time to symptom resolution [12].

In Tran et al.'s study, treatment of viruses with AZT before disease considerably restricted the replication of progeny virus; however, administration of AZT after infection had no effect on this procedure. Thereafter, the steps prevented by AZT during virus attacks were discovered. While the connection of viruses to the cell surface was not affected by AZT, internalization into host cells during the initial stage of disease was inhibited.

Recently, it has been demonstrated that AZT can target matured progeny virus from the host cells and inactivate their endocytic movement. This inhibitory mechanism has not been perceived for other anti-influenza drugs, which signifies the probable role of AZT, not only before but also after the influenza virus infection. With regard to the mechanism of in vitro observations, AZT was intranasally given to mice infected with the influenza A (H1N1) pdm09 virus. Hypothermia caused by infection was alleviated, and viral load in the lungs were effactually mitigated by the intranasal treatment with AZT. The entry of IAV into cells is mediated by interactions with lectin receptors. It is possible that AZT hampers the interaction between the virus and lectin receptor to prevent internalization [13].

In a former survey, AZT and oseltamivir combination was compared with oseltamivir monotherapy in a deadly BALB/c model of influenza A (H1N1) pdm09 infection. In groups of 14–16, Mice were orally given oseltamivir alone or in combination with AZT. All the mice received 10 mg/kg of oseltamivir once daily for five days, starting at day two after vaccination, while for AZT, a single dose of the drug (100 mg/kg) was administered intraperitoneally at day three post-immunization.

Based on the survival rates, lung viral titers, and pro-inflammatory cytokine levels, the combination therapy demonstrated no additional clinical/virological superiority over oseltamivir monotherapy [14]. Treatment with the oseltamivir-AZT combination was explored to be more effective than therapy with oseltamivir alone, particularly in rapid recovery and inhibition of influenza-related complications and in patients whose lives are at risk [15].

2.2. Ebola virus

2.2.1. Structure

EBOV, another enveloped negative-sense RNA viruses from the family of *Filoviridae* and order *Mononegavirales*, is identified by its filamentous morphology. The genus *EBOV* is categorized into five distinct species, including *Bundibugyo ebolavirus* (BDBV), *Sudan ebolavirus* (SUDV), *Taï Forest ebolavirus* (TAFV; previously known as *Cote d'Ivoire Ebolavirus*), *Reston virus* (RESTV), and EBOV (formerly known as the *Zaire ebolavirus*). Depending on genetic similarities, the EBOV has a close relationship with the *Marburg virus* [16] and has a distinctive thin filamentous structure with a width of 80 nm and a length of ~14 μm [17]. The filoviral genome is composed of a nonsegmented, negative single-stranded linear RNA molecule, which comprises 1.1% of the total virion mass. The size of the genome is estimated to be ~19 kb for EBOV [18].

The filoviral genome is composed of seven genes organized from the 3′ end to the 5′ end. These genes are nucleoprotein (NP) and viral structural proteins VP35, as well as VP40, GP, VP30, VP24, and RNA-dependent RNA polymerase gene (L) [19]. NP, VP35, VP30, and L have the ability to directly bind to the negative-sense RNA genome and are able to form the ribonucleoprotein complex. The remaining three proteins entail GP (the major membrane spike protein), VP40 (the major matrix protein), and VP24 (the minor matrix protein), which the two last interact with both the viral envelope and the nucleocapsid, as bridging molecules [20,21].

When transcribed and translated, the EBOV GP gene produces two proteins, sGP and GP; the former is the first small polypeptide product of the GP gene, and the latter is the full-length larger gene product and produced by inserting an adenosine residue during transcription through RNA editing. The mature protein has two subunits, an N-terminal GP1, and a C-terminal GP2, which are connected by a disulfide bond. The first subunit, GP1, is composed of varied N- and O-glycosylations. The mature protein is observed as homo-trimers on the surface of viral particles, while GP2 mediates viral entry into target cells (GP1 function) and release of viral ribonucleoprotein from endosome to the cytoplasm for replication (GP2 function) (Fig. 4) [22].

2.2.2. Effects of AZT against Ebola virus

AZT has been demonstrated to have an in vitro anti-EBOV activity,

but in animal models, the results were inconclusive or inconsistent. AZT is a well-known antibiotic and is frequently prescribed for treating several bacterial infections. These effects seem to come from the amplification of the systemic antiviral response mediated by the IFN (interferon) pathway [24,25]. AZT has formerly been evaluated as an effective treatment for EBOV in vitro, and in small animal models, it has indicated potent in vitro prevention of the EBOV.

In a mouse model, 100 mg/kg of AZT was used twice daily to treat animals. The results showed a 60% survival rate in comparison to 20% for the control group ($P = 0.02$). The repetition of the test under identical conditions exhibited no statistically significant results. Treatment with 210 mg/kg oral AZT PO once daily showed no survival rate. Moreover, no positive outcomes were observed after an efficacy screening by the use of several doses of AZT on guinea pigs [26].

In an earlier investigation, two sets of three-drug combinations remarkably ameliorated the effectiveness of each drug against the EBOV infection, not only in vitro but also at clinically relevant. In one of the sets, chloroquine was combined with maprotiline, which combination elevated AZT antiviral activity against EBOV fivefold (Fig. 5) [25].

In another survey, following a combinatorial screening using pseudo virion and mini-genome replicon systems, several drugs, including AZT, were identified with some activities against EBOV. Studies have attributed the AZT inhibitory effect on EBOV to its innate cationic amphiphilic structure, which changes the homeostasis of later endosomal vesicles similar to tamoxifen. It has also been denoted that AZT may hinder the EBOV infection by disturbing the phospholipid metabolism and hemostasis LE/Lys calcium [27].

3. Antiviral effects of AZT against enveloped positive-sense RNA viruses

3.1. Zika virus

3.1.1. Structure

Zika virus (ZIKV), an enveloped positive-sense RNA virus, is a family member of the *Flaviviridae* and belongs to the *Flavivirus*, the largest genus of the *Flaviviridae* family comprising of 53 various species. The genus flavivirus is categorized into three (non-vector, tick-borne, and mosquito-borne) clusters. For a decade, five groups of mosquito-borne flaviviruses, i.e., ZIKV, Dengue (DENV), West Nile (WNV), Yellow fever (YFV), and Japanese encephalitis (JEV) viruses, have become a major public health concern in virtue of their drastically increased prevalence worldwide [29].

In 1947, the first isolation of ZIKV (strain MR 766) was made from serum samples of a rhesus monkey in Uganda's Zika Forest. The first human case of ZIKV infection was detected in Africa in the 1950s and in Asia afterwards, though it remained in these areas until the occurrence of a large outbreak in Yap Island in 2007. Later in 2013 and 2014, the outbreak continued in French Polynesia, New Caledonia, and the Cook Islands [30,31]. ZIKV is spread to humans mainly through the bite of infected *Aedes aegypti* and *Aedes albopictus* and can infect various tissues and organs [32].

The ZIKV genome is composed of a single, positive-sense molecule of RNA of 10.8 kb, a single open reading frame (ORF) of 10 kb, and about 100 and 420 nucleotides in 5′ and 3′ untranslated regions (UTRs), respectively (Fig. 3). After coding for a large polyprotein precursor of 3423 amino acids, the ORF is cleaved both co-translationally and post-translationally into three structural (C, prM, and E) and eight nonstructural (NS1, NS2A, NS2B, NS3, S4A, 2K, NS4B, and NS5) proteins (Fig. 3) [29].

3.1.2. Effects of AZT against Zika virus

In spite of the fact that ZIKV is a serious menace to global health, no vaccines or effective therapeutics have hitherto been developed. AZT has been shown to promisingly suppress ZIKV infection in vitro. In response to this infection, the antibiotic upregulates the expression of

host type I and III IFNs and a number of their downstream IFN-stimulated genes (ISGs), i.e., the expression of melanoma differentiation-associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I), two pattern recognition receptors (PRRs) caused by ZIKV infection. AZT also elevates the levels of phosphorylated TANK-binding kinase 1 (TBK1) and IFN regulatory factor 3 (IRF3) so that the TANK-binding kinase 1 phosphorylation is upregulated independently without inducing the IFN regulatory factor 3 phosphorylation.

Of note, after ZIKV infection, AZT could upregulate the expression of C-X-C motif chemokine ligand 10 (CXCL10), as well as that of interleukin 28 and interleukin 29 in Vero cells. This result supports the findings that IFN-γ is able to be induced by ZIKV infection and inhibit Dengue in Vero cells. Moreover, after the infection, ISGs upregulation by AZT was observed to be lower compared to IFN-III upregulation in Vero cells. Considering such evidence, it seems that other mechanisms are involved in the inhibitory function of AZT [33].

Screening of 2177 drug compounds against the Flavivirus Zika revealed the ability of AZT to decrease the proliferation of the virus and the cytopathic effects induced by the virus in glial cell lines and human astrocytes. An in vitro study has explored that AZT efficiently suppresses ZIKA infection through targeting a late stage in the life cycle of the virus [34].

An in vitro recently published data has suggested that AZT is capable of mitigating the infection rate of U87, a human glioblastoma astrocytoma cell line, in order, not only to rescue cell viability but also reduce viral production. While the AZT mechanism of action in preventing the ZIKV infection of target cells and viral production has not been identified, there are similar results achieved using ZIKV-infected neural cells derived from a human pluripotent stem cell (hPSC) [35].

Using both viral internalization and ZIKV replicon assays, Li et al. [33] displayed that AZT can inhibit ZIKV at the late stage of viral replication. AZT can also elevate the expression of multiple ISGs, including IFITM3 (IFN-induced transmembrane protein 3), *RASD2* (GTP-binding protein, Rhes, a protein encoded in humans by the *RASD2* gene), and *MX1* (a GTPase that is a part of the antiviral response), which all are famous for their anti-ZIKV functions [36].

Li et al. also uncovered that AZT, an antibiotic with extra anti-inflammatory features, can upregulate PRRs, IFN-I/III, and ISGs activated by ZIKV and/or other viruses and the natural IFN-I/III responses of the host to ZIKV, thereby suppressing infection. The results of their study reflect the activity mechanism of AZT against ZIKV, as well as affirm its potential as a candidate for clinical testing in the future [33].

The nucleotide sensor Zipcode binding protein 1 (ZBP1), a gene upregulated by AZT following ZIKV infection, has been found to be able to suppress ZIKV infection through inducing the metabolite itaconate [37]. AZT has been demonstrated to be able to hinder autophagy in macrophages. As autophagy has a vital function during ZIKV replication, the impacts of AZT on autophagy during ZIKV infection could be a subject for our next investigation [38].

The AZT efficacy was tested by Bosseboeuf et al. on Vero cells infected with ZIKV at a concentration that can be reached in vivo in amniotic fluid. They added a single or multiple dose(s) of 50 mg/L of AZT to the infected cells. They then assessed ZIKV replication by both immunofluorescence assay (IFA) and measuring viral RNA loads at various times up to 96 h post-infection. Their results revealed that ZIKV replication could be prevented by adding multiple doses of 50 mg/L of AZT and approved that AZT has the in vitro activity against ZIKV [39].

3.2. SARS-CoV

3.2.1. Structure

SARS was initiated from Guangdong Province of Foshan in China and spread rapidly to 33 regions of five continents, from November 2002 to July 2003 [40–42]. SARS promptly escalated across the world, and following its pandemic, almost 8500 people were infected, giving rise to more than 800 deaths [43]. SARS-CoV, the main causative agent of

human CoV group 2b, has animal's zoonotic transmission. The SARS-like-CoV, SL-CoV, is originated from animal hosts and has a homologous nucleotide (< 99%) with SARS-CoV.1 [44–47].

The term CoV is derived from the crown-like spikes located on the exterior layer of the virus. In the middle of the 1960s, two human CoVs were discovered. Both viruses, the human alpha CoV229E (HCoV-229E) and beta coronavirus OC43 (HCoV-OC43), were recognized as the causes of normal cold or mild-to-moderate respiratory diseases [48,49]. Later, during 2004 and 2005, two additional CoVs, namely the alpha CoVNL63 and the beta CoVHKU1, were explored. In the same years, a human beta CoV was identified. This highly dangerous new virus was capable of inducing lethal diseases such as SARS-CoV [43].

CoVs are members of the Nidovirales order and a family of positive-sense single-stranded RNA viruses with helical nucleocapsids and 80–160 nm in diameter [44]. In humans, CoVs cause the common cold together with SARS-CoV, whereas in animals, these viruses cause mild-to-serious intestine inflammation, as well as respiratory, neurologic, and systematic diseases [50]. In newborns, a CoV-like agent relevant to necrotizing enterocolitis has been identified [51]. Animals are infected with group 1 CoVs, humans with group 2, and birds with group 3 [49].

Polycistronic RNA of CoV genome entails 5′ methylated caps and 3′ polyadenylated tails. The partial overlap of 5′ terminal open reading frame ORF1a with ORF1b encodes two large replicase polyproteins (pp1), named pp1a and pp1ab. Nonstructural proteins, such as RNA polymerase and helicase, are important enzymes within the transcription and replication of CoVs. These enzymes are induced by the aforesaid polyproteins as a consequence of their cleavage by multiple proteases, i.e., 3C-like serine and papain-like cysteine proteases. The region downstream of ORF1b consists of four structural proteins (envelope [E], spike [S], membrane [M], and nucleocapsid [N]), which are a key player in virus-cell receptor assembly of the virion and have pathogenesis and immunomodulatory impacts Fig. 2 [53].

3.2.2. Effects of AZT against SARS-CoV

Owing to the antiviral properties, AZT has been used for patients with the CoVs, SARS-CoV, or MERS-CoV. Moreover, it possesses anti-inflammatory features such as the inhibition of interleukin 1β, interleukin 2, tumor necrosis factor, and granulocyte-macrophage colony-stimulating factor (GM-CSF). AZT can also restrict T cells through impeding calcineurin signaling and mammalian target of rapamycin activity and NFκB activation. The particular target of AZT is granulocytes, where it concentrates on lysosomes to achieve various functions, viz affecting bond, accumulation, degranulation, and apoptosis of neutrophils [54].

In a cohort study conducted by Zhao et al., clinical and laboratory characteristics of 190 patients who were randomly allocated to four treatment regimens were explained. Based on their results, the combined use of interferon (IFN), antibiotic treatment, and a high dose of immunoglobulins showed no remarkable effect on the disease. Therefore, in addition to AZT, quinolone was selected as the main diet for treating abnormal pneumonia. They found that in cases that COVID is not sensitive, a defensive impact may contribute to the advancement of secondary infections. These authors concluded that a mixture of the initial use of high-dose steroids with a quinolone and AZT could be the best outcome, which not only declined the frequency of acute respiratory distress syndrome (ARDS) but also diminished mechanical ventilation and mortality [55].

3.3. SARS-CoV-2

3.3.1. Structure

Coronaviruses belong to the genus Betacoronavirus of the subfamily Coronavirinae of the Coronaviridae family. They have been categorized into three known species: SARS-CoV, Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV), and the recently identified novel

Coronavirus (SARS-CoV-2) [56]. Coronaviruses have a large positive-stranded RNA genome of 25–32 kb with at least six small alternative-frame open reading frames (ORFs) [57]. Comparison of the alignment results shows Corona viral genome encodes four structural proteins, including spike glycoprotein (S), nucleocapsid (N), envelope (E), and polyprotein (P) [58]. According to the protein alignment, OFR8 and ORF10 of SARS-CoV-2 are different from the other Betacoronavirus members, while other ORFs are conserved [59].

The host cell membrane fusion mediated by the SARS-CoV-2 spike glycoprotein, thus, plays a critical role in virus infectivity and pathogenesis [60]. The spike trimer protein consists of two main subunits, including three S2 proteins located in the central helical stalk and S1 that covered S2 subunits. The S1 subunit harbors two domains, including the N-terminal domain (NTD) and receptor-binding domain (RBD) [61].

Many antiviral drugs target the spike-angiotensin-converting enzyme 2 (ACE2) receptor interaction, directly contributing to cell entry [60]. Two different conformational forms of S1 are detected during molecular studies, open and close. The S1-ACE2 interaction occurred in open form; thus, virus infection is initiated by interactions between the RBD and ACE-2 [59]. Therefore, targeting both RBD and ACE-2 is considered as a way to inhibit the infection of the host cells. Nowadays, many attempts have been made to design and discover various RBD blockers as well as ACE-2 inhibitors.

3.3.2. Effects of AZT against SARS-CoV-2

AZT has become a subject of paramount importance owing to its antiviral and immunomodulatory activities toward SARS-Cov-2 infection. Various mechanisms have hitherto been defined for the antiviral effect of AZT. For instance, it has been described that for host-cell entry, the SARS-CoV-2 viral spike protein needs to bind to ACE2. According to virtualized mechanical modeling techniques, AZT presumably interfere because of its affinity with the binding interaction point of the spike protein and ACE2. Furthermore, it may prevent a viral cofactor binding site in view of its significant molecular resemblance with the host-cell ganglioside GM1, binding the ganglioside binding domain of the spike protein [62].

The antiviral effects of AZT are speculated to arise from its interference with receptor-mediated binding, viral lysosomal escape, and intracellular cell-signaling pathways, as well as to result from the enhancement of type I and III interferon expression. AZT is able to decline mucus production and to enhance epithelial barrier thickness in in vitro models with respiratory epithelial cells. It could also diminish the activity of matrix metalloprotease (MMP) following challenge with bacterial lipopolysaccharides, which decrease inflammatory signaling and contribute to the retention of cell integrity and the function of epithelial barrier [63].

AZT has been indicated to have indirect antiviral effects. In infected host cells, AZT induces intracellular mRNA expression of antiviral genes, IFN-stimulated genes, and IFN production, which are possibly increase the cellular antiviral response mediated by the IFN pathway and maintain balance in the initial innate immune response [62].

Human cytosolic pH is under strict control and kept in a narrow range. A decrease of cytosolic pH value makes it easier for the virus to attach to ACE2. Recent structural studies showed that the cytosol pH plays an important role in virus penetration; the low cytosolic pH is related to the increase of the ACE2-related cell penetration, as virus penetration is associated with the change of cytosolic pH. Angiotensin II increasing cytosolic pH by effects on sodium (Na+)/hydrogen (H+) exchanger (NHE) and increasing Na+. NHE, a powerful intracellular pH regulator ion pump, is abundant in microtubules and is involved in regulating intracellular and extracellular pH [63].

Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARBs) by convert angiotensin II to angiotensin 1–7, increase the ACE2 level. So, ACEI and ARBs increase the viral load by lowering the cytosolic pH and increasing the ACE2 level [62].

In the last decade, the effect of AZT on Trans-Golgi network pH has been meticulously investigated in the cystic fibrosis (CF) model. The first model was developed by Poschet et al.; this proposed model relied on having substantial knowledge about the effect of cytosolic pH on the glycosylation of human ACE2 and other proteins [63]. *In silico* techniques are still developing technology, but some attempts have been made to quickly identify promising drug candidates against SARS-Cov-2, including AZT [64]. Due to accidental molecular similarity between AZT and the sugar moiety of GM1 (a lipid raft ganglioside acting as a host attachment cofactor for respiratory viruses, landing platform for the SARS-CoV-2 spike protein), AZT interacts with the ganglioside-binding domain of the SARS-CoV-2 spike protein. A conserved amino acid sequence that is known as QFN (Q-134/F-135/N-137) is responsible for interaction GM1 or/and AZT with the tip of the S1 protein, as AZT show a direct antiviral activity against SARA-CoV-2 [64].

Clear, well-published, and robust evidence supports the idea of the indirect anti-SARS-Cov-2 activity of AZT. In a study performed by Poschet et al., they found that 100 μm AZT can activate the furins. Furin is a proprotein convertase located in the trans-Golgi network and procced in acidic condition and in the active form break the specific precursor proteins that contain N- terminal "Arg-X-X-Arg" [65]. The acid –pH-dependent activation of furin is affected by AZT treatment [18]. Recently, Poschet et al., reported that the activity of furin markedly reduced in the presence of AZT, suggesting the existence of a controlling mechanisms that influenced organeller pH and reduce the furin activity. During the loss of furin in cell surface the furin-related cell entry decreased (Fig. 1) [65,66].

Some studies demonstrated that AZT shows activities against SARS-CoV-2 and interferes with the viral cycle [67]. It is also mentioned that AZT therapy have some positive effects on the hyperinflammatory state due to its inhibition of NF-κB and other proinflammatory signaling pathways [68]. Touret et al. reported the effects of AZT on a specific cell line consisting of Vero E6 cells with a MOI of 0.002 of SARS-CoV-2 and evaluated that AZT has a EC_{50} of 2.12 μM, an EC_{90} of 8.65 μM and a CC50 > 40 μM, with a selectivity index > 19 [69].

Andreani et al. have been experimentally demonstrated that AZT has a synergistic effect against SARS-CoV-1 and 2 in combination with hydroxychloroquine [70]. In Vero E6 cells, the cytopathic effect was reported, at the multiplicity of infection (MOI) 0.25 the viral replication significantly reduced in the presence of hydroxychloroquine in combination with AZT at 5 μg and 10 μg, respectively. The viral suppression was high in 5 μg and 10 μg AZT, 97% and 99.1%, respectively [70].

Although some studies have suggested the effective antiviral property of AZT, whereas others have argued that clinical use of AZT did not improve clinical outcomes (alone and in combination with hydroxychloroquine). A randomized clinical trial conducted by Furtado et al. noted several unexpected findings that lead the author to suggest that clinical use of AZT in combination with hydroxychloroquine did not improve clinical outcomes [71].

Furtado et al. reported that AZT does not affect COVID-19 mortality; mortality was similar in patients who had received AZT (214 patients, 42%, n = 90) and who had received no further treatment (control group with only hydroxychloroquine, 183 patients, 40%, n = 73). More recently, a randomized clinical trial indicated improved clinical outcomes and survival in patients admitted to hospital with COVID-19 is not observed by AZT treatment [72]. Although AZT provides a good safety profile in the COVID-19 treatment, these findings provide the support that immunomodulatory effects of AZT in patients admitted to hospital with COVID-19 are insufficient and may be a benefit in COVID-19 alone with high lung infection [67,72].
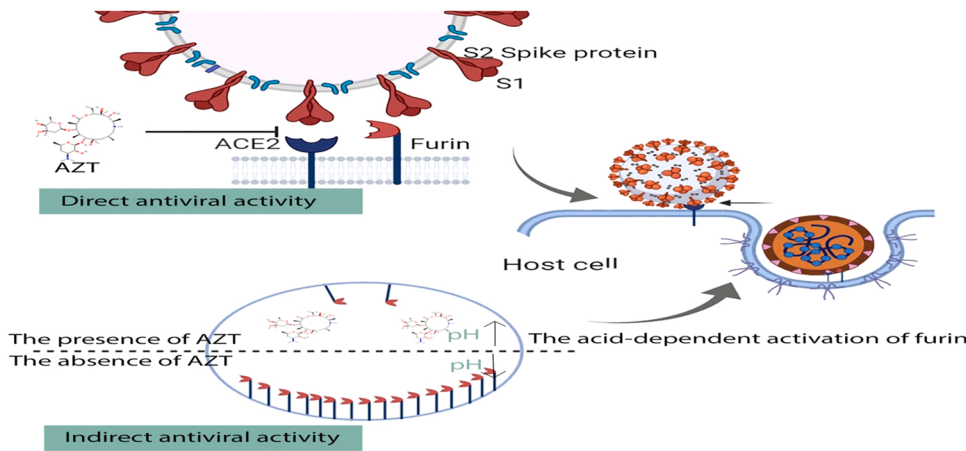
Fig. 1. Structure and mechanisms of action of azithromycin against SARS-CoV-2 cell interaction. Virus entry occurred by spike-ACE2 interaction. ACE-2 is an S1 particular receptor that can be inhibited by AZT in a direct inhibition pathway. After the S1-ACE2 connection, cell penetration is facilitated by the furin system, in which is blocked by AZT in an indirect inhibition pathway. In fact, the acid-dependent activation of furin reduced in the presence of AZT. Furin activity decreased indirectly by AZT during organelle pH alteration. Furin system cleavages S1 subunit from spike protein.
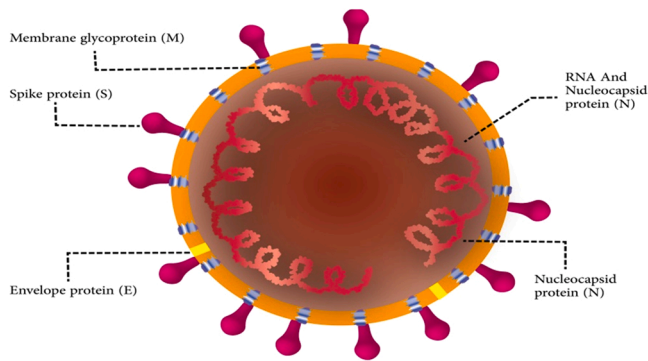

Fig. 2. Schematic model of the CoV. The helical nucleocapsid is covered by a lipid bilayer comprising the envelope protein (E), the spike protein (S), the membrane glycoprotein (M), as well as nucleocapsid protein (N), which is related to the viral RNA. In view of COVID, the lipid envelope arises from intracellular membranes [52].

and meningitis, and pneumonia, as well as hand-foot-mouth diseases [73]. The two aforementioned viruses are small, nonenveloped, positive-stranded RNA viruses having a genome size of ~7.2–7.5 kb and packed in an icosahedric capsid of 30 nm. Their viral genome is comprised of a single gene whose translated protein is cleaved by proteases encoded virally in order to produce 11 proteins. VP1, VP2, VP3, and VP4 consist of the viral capsid covering the RNA genome, and the eight remaining nonstructural proteins are responsible for the viral genome replication and assembly. The first three proteins. i.e., VP1, VP2, and VP3 are involved in the antigenic diversity of the virus, whereas VP4 protein connects the RNA core to the capsid. Each of the

4. Antiviral effects of AZT against non-enveloped positive-sense RNA viruses

4.1. Enterovirus and Rhinoviruses

4.1.1. Structure

Enterovirus (EVs) and Rhinoviruses (RVs), family members of *Picornaviridae,* are viruses that often cause acute and chronic diseases, such as heart disease, poliomyelitis, severe bronchiolitis encephalitis,
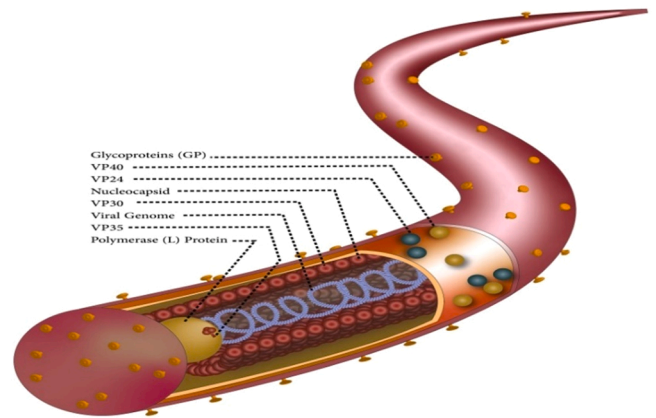

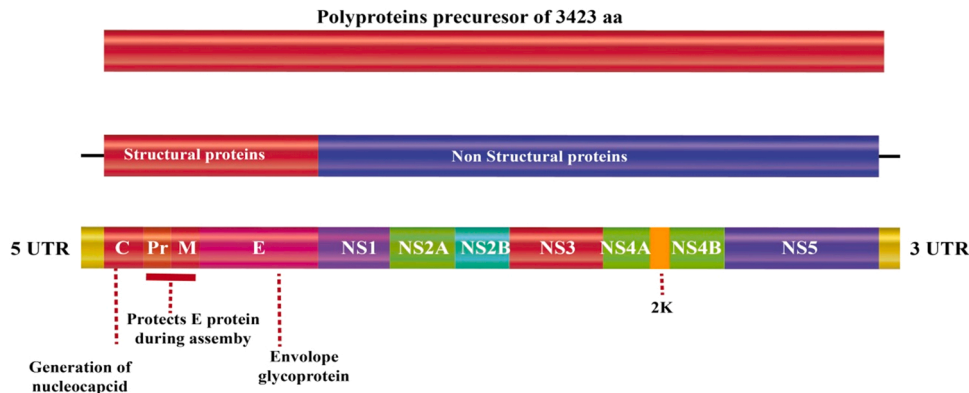
Fig. 4. The filoviral genome [23].

Fig. 3. The detailed structure of the ZIKV genome. ZIKV genomic RNA is capped but lacks poly A tail. The viral RNA codes for a polyprotein that is co-translationally cleaved to yield 11 proteins: three structural proteins (C prM/M and E) and eight non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B, and NS5).
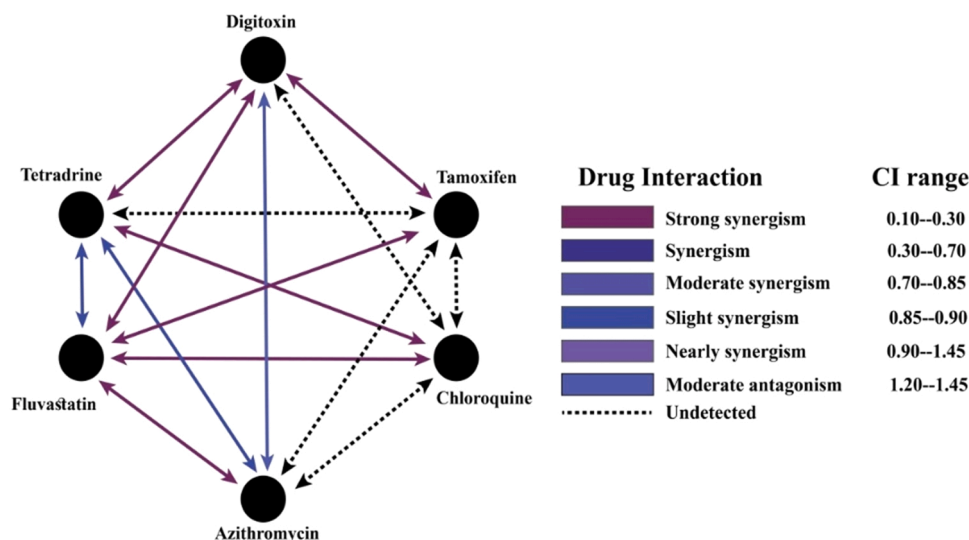
Fig. 5. The polygonogram of the synergistic antiviral effect of candidate drug combinations in an EBOV pseudovirion model [27]. Although the direct antiviral inhibitory effect of macrolide is not yet known, it is speculated that the antiviral effects of this antibiotic arise from its capability of amplifying systemic antiviral response mediated by the IFN pathway [28].

four capsid proteins has 60 copies, offering the virion an icosahedral structure with a canyon in VP1, which acts as the attachment site to cell surface receptors (Fig. 6) [74].

EV receptors encompass poliovirus receptor, Neclin-5 (Necl-5), intracellular adhesion molecule-1 (ICAM-1), coxsackie-adenovirus receptor (CAR), decay-accelerating factor (DAF), low-density lipoprotein (LDL), SCARB2, and integrin receptors. However, the receptor of most EVs is unknown because the majority of experimental studies have investigated model EV types. Non-protein factors, e.g., heparan sulfate and sialic acid, also mediate EV infection [75].

Cellular proteins such as cellular receptors, including ICAM-1, for human RVs and viral proteins that are highly conserved among serotypes (such as protease, polymerase, and hydrophobic pocket in VP1) might portray potential targets for inhibitors acting against different serotypes, and therefore having a broad-spectrum activity. Furthermore, compounds, especially those binding to the three serotypes as mentioned above, have been established to prevent multiple serotypes in vitro (Fig. 7) [73].

4.1.2. Effects of AZT against Enterovirus and Rhinoviruses

The macrolide antibiotics spiramycin (SPM) and AZT have been indicated to possess antiviral activities against EV-A71. Based on animal experiments, the anti-infection efficacy of AZT is stronger than spiramycin (SPM); for instance, in a mouse model severely infected with

EV-A71, AZT could remarkably alleviate the disease symptoms and enhance the survival rate. AZT is also a potential treatment alternative for EV-A71-induced hand-foot-mouth diseases. Moreover, it has been affirmed that this drug is safe for infants and children, making it more promising (Fig. 8) [76].

In a survey on young children, AZT has been reported to decline the occurrence duration of asthma-like symptoms, which highlights the role of this drug in the acute management of exacerbations. The mean duration of the occurrence after treatment was 3.4 and 7.7 days for children receiving AZT and placebo, respectively. AZT resulted in a 63.3% reduction of the occurrence, which was notable. Following the early initiation of treatment, the effect size raised, which indicates that if treatment begins before day six of the occurrence, the occurrence duration reduces up to 83%, but on or after day six, the duration decreases up to 36% (p < 0.0001) [77].

In a randomized, placebo-controlled trial on 6–11-month-old Indian infants, the effect of AZT on the mOPV3 immunogenicity was evaluated. In brief, 754 infants without serum neutralizing antibodies to type 3 poliovirus were randomly selected 1:1 and given oral AZT (10 mg/kg) or placebo for three days, starting two weeks before administrating a single dose of mOPV3. At the time of vaccination (day zero), EVs were present in 107 out of 367 (29%) seroconverted infants and in 149 of 337 (44%) who were not seroconverted. AZT administration had no significant effect on the incidence of any EV species, and infection with various EV species were prevalent [78].

The potential of AZT was examined in the study of Schögler et al. for the induction of antiviral mechanisms in the bronchial epithelial cells of CF. To this end, initial bronchial epithelial cells, which were collected from CF and control children, were infected with RVs following AZT pretreatment. AZT could lessen the replication of RVs in CF cells with no induction of cell death, as well as could raise RVs-induced PRRs, i.e., mRNA levels of IFN and ISG. Moreover, AZT stimulated antiviral responses but could not inhibit virus-induced pro-inflammatory responses [79].

In another study conducted by Ling et al., it was hypothesized that both the small and large airways could be infected with RVs, leading to significant inflammation. The impact of AZT on viral replication, apoptosis induction, and inflammation was scrutinized. Their results revealed that RV infection has the ability to infect both upper and lower airway epithelial cells, ultimately resulting in cell death and inflammation. Preventive treatment with AZT was also explored to diminish different detrimental responses [80].
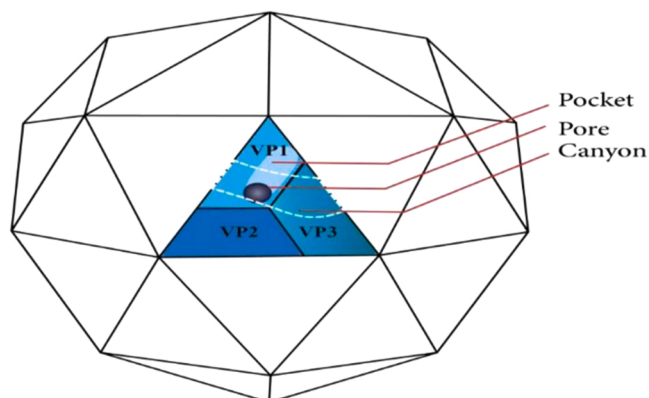

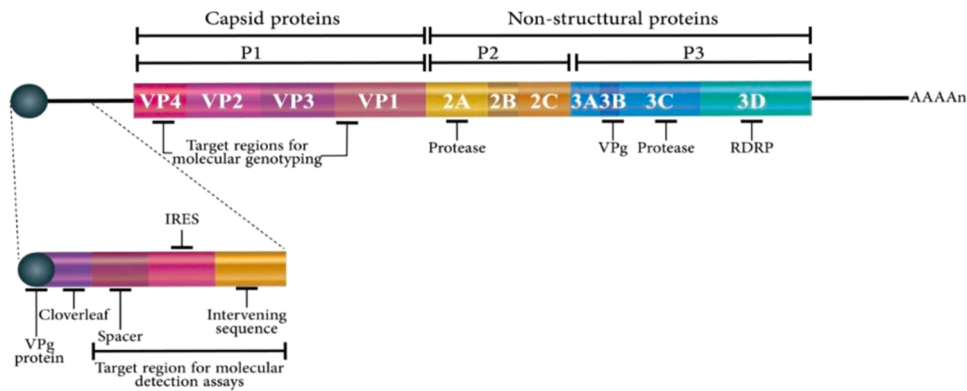**Fig. 6.** The structure of *Enterovirus* and *Rhinoviruses* [73].

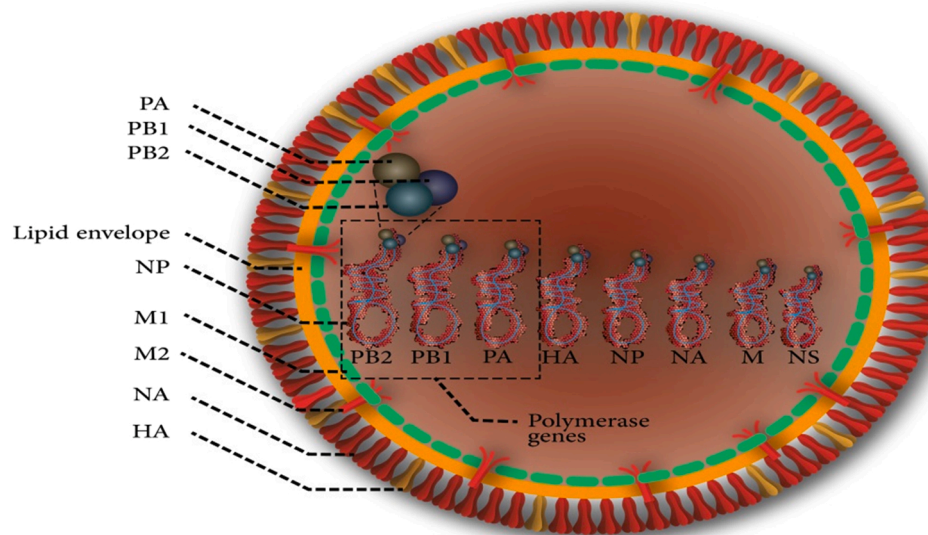**Fig. 7.** The genomic structure of *Rhinoviruses* [74].

Fig. 8. Type A and B influenza.

In the experimental models of asthma exacerbation, Menzel et al. uncovered that AZT could augment RV-induced IFN-β through cytosolic MDA5. The MDA5 knockdown by siRNA demonstrated that the MDA5 is involved in IFN-inducing effects by AZT in vitro. However, AZT induced IFN-β protein in vivo, which restored a decreased lung IFN response, particularly in exacerbating allergic mice. This behavior was likely due to the induction of ISGs and MDA5 [81].

Through the induction of antiviral gene mRNA and protein, the anti-rhinoviral potential of macrolides was investigated by Gielen et al. Using the macrolides AZT as well as RV 1B and RV 16, they pre-treated and infected primary human bronchial epithelial cells, respectively. AZT could considerably enhance IFNs induced the two RVs and also could increase IFN-stimulated gene mRNA expression and protein production. AZT, however, remarkably declined RV replication and release, but pre-treatment with the drug had no significant reduction in RV-induced interleukin 6 and interleukin 8 protein and mRNA expression. They finally reached the conclusion that AZT possesses anti-rhinoviral activity in bronchial epithelial cells and also maximizes the production of ISGs during RV infection [82].

5. Conclusion

The prevalence and intensity of viral outbreaks are increasing globally, and the economic costs of these outbreaks are going up. COVID-19 is an example which made its way to the top of the human prime concerns in a short time and this threat was the motivating force for researchers to discover efficient treatments against the virus. The proper way to achieve an efficient therapy against a virus is to study the structure of the virus and discuss the drug's mechanism of action on the virus. In this study, we reviewed the structure of some RNA viruses and discussed the effects of AZT against them. Although many studies have reported the use of AZM in the treatment of COVID-19, recently, WHO announced the discontinuation of AZT use in treating SARS-CoV-2 infection. In vitro studies showed that AZT can suppress the ZIKV infection and EBOV activity. In addition, AZT anti-rhinoviral activity in bronchial epithelial cells and remarkable decrease in RV replication has been confirmed. AZT can also reduce the duration of EV-caused asthma-like symptoms and IAV caused hypothermia. Recent studies show that in addition to antiviral effects, AZM can also have anti-cancer activities, which could be explored by other researchers in the future. Furthermore, since there is not any study about the antiviral effects of AZM against DNA viruses, we recommend that the researchers focus on this field. The synergistic and antagonistic effects of AZM with other drugs on viruses are also suggested.

Ethical approval

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Informed consent

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Consent for publication

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CRediT authorship contribution statement

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Conflict of interest statement

Authors declare that they have no competing interests.

Data availability

All the data in this review are included in the manuscript.

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