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REVIEW

Azithromycin in the treatment of COVID-19: a review

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ABSTRACT

Introduction: SARS-CoV-2 is a novel virus that causes coronavirus disease-19 (COVID-19). Antiviral and immunomodulatory agents have been proposed as potential treatments. Azithromycin exhibits both properties and therefore may play a role.

Areas covered: This article reviews the pharmacology, pharmacokinetics, clinical efficacy, and safety of azithromycin in viral infections, with emphasis on COVID-19. A literature search of PUBMED was conducted on May 30th and updated on July 28th.

Expert opinion: Azithromycin presents *in vitro* activity against SARS-CoV-2 and could act in different points of the viral cycle. Its immunomodulatory properties include the ability to downregulate cytokine production, maintain epithelial cell integrity or prevent lung fibrosis. Azithromycin use was associated with a reduction in mortality and ventilation days in other viral infections. These properties could be beneficial throughout the COVID-19. However, the evidence of its use is scarce and of low quality. Azithromycin has been assessed in retrospective observational studies mainly in combination with hydroxychloroquine, which has shown to provide no benefit. This macrolide presents a well-known safety profile. Upcoming clinical trials will determine the role of azithromycin in the COVID-19 (including the stage of the disease where it offers the greatest benefits and the effect of its combination with other drugs).

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1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the coronavirus disease-19 (COVID-19) [1,2]. According to the WHO, this virus has been declared pandemic, and to date (7th August), a total of 18,902,735 diagnosed cases and 709,511 deaths have been confirmed [3].

The treatment of choice for this new disease remains unknown, so effective and safe treatments shall be found urgently. The major therapeutic strategies in COVID-19 have been deeply revised in recent reviews [1,2]. The use of antiviral agents or the modulators of the immune function are some of the proposed options [1]. Lopinavir/ritonavir, hydroxychloroquine/chloroquine, remdesivir, ivermectin, favipiravir, umifenovir, camostat, nitazoxanide, minocycline, corticosteroids, tocilizumab, sarilumab, siltuximab, anakinra, interferons (IFN), adalimumab, and baricitinib/ruxolitinib have been studied, with conflicting results [1,2,4–7].

Within antiviral drugs, three mechanisms of action stand out [8]. Firstly, some drugs could act on the virus recognition and cellular entry (mainly targeting human angiotensin-converting enzyme 2 receptor [hACE2], the spike protein and type II transmembrane protease serine 2 [TMPRSS2]) [8]. This protease is pivotal to cellular entry, as it processes the SARS-CoV-2 to expose a cell-membrane fusion peptide [1]. Secondly, other drugs could hamper viral uncoating and replication (mainly acting on virus proteinases) [8]. Thirdly, other drugs could act on other viral structural proteins as envelope protein E or membrane protein M [8].

Another potential class of adjunctive therapies consists of drugs directed against key inflammatory cytokines or other aspects of the innate immune response [2]: interleukin 6 (IL-6) (tocilizumab, sarilumab, siltuximab), IL-1 (anakinra), tumor necrosis factor alpha (TNF-α, adalimumab), IFN (α, β, and γ), and Janus associated kinase inhibitors (baricitinib and ruxolitinib) [1,2].

In general, the quality of the studies was low, with poorly designed studies (mostly retrospective observational), biased conclusions, and unproven hypotheses [1]. Among the clinical trials carried out, only remdesivir and corticosteroids have improved clinical outcomes in hospitalized patients with COVID-19 [6,8]. Remdesivir was superior to placebo in shortening the time to recovery (11 vs. 15 days), although no differences in 14-day mortality were found [8]. In the RECOVERY trial, the use of dexamethasone was associated with a lower 28-day mortality (22.9 vs. 25.7%) in those patients requiring oxygen support or mechanical ventilation [6].

Azithromycin has been proposed as a potential therapy for the treatment of SARS-CoV-2 pneumonia given its antiviral and immunomodulatory activity with a well-known safety profile [9,10]. Nevertheless, its role in the treatment of COVID-19 remains unclear.

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We present an overview of the potential usefulness of azithromycin in the treatment of COVID-19. In this review, we discuss the pharmacology, pharmacokinetics, clinical efficacy, and safety of azithromycin in viral infections, with a special emphasis on COVID-19.

2. Data sources

A literature search of PUBMED was conducted on May 30th and was updated on July 28th. We included the search terms 'azithromycin', 'SARS-CoV-2,' 'COVID-19,' 'immunology,' 'immunomodulatory,' 'cytokine release syndrome,' and 'acute respiratory distress syndrome.' Results were limited to articles in English. Other citations were identified in references of available literature and from bioRxiv, medRxiv, and ClinicalTrials.gov.

3. Pathogenesis of COVID-19

SARS-CoV-2 enters the cell mainly via hACE2 through glycosylation [7,11]. In this process, SARS-CoV-2 is dependent upon plasmatic membrane components as gangliosides (especially GM-1), which act as attachment cofactors within lipid raft membrane platforms [11]. Dual recognition of both hACE2 and gangliosides by the spike protein is therefore needed [11].

Once this process has occurred, SARS-CoV-2 subsequently penetrates into the cell through endocytosis [7]. Thereafter, lysosomal proteases such as cathepsins, TMPRSS2, and furins must activate the fusion process by cleaving coronavirus surface spike proteins [7].

Infection triggers the host's immune response. The replication and release of the virus in alveolar epithelial cells causes the host to undergo pyroptosis and release pathogen-associated molecular patterns [12]. These molecules are recognized through Toll-like receptors by surrounding epithelial cells, endothelial cells, and alveolar macrophages, which present the foreign antigen to CD4+-T-helper (Th1) cells [12]. These processes set off the generation of other pro-inflammatory cytokines and chemokines such as IL-1, IL-2, IL-6, IP-10, monocyte chemoattractant protein, IFN-γ, TNF-α, granulocyte-colony stimulating factor (G-CSF), macrophage inflammatory protein 1α and 1β (MIP1α and MIP1β) [12,13]. The secretion of these substances attracts other immune cells as monocytes, macrophages, and T lymphocytes from the blood, promoting further inflammation [12].

Although most patients are able to clear the infection in the lungs, some will develop a dysfunctional immune response leading to a 'cytokine storm' [12]. The development of such cytokine release syndrome, characterized by an uncontrolled increase in the proinflammatory cytokines, has been associated with disease severity and prognosis [7,12,13]. This syndrome causes multi-organ damage. The respiratory failure is a consequence of lung fibrosis development and acute respiratory distress syndrome (ARDS), which is the leading cause of mortality of this virus [12,13]. In the specific scenario of ARDS, cytokines may cause epithelial and capillary endothelial damage [14]. COVID-19 has also been associated with a large number of cardiovascular complications, including myocarditis, type I and II myocardial infarction, arrhythmias, pulmonary edema, and acute heart failure [4,15]. The potential mechanisms of myocardial injury are the presence of cytokine storm, supply-demand imbalance, myocarditis related to viral invasion, plaque rupture, disseminated intravascular coagulation, coronary microvascular damage from thrombosis, hypoxemia, and multi-organ failure [4,15,16].

In order to facilitate the therapeutic approach of COVID-19, a 3-stage classification system has been proposed [17]. The first stage is usually mild with nonspecific symptoms [17]. In this phase, antiviral therapy may reduce the duration of symptoms, minimize contagiousness, and prevent progression of severity [17]. In the second stage, patients may develop viral pneumonia needing in most cases hospitalization [17]. The treatment consists of supportive measures and antiviral therapy [17]. Finally, few patients will transition into the third and most severe stage, where an extra-pulmonary hyperinflammatory syndrome develops [17]. At this point, the use of immunomodulatory agents could be useful to try to reduce systemic inflammation [1,2,,7,17].

4. Useful features of azithromycin in the treatment of COVID-19

4.1. Pharmacology

Azithromycin is an antibiotic that belongs to the macrolide family used in a wide variety of bacterial diseases [9]. Beyond its antibacterial activity, this macrolide has shown antiviral and immunomodulatory activities that could be of interest in viral infections, including COVID-19.

In Figure 1, the proposed antiviral and immunomodulatory mechanisms of action of azithromycin in the treatment of COVID-19 were described.

Azithromycin could act on SARS-CoV-2 binding to respiratory cells. Its intracellular accumulation led to an increase in the pH that may impair trans-Golgi network (TGN) and lysosome functions [18,19]. Poschet et *al*. found that the treatment of CF bronchial epithelial cells with 100 μM for 1 h and 1 μM of azithromycin for 48 h led to an increase in TGN pH from 6.1 ± 0.2 to 6.7 ± 0.1 [19]. Authors postulated that this increase in pH in TGN may alter glycosylation of hACE2 and other proteins [19].

Using molecular dynamic simulations, another direct antiviral mechanism of this macrolide was theorized [11]. Azithromycin resulted in a ganglioside-mimic given its similar volume and analogous chemical features than GM1. Since the spike protein of SARS-CoV-2 displays a ganglioside-binding site, azithromycin might inhibit SARS-CoV-2 infection by binding to this site. This would prevent the virus spike protein to reach gangliosides on the host plasma membrane, which is involved in SARS-CoV-2 pathogenesis [11]. In addition, azithromycin may interfere in the spike protein/CD147 interaction or CD147 expression [20].

The increase in the lysosomal pH by azithromycin may also alter the endocytosis process and lysosomal proteases function (cathepsins or furins), which may difficult the fusion process [9,18,19,21]. Poschet et *al*. found that 100 μM of azithromycin could normalize the excessive processing and activation of furins [19]. Given that SARS-CoV-2 has been shown to present a furin-like cleavage site in the spike protein, the reduction in the activation of furins by azithromycin could prevent the entry of SARS-CoV-2 into human epithelial cells [1,19].

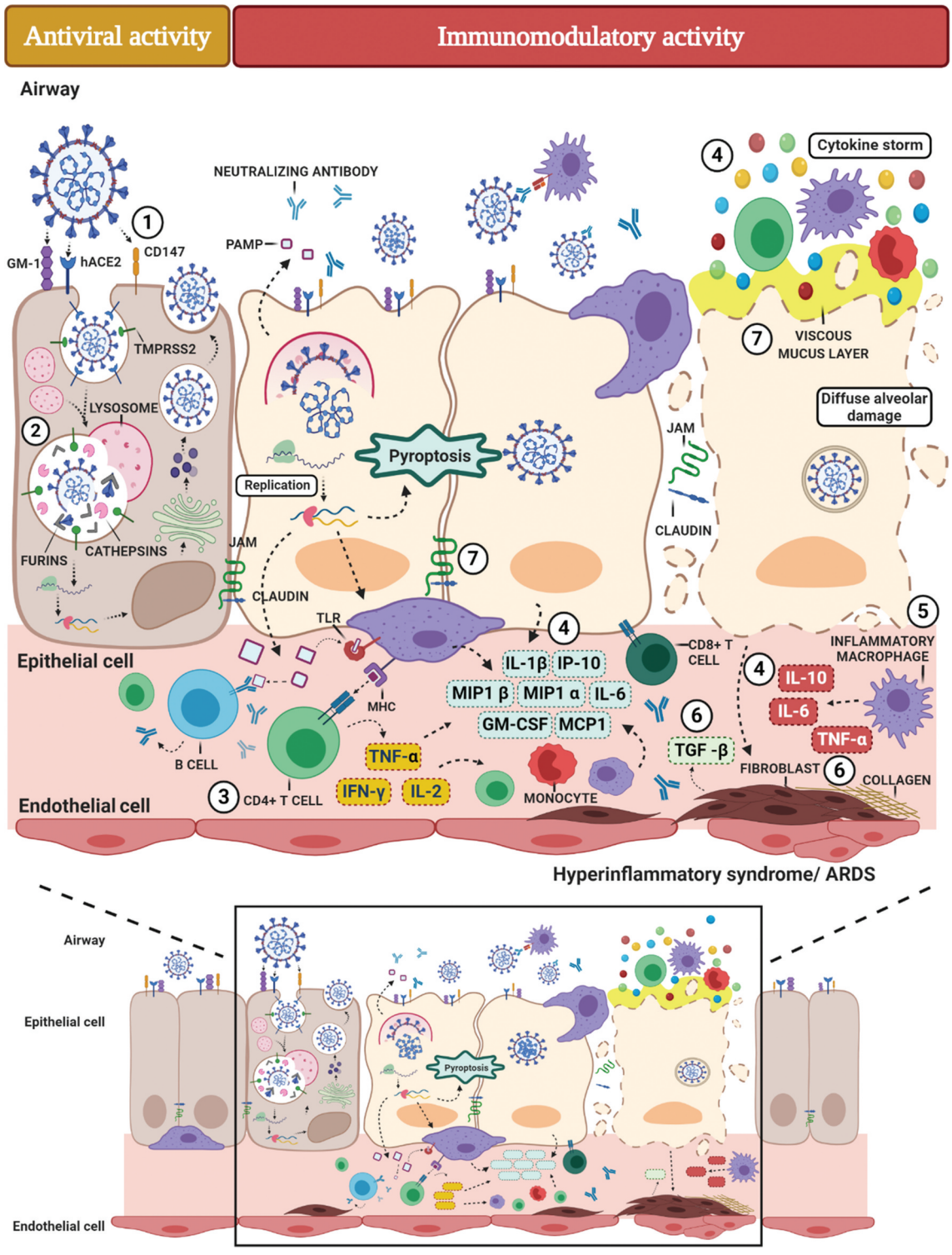

Figure 1. Potential mechanisms of action of azithromycin in the treatment of COVID-19.
1. *SARS-CoV-2 binding:* the increase in the pH of Trans-Golgi network may alter hACE2 glycosylation. Azithromycin resulted in a ganglioside-mimic given its similar volume and analogous chemical features than GM1. Since the spike protein of SARS-CoV-2 displays a ganglioside-binding site, azithromycin might inhibit SARS-CoV-2 infection by binding to this site. It may also interfere with ligand CD147 receptor interactions.
2. *Membrane fusion, endocytosis, and lysosomal protease activation:* the increase in lysosomal pH impairs the endocytosis process and the action of essential lysosomal proteases, as cathepsins or furins, implicated in the cleavage of the spike protein of SARS-CoV-2.
3. *Lymphocytes:* suppression of CD4+ T-cell activation.
4. *Reduction of pro-inflammatory cytokines and chemokines production:* IL-1β, IL-6, IL-8, IL-12, IFN-γ, IP-10, TNF-α, and GM-CSF.
5. *Alveolar macrophages:* shift in the polarization to anti-inflammatory phenotype and increase apoptosis.
6. *Fibroblasts:* antifibrotic activity: inhibition of fibroblast proliferation, collagen production reduction, decrease transforming growth factor TGF-β production, inhibition of TGF-β induced pro-fibrotic gene stimulation.
7. *Epithelial cells:* stabilization of the cell membrane, increase in the transepithelial electrical barrier and induction of the processing of the tight junction proteins claudins and junctional adhesion molecule-A. Decrease mucus hypersecretion, which may improve mucociliary clearance.

4.2. Pharmacokinetics in lung infections

Although a 37% of oral bioavailability has been described, the extensive tissue accumulation offsets its sub-optimal absorption [9]. Azithromycin accumulates in epithelial cells, fibroblasts, lymphocytes, and alveolar macrophages where, compared to serum, 400 to 1,000-fold higher concentrations can be achieved [9]. The chemotactic drug delivery further increases local drug concentrations, as blood phagocytes and other cells that migrate into infected and inflamed tissues release accumulated azithromycin [9,22]. As a consequence, azithromycin presents a long half-life of 68–79 h [22].

This drug presents an excellent lung tissue penetration and sustained drug concentrations [9,22,23]. Following 500 mg once daily (OD) for 3 days, a C_{max} of 0.72–0.83 μg/mL in bronchial washing and 8.93–9.13 μg/mL in lung tissue was found [23,24]. After a single oral dose of 500 mg, peak concentrations were 1.2–2.18 μg/mL in the epithelial lining fluid and 194 μg/mL in alveolar macrophages [25,26].

Azithromycin can be given either 500 mg OD for 3–5 days or 500 mg on day 1 followed by 250 mg OD on days 2–5 [23]. However, the optimal dosage in viral infections remains unknown. In community-acquired pneumonia (CAP) IDSA guidelines several regimens are recommended, based on severity: for outpatients, 500 mg 1 day and 250 mg thereafter for 3–5 days, whereas for severe patients, 500 mg OD for 5 days is advised [27]. In the RECOVERY trial, which is evaluating the potential role of azithromycin in COVID-19, 500 mg OD during 10 days is being studied [28].

4.3. Antiviral activity

Azithromycin has shown *in vitro* activity against a wide variety of viruses (Zika, Ebola, rhinovirus, enterovirus, influenza), with a wide range of 50% effective concentration (EC_{50}), depending on cell culture and multiplicity of infection (MOI) [24,29–32]. In the case of Zika virus, azithromycin was assessed in Vero, Huh7, A549, U87, and Hela cells 12 h pre-treatment with an MOI of 0.1, showing an EC_{50} of 1.23–6.59 μM [24]. As for Ebola, the assay was performed with Hela cells 1–8 h pre-treatment (MOI unknown), with EC_{50} of 0.69–2.79 μM [24]. In rhinovirus infection, azithromycin was added 24 h pre-treatment in human bronchial epithelial cells with an MOI of 1.0. In these conditions, although azithromycin EC_{50} was not calculated, rhinovirus replication was inhibited at 10 and 50 μM [24,33].

In infections caused by zika and rhinovirus azithromycin upregulated virus-induced type I and III interferon responses that reduced viral replication, suggesting that rather than antiviral activity immunomodulatory actions may be involved [30,31,34,35]. In mice with influenza A(H1N1) pneumonia pretreatment with azithromycin was associated with a reduction in viral load and relieved hypothermia [29].

Macrolides have shown *in vitro* activity against SARS-CoV-2. Bafilomycin A decreased the entry of pseudovirions by 99% compared to the control group [36]. In Vero E6 cells with an MOI of 0.002, azithromycin showed an EC_{50} of 2.12 μM, an EC_{90} of 8.65 μM, and a 50% cytotoxic concentration >40 μM, with a selectivity index >19 [37]. On the contrary, another study performed in Vero E6 cells with an MOI of 0.25 azithromycin alone did not show any antiviral activity [38]. However, the combination of hydroxychloroquine at 5 μM with azithromycin at 5 μM and 10 μM was found to be synergistic and significantly inhibited viral replication [38]. The different MOI among the two studies may have accounted for these differences. Caution is advised when interpreting these results given the different MOI, cell lines, incubation times, and analytical methods [24].

The aforementioned therapeutic regimens of azithromycin could achieve therapeutic concentrations in the lung to be effective against SARS-CoV-2 [24,38]. Based on previously described pharmacokinetic and *in vitro* data, C_{max}/EC_{50} ratios of 91.5 in alveolar macrophages or 4.3 in lung tissue could be achieved [24,38]. In the study of Andreani et *al*. authors concluded that the observed synergy with hydroxychloroquine was observed at concentrations achieved *in vivo* in the lungs [38].

4.4. Immunomodulatory activity

Azithromycin exerts its immunomodulatory effects on different points of the inflammatory cascade, modulating cell functions, and cell signaling processes [9,39,40].

In airway epithelial cells, macrolides can maintain cell integrity by stabilizing the cell membrane, increasing the transepithelial electrical barrier, and inducing processing of the tight junction proteins claudins and junctional adhesion molecule-A [39,41,42]. They can also decrease mucus hypersecretion *in vitro* and in vivo, even when not produced by bacteria, which may improve mucociliary clearance [39,43,44]. Furthermore, azithromycin use directly relaxed precontracted airway smooth muscle cells [9].

In vitro, this macrolide can decrease the hypersecretion of pro-inflammatory cytokines and chemokines by acting in many inflammatory cells as monocytes, macrophages, and fibroblasts [9]. Its use has been related to a reduction of IL-1β, IL-4, IL-5, IL-6, IL-8, IL-12, IFN-γ, IP-10, TNF-α, and GM-CSF [9,39,40]. Azithromycin shifted the polarization of alveolar macrophages to their alternative activated anti-inflammatory M2 phenotype leading to attenuated Th-1 cell responses [9]. It also increased the phagocytosis of apoptotic bronchial epithelial cells by macrophages [45]. In lymphocytes, azithromycin has shown to suppress CD4 + T-cell activation [46]. On the contrary, azithromycin can increase the release of an anti-inflammatory cytokine (IL-10) related to the reparation of inflamed tissues [9,39].

In mice, the treatment with azithromycin reduced mortality in pneumococcal pneumonia, and viral bronchiolitis [47,48]. These findings were found even in the setting of macrolide-resistant strains, suggesting that the immunomodulatory properties, including the aversion of cytokine storm, may explain these benefits. Azithromycin reduced the accumulation of inflammatory cells (macrophages, lymphocytes, and neutrophils) in bronchoalveolar lavage and in lung tissue [47]. In addition, it downregulated the expression of chemokines (G-CSF, CCL3/MIP-1α, CCL4/MIP-1β) and cytokines (IL-1β, IL-6, IL-12, TNF-α, and IFN-γ) in the lung [47].

In fibroblasts, macrolides have demonstrated *in vitro* to inhibit fibroblast proliferation, collagen production, and to

Table 1. Clinical efficacy of azithromycin in viral infections.

Ref.	Design and location	Severity, % or mean value	Virus	Treatment regimen (mg)	Number of patients	Main results
Lee et *al* [53].	Multicenter, randomized open-label controlled trial, China	Supplemental oxygen: 32 Mechanical ventilation: 4	Influenza A (H3N2) (H1N1) Influenza B	OR 500 OD for 5 days	AZT + OST: 25 OST: 25	At day 10: IL-6: −83.4% vs. − 59.5 %, P = 0.017 IL-17: −74.0 % vs. −34.3 %, P = 0.011 CXCL9/MIG: −71.3 % vs. −56.0%, P = 0.031 CRP: −77.5% vs. −48.2 %, P = 0.171
Kakeya et *al*. [54]	Multicenter, randomized open-label clinical trial, Hong-Kong	Not reported	Influenza A (H1N1)	OR 2,000 extended-release single-dose	AZT + OST: 56 OST: 51	Improvement in sore throat at day 2 (P = 0.03) Decrease in the maximum temperature on day 4 (P = 0.037) Maximum temperature on day 3–5 significantly lower (P = 0.048)
Martin-Loeches et *al* [55].	Multicenter, prospective observational cohort study, Spain	ICU admission: 100 APACHE II: 14.3	Influenza A (H1N1)	NR	Macrolides: 190 *CLT: 99 (52.1)* *AZT: 90 (47.4)* No macrolides: 543	ICU mortality rate: aOR: 0.89 (95% CI 0.53–1.49) ICU mortality rate in mechanically ventilated: aOR: 0.77 (95% CI 0.44–1.35)
Ishaqui et *al*. [56]	, retrospective observational cohort study, Saudi Arabia	Lymphocytes: 240 cell x10^9 Albumin: 4.1 g/dL	Influenza A (H1N1)	OR/IV 500 (duration unknown)	AZT + OST: 102 OST: 227	Secondary bacterial infections: aOR: 0.285 (95% CI, 0.10–0.81) Respiratory support during hospitalization: aOR: 0.28 (95% CI, 0.09–0.786) Length of hospital stay: aOR: 0.21 (95% CI, 0.14–0.31) Influenza symptom severity score day 5: aOR: 0.67 (95% CI, 0.57–0.87)
Arabi et *al* [57].	Multicenter, retrospective observational cohort study, Saudi Arabia	SOFA: 9 Mechanical ventilation: 61.8	MERS-CoV	NR	Macrolides: 136 *AZT: 97 (71.3)* *CLT: 28 (20.6)* *ERT: 22 (16.1)* No macrolides: 213	90-day mortality: aOR: 0.84 (95% CI 0.47–1.51) RNA clearance: aHR: 0.88 (95% CI 0.47–1.64)

aOR: adjusted odds ratio; APACHE II: Acute Physiology and Chronic Health Disease Classification; AZT: Azithromycin; CLT: Clarithromycin; CRP: C-reactive protein; ERT: erythromycin; IL: interleukins; IV: intravenous; MERS-CoV: Middle East Respiratory Syndrome coronavirus; NR: not reported; OD: once daily; OR: oral; OST: oseltamivir; SOFA: Sequential Organ Failure Assessment.

decrease transforming growth factor (TGF-β) levels [49]. In a murine model of acute lung injury caused by bleomycin, azithromycin significantly reduced fibrosis and restrictive lung function pattern [50]. Once fibrosis has been established, azithromycin could also have antifibrotic and proapoptotic effects on primary fibroblasts [51]. Therefore, in the late fibroproliferative-fibrotic phase of ARDS azithromycin may suppress lung fibrosis [14].

Although specific data are lacking, this macrolide exhibits immunomodulatory properties that could be beneficial in the treatment of COVID-19.

4.5. Clinical efficacy

Macrolides have shown their clinical efficacy in a wide variety of respiratory viral infections [52]. In particular, azithromycin has been studied in influenza and Middle East Respiratory Syndrome coronavirus (MERS-CoV) infections [53–57]. The clinical efficacy of azithromycin in these infections is summarized in Table 1.

Lee et *al*. concluded that in hospitalized patients with influenza A pneumonia, the addition of azithromycin to oseltamivir significantly reduced the synthesis of proinflammatory cytokines, with a trend toward a faster symptom resolution [53]. Kakeya et *al*. assessed the treatment with azithromycin and oseltamivir initiated within 48 h of the onset of symptoms in patients with mild influenza A pneumonia. The addition of azithromycin significantly increased the resolution of fever and sore throat, without differences in the expression levels of cytokines and chemokines [54]. The low baseline values of these substances, however, may have affected the outcomes. These studies were not exempt from limitations, since they were open-label clinical trials with a small number of patients included. Subjective outcomes were analyzed, which does not seem to be the most appropriate measure in an open-label trial.

On the contrary, Martin-Loeches et *al*. did not show a survival benefit of macrolides in the treatment of influenza A pneumonia in critically ill patients [55]. However, this was a secondary analysis of an observational study and, importantly, both clarithromycin and azithromycin were included. Given that clarithromycin has shown less immunomodulatory activity, the potential benefits of azithromycin in this setting may have been underestimated [39,40].

Recently Ishaqui et *al*. demonstrated that the addition of azithromycin (initiated 6–8 h after diagnosis) significantly improved meaningful clinical outcomes as the length of stay or the need for respiratory support during hospitalization [56]. Although groups were well balanced in admission and adjusted in the multivariate model, it was a retrospective observational study so other confounding factors may have also been present.

In the study of Arabi et *al*. in MERS-CoV infection, macrolides were not associated with a significant benefit in 90-day mortality [57]. Again, it was a secondary analysis of an observational study and macrolides were grouped.

The immunomodulatory action of azithromycin improved important clinical outcomes in other settings, which could be applicable to COVID-19 [9].

In the treatment of CAP, including those admitted to the intensive care unit (ICU), its use is recommended in guidelines in combination with beta-lactams, especially in critically ill patients [27,58]. In ICU patients, macrolide use was associated with a significant reduction in mortality, even in the setting of macrolide-resistant strains, suggesting that the immunomodulatory properties may account for this difference [44,48].

In patients with ARDS, azithromycin use presented important clinical benefits. In a secondary analysis of a multicenter, randomized controlled clinical trial, 235 patients with acute lung injury (mainly due to pneumonia) were included [59]. After adjusting for confounding factors, the treatment with macrolides was associated with a reduction in the time to successful ventilator discontinuation (HR 1.93 [95% CI 1.18–-3.17]) and 180-day mortality (HR 0.46 [95% CI 0.23–0.92]). These differences may be due to immunomodulatory properties as were not seen with fluoroquinolones or cephalosporines. A single center, retrospective, propensity-score matched analysis included 124 patients with moderate-severe ARDS (due to pneumonia and sepsis) [14]. The adjunctive therapy with azithromycin was associated with a shorter time to successful discontinuation of mechanical ventilation (HR 1.74 [95% CI 1.07–2.81]) and a reduction in 90-day mortality (HR 0.49 [95% CI 0.27–0.87]).

These benefits may be translated into patients with COVID-19, as a recent study showed that the cytokine profile in plasma (IL-1β, IL-1RA, IL-6, IL-8, IL-18, and TNFα) of severe COVID-19 patients did not differ from that found in other ARDS and sepsis of other causes [60].

Concerning the data on SARS-Co-2 pneumonia, all the available evidence on the use of azithromycin is summarized in Table 2.

In March, Gautret et *al*. showed that the early treatment with hydroxychloroquine presented superior virological clearance compared to standard of care [10]. Moreover, the addition of azithromycin further improved the activity of hydroxychloroquine alone. However, this study presents many limitations. This was a non-randomized open-label clinical trial that only included 36 patients. Only six patients were treated with hydroxychloroquine and azithromycin without an adequate control group. From a total of 26 patients treated with hydroxychloroquine, 6 were lost in follow-up: 3 because were transferred to ICU, 1 died, 1 decided to leave and 1 stopped the treatment due to nausea. Finally, baseline clinical data were lacking, and no clinical outcomes or safety data were reported. The International Society of Antimicrobial Chemotherapy raised concerns as they believed that did not meet the society's expected standard [61].

These authors subsequently expanded the number of patients evaluating this combination [62,63]. Given that they included those admitted to the infectious disease ward or treated in day-care hospital, disease presentation was mild. Overall, clinical, and viral outcome was positive. On the contrary, Molina et *al*. challenged these results in sicker patients as this strategy was not associated with any clinical benefit or antiviral activity [64]. In all these studies, unfortunately, the lack of control group prevents the attribution of any effects.

Other studies have analyzed this strategy in outpatients. Guerin et *al*. assessed the time to clinical recovery of

Table 2. Clinical studies of azithromycin for the treatment of COVID-19.

Ref.	Design and location	Comorbidities, %	Severity, %	Days from symptoms onset	Treatment regimen (mg)	Number of patients	Main results
Gautret et *al* [10].	Multicenter, open-label, non-randomized CT, France	HBP: NR Diabetes: NR Obesity: NR	Asymptomatic: 16.7 URTI symptoms: 61.1	4	500 day 1, 250 OD days 2–5	 HCQ: 20 HCQ + AZT: 6 SOC: 16	*PCR negative day 6 (P = 0.001)* 57.1 % 100% 12.5%
Gautret et *al* [62].	Single center, retrospective, observational study, France	HBP: 16 Diabetes: 11 Obesity: 5	Asymptomatic: 5.0 URTI symptoms: 41.2 NEWS low (0–4): 92	5	500 day 1, 250 OD days 2–5	HCQ + AZT: 80	*Day 7*: 81% clinical cure 3.8% transferred to ICU 83% PCR negative
Million et *al* [63].	Single center, retrospective, observational study, France	HBP: 14 Diabetes: 7 Obesity: 6	NEWS low (0–4): 95	6	500 day 1, 250 OD days 2–5	HQC + AZT: 1,061	*Day 7*: 91.7% clinical and virological cure 0.9 % transferred to ICU 0.8% died
Molina et *al* [64].	Single center, retrospective, observational study, France	HBP: NR Diabetes: NR Obesity: 18 Cancer: 46	NR	NR	500 day 1, 250 OD days 2–5	HCQ + AZT: 11	*Day 5*: 9% died 18.2% transferred to ICU *Day 6*: 80% PCR positive
Guerin et *al* [65].	Single center, retrospective, observational study, France	HBP: 12.8 Diabetes: 3.4 Obesity: 13.6	Outpatients	1 (41%) 15 (57.9%) 40 (1.1%)	500 day 1, 250 OD days 2–5	 HCQ + AZT: 20 AZT: 34 SOC: 34	*Time to clinical recovery, median (range):* 7 (2–40) 7 (3–48) 27 (6–48)
Barbosa et al [66].	Open label, controlled non-randomized trial, Brazil	HBP: 26.5 Diabetes: 13.4 Obesity: 7.7	Outpatients	5.2 ± 3.1	500 OD 5 days	 HCQ+AZT: 412 SOC: 224	*Need for hospitalization* 1.9% 5.4%
Mahevas et *al* [68].	Multicenter, retrospective, propensity-score matched observational study, France	HBP: 51 Diabetes: 9 Obesity: 26	>50% extend on CTS: 33	7	500 day 1, 250 OD days 2–5	 HCQ: 84 *HCQ + AZT: 15* SOC: 89 *AZT: 26*	*21-day mortality % and HR:* HCQ: 11, 1.2 (95% CI 0.4–3.3) Control: 9. Reference
Magagnoli et *al* [69].	National retrospective, propensity-score matched observational study, USA	HBP: NR Diabetes: 67.7 BMI: 29.8 Charlson: 2.3	Albumin < 2.8 g/dL: 17.6 Heart rate >100 lpm: 15.5	NR	NR	 HCQ: 198 HCQ + AZT: 214 SOC: 395 *AZT: 91*	*In-hospital mortality % and aHR:* 19.2, 1.83 (95% CI 1.16–2.89) 22.9, 1.31 (95% CI 0.80–2.15) 9.4. Reference
Geleris et *al* [70].	Single center, retrospective, propensity-score matched observational study, USA	HBP: 52 Diabetes: 36 Obesity: 41	Median values: Pao_2/Fio_2: 248 mmHg Oxygen saturation: 94% Heart rate: 98 bpm Ferritin: 665 ng/ml	NR	500 day 1, 250 OD days 2–5	HCQ: 811 *HCQ + AZT: 486* SOC: 274 *AZT: 102*	*Time to intubation or death HR*: HCQ: 1.04 (95% CI 0.82–1.32) AZT: 1.03 (95% CI 0.81–1.31)
Rosenberg et *al* [71].	Multicenter, retrospective, observational study, USA	HBP: 57 Diabetes: 7 Obesity: 43	ICU: 12.8 Mechanical ventilation: 9.5	NR	500 OD. Unknown duration	 HCQ + AZT: 735 HCQ: 271 AZT: 211 SOC: 221	*In-hospital mortality aHR:* 1.35 (95% CI 0.76–2.40) 1.08 (95% CI 0.63–1.85) 0.56 (95% CI 0.26–1.21) Reference

(*Continued*)

Table 2. (Continued).

Ref.	Design and location	Comorbidities, %	Severity, %	Days from symptoms onset	Treatment regimen (mg)	Number of patients	Main results
Arshad et *al*. [72]	Multicenter, retrospective, propensity-score matched observational study, USA	HBP: 65.4 Diabetes: 37.6 Obesity: 52.3	ICU: 24.2 Mechanical ventilation: 17.6 Oxygen saturation: 92%	NR	500 day 1, 250 OD days 2–5	HCQ + AZT: 783 HCQ: 1,202 AZT: 147 SOC: 409	*In-hospital mortality % and HR* 20.1. 0.294 (95% CI 0.218–0.396) 13.5. 0.340 (95% CI 0.254–0.455) 22.4. 1.05 (95% CI 0.682–1.616) 26.4. Reference
Cavalcanti et *al*. [73]	Multicenter, randomized, open-label, controlled trial, Brazil	HBP: 38.8 Diabetes: 19.1 Obesity: 15.5	ICU: 13.8	7	500 OD 7 days	HCQ + AZT: 217 HCQ: 221 SOC: 227	*7-level ordinal outcome at 15 days and HR* 1 (1–2). 0.99 (95% CI 0.57–1.73) 1 (1–2). 1.21 (95% CI 0.69–2.11) 1 (1–2). Reference

aHR: adjusted Hazard Ratio; AZT: Azithromycin; BMI; body mass index, kg/m^2; CI: confidence interval; CLT: clarithromycin; CT: Clinical trial; CTS: computed tomography scan; HBP: high blood pressure; HCQ: hydroxychloroquine; HR: hazard ratio; ICU: intensive care unit; MCR: macrolide; NEWS: National Early Warning Score; NR: not reported; OD: once daily; PCR: polymerase chain reaction; SOC: standard of care; URTI: upper respiratory tract infections.

azithromycin alone and its combination with hydroxychloroquine compared to standard of care in outpatients [65]. Both treatments accelerated recovery both in the global cohort and after adjusting in a case–control analysis compared to the control group. No significant differences were found when azithromycin monotherapy and combination therapy were compared (P = 0.26). Caution is advised given the small sample size and the outcome was a subjective measure. Other limitations include that the time of treatment initiation from symptom onset was day 1 in 41% of patients, while the rest initiated within 15 days except one in the azithromycin alone group in day 40.

Barbosa et *al*. evaluated the need for hospitalization in outpatients treated with combination therapy [66]. Patients with flu-like symptoms were referred to telemedicine service, where combination therapy was offered. Those who refused to initiate this treatment were considered the control group. The treatment group was associated with a reduction in the need for hospitalization of 3.5%. Moreover, among those in the treatment group, patients treated before day 7 of symptoms onset required less hospitalization (1.2% vs. 3.2%, P < 0.001). Again, this was a pre-print open-label study and was performed by a telemedicine health-care team, so it may not be applicable to other settings.

A recent review concluded that the combination of hydroxychloroquine and azithromycin should be used in symptomatic high-risk outpatients [67]. According to this study, early outpatient illness is very different than the later disease, and in this setting, the combination therapy could confer important clinical benefits [67].

Mahevas et *al*. assessed the efficacy of hydroxychloroquine in 173 hospitalized patients showing no effect in any outcomes [68]. Patients with organ failure, ARDS or ICU at admission and those treated with other experimental therapies (remdesivir, tocilizumab, or lopinavir/ritonavir) were excluded. Given that the objective of the study was the evaluation of the efficacy of hydroxychloroquine, the outcomes of azithromycin alone or in combination were not analyzed. Azithromycin was administered in 15 (18%) patients in the treatment group and 26 (29%) in the control group. Among those treated with azithromycin alone, 5 (19.2%) died and 6 (23.1%) were transferred to the ICU. These patients, however, were not further analyzed nor included in the propensity-score analysis and no data about their baseline and clinical demographics were detailed.

In patients hospitalized at Veterans Health Administration medical centers, Magagnoli et *al*. demonstrated a higher risk of mortality in hospitalized patients treated with hydroxychloroquine alone after propensity-score adjustment [69]. However, this finding was not observed with combination therapy. The risk of mechanical ventilation was similar among hydroxychloroquine alone (aHR 1.19 [95% CI 0.78–1.82]) and hydroxychloroquine/azithromycin groups (aHR 1.09 [95% CI 0.72–1.66]) when compared to the no-hydroxychloroquine group. The use of other therapies was not assessed and no information about ICU status at admission was reported.

Geleris et *al*. included 1,085 hospitalized patients in a propensity-score matched analysis in New York [70]. Patients who died or were intubated within 24 hours after presentation were excluded. Azithromycin was used in both groups (59.9% in the treatment group and 37.2% in the

control group). Other agents as tocilizumab/sarilumab or remdesivir were allowed (data on corticosteroids were not shown). In the multivariate analysis, hydroxychloroquine or azithromycin use was not associated with the composite primary endpoint.

Rosenberg et *al*. showed a trend toward reduced mortality in the azithromycin alone group, after adjusting for multiple factors [71]. Unlike other studies, patients admitted to the ICU were not excluded. In the estimated direct-adjusted model, 21-day mortality was 22.5% (95% CI 19.7–25.1) in the combination group, 18.9% (95% CI 14.3–23.2) in the hydroxychloroquine alone group, 10.9% (95% CI 5.8–15.6) in the azithromycin group and 17.8% (95% CI, 11.1–23.9) in the control group. When hydroxychloroquine and azithromycin monotherapy groups were compared, no differences were observed in mortality (aHR 1.92 [95% CI 0.99–3.74]), although it was in the limit of significance. This is the only studied that found a potential mortality benefit of the use of azithromycin alone in hospitalized patients.

Arshad et *al*. demonstrated that the use of hydroxychloroquine reduced the in-patient hospital mortality by 66% and by 71% when combined with azithromycin, whereas azithromycin alone did not provide any advantages [72]. However, the significant differences in the use of corticosteroids (patients treated with hydroxychloroquine received more corticosteroids) among different groups could have biased the obtained results [72].

In the biggest conducted randomized-controlled clinical trial in hospitalized patients with mild to moderate COVID-19, the use of hydroxychloroquine and azithromycin or hydroxychloroquine alone did not improve clinical status at 15 days compared to standard of care [73]. Unfortunately, although the safety of azithromycin alone was assessed, the clinical efficacy of the treatment with this macrolide was not described.

These results in hospitalized patients must be interpreted with caution given the many limitations of the included studies. Some of them presented low sample sizes so were underpowered. Only one of the studies was a placebo-controlled randomized clinical trial, so most of them were not designed to assess the efficacy of these regimens. Despite the efforts to control for confounding factors, in observational studies, even the best adjustment methods can miss major systematic biases [74]. Among confounding factors, the use of other therapies such as antivirals (remdesivir), immunomodulators (especially corticosteroids), and anticoagulation therapy were not either described or adequately controlled. This is of utmost importance given recent evidence showing clinical improvements with the use of remdesivir, corticoids, or anticoagulation therapy [6,75]. Another important issue is that azithromycin was given alone, when reported, in 29–37% of patients in the control groups. Given the potential benefits associated with this macrolide, this may have also been a potential confounding factor. The time from the onset of symptoms until the initiation of treatment is another important issue. Only two studies reported these data and treatments were initiated late (7 days) [68]. This could have underestimated the efficacy of the treatment since it was not initiated when it should be more active.

The available guidelines (WHO, IDSA) suggest that this macrolide should not be used in combination with hydroxychloroquine outside of the context of clinical trials, due to the lack of high-quality evidence in favor and concerns about their potential side effects [76,77]. Unfortunately, the question about its use in monotherapy or in combination with other drugs remains unanswered.

Given these limitations, the future results of clinical trials are essential to establish the role of azithromycin in COVID-19. According to the information available on ClinicalTrials.gov, to date 44 clinical trials are recruiting patients to evaluate azithromycin in a wide variety of scenarios (outpatients, inpatients, in ICU, combined with hydroxychloroquine or other drugs) [78]. Among them, the RECOVERY trial should be highlighted. After the results of dexamethasone, lopinavir/ritonavir and hydroxychloroquine, the arm of azithromycin is still being studied in hospitalized patients with COVID-19 pneumonia with a regimen of 500 mg intravenously or by mouth (or nasogastric tube) OD for 10 days [28]. Those patients with a known prolonged QTc interval, hypersensitivity to macrolides, or that are also receiving chloroquine or hydroxychloroquine will be ineligible for randomization [28].

4.6. Safety data

Azithromycin is considered to be safe, with a low risk for severe adverse events [79]. The most frequently reported adverse events were gastrointestinal (nausea and abdominal pain), central and peripheral nervous system (headache or dizziness), hepatotoxicity, and the development of antibacterial resistance [9]. Its use, as occurs with other macrolides, has been related to QTc interval prolongation, torsade de Pointes (TdP), ventricular tachycardia, and sudden cardiac death [79]. The proarrhythmic mechanism of azithromycin is thought to be due to intracellular sodium overload [80]. A study showing an increased risk of cardiovascular death prompted the FDA to introduce a black box warning [9]. However, in a Cochrane review, macrolide use was not associated with a higher risk of cardiac disorders when compared to placebo (OR 0.87 [95% CI 0.54–1.40]) [81]. In other systematic reviews and meta-analysis, macrolides did not increase the risk for short-term arrhythmia (OR 1.19 [95% CI 0.89–1.61]) nor 30-day mortality (OR 1.22 [95% CI 0.94–1.60]) [79]. A recent retrospective cohort study showed that the use of azithromycin was associated with a significantly increased risk of cardiovascular death when compared to amoxicillin (HR 1.82 [95% CI 1.23–2.67]) but not sudden cardiac death (HR 1.59 [95% CI 0.90–2.81]) [82]. Nevertheless, the global incidence of event rates was low, other confounding factors may have been present given the underlying differences among cohorts and causality cannot be established due to its retrospective nature.

SARS-CoV-2 pneumonia, similar to has been seen in other viral (influenza, respiratory syncytial or other viruses) and bacterial pneumonia, can also lead to myocardial injury [4,15,83]. In the setting of CAP, the use of azithromycin was related to a lower 90-day mortality risk in elderly patients, with a 0.6% increase in the risk of myocardial infarction, showing a net benefit of its use [84]. Another clinical trial did not find an

Table 3. Cardiovascular safety data on the use of azithromycin alone or in combination for the treatment of COVID-19.

Ref.	Design and location	Treatment regimen (mg)	Number of patients	ΔQTc (ms)	Clinical outcome (arrhythmia, TdP)	Treatment discontinuation
Million et *al* [63].	Single center, retrospective, observational study, France	500 day 1, 250 OD days 2–5	HQC + AZT: 1,061	ΔQTc> 60: 0.8% QTc> 500: 0%	None	3 (abdominal pain, urticaria, erythematous and bullous rash)
Guerin et *al* [65].	Retrospective, observational study, France	500 day 1, 250 OD days 2–5	HCQ + AZT: 20 AZT: 34 SOC: 34	None	None	None
Barbosa et *al* [66].	Open label, controlled non-randomized trial, Brazil	500 OD 5 days	HCQ+AZT: 412 SOC: 224	None None	None	None
Mahevas et *al* [68].	Multicenter, retrospective, propensity-score matched observational study in France	500 day 1, 250 OD day 2–5	HCQ: 84 *HCQ + AZT: 15*	ΔQTc > 60: 8.3 % None	1.2% atrioventricular block None	8 (10%) None
Rosenberg et *al* [71].	Multicenter, retrospective, observational cohort study, USA	OR/IV 500 OD. Duration NR	HCQ + AZT: 735 HCQ: 271 AZT: 211 SOC: 221	81 (11.0%) 39 (14.4%) 15 (7.1%) 13 (5.9%)	*Cardiac arrest:* *Arrhythmia* 15.5% 20.4% 13.7% 16.2% 6.2% 10.9% 6.8% 10.4%	NR
Arshad et al. [72]	Multicenter, retrospective, observational study, USA	500 day 1, 250 OD days 2–5	HCQ + AZT: 783 HCQ: 1,202 AZT: 147 SOC: 409	NR	4% cardiac arrest 8% cardiopulmonary arrest	NR
Cavalcanti et al. [73]	Multicenter, randomized, open-label, controlled trial, Brazil	500 mg OD 7 days		*ΔQTc > 80 ms within 7 days:*	*Any adverse event* *Arrhythmia*	NR
			HCQ + AZT: 217 AZT: 50 HCQ: 221 SOC: 227	17/116 (14.7%) 0/6 (0%) 13/89 (14.6%) 1/58 (0.7%)	94 (39.3%) 3 (1.3%) 9 (18.0%) 0 (0%) 67 (33.7%) 3 (1.5%) 40 (22.6%) 1 (0.6%)	
Saleh et al [80].	Multicenter, prospective, observational study, USA	OR/IV 500 OD 5 days			*Ventricular arrhythmia*	4.2% due to QTc prolongation
			HCQ + AZT: 119	Mean Δ: 27.5 ± 44.3 QTc> 500: 9.2%	5.0%	
			HCQ: 82	Mean Δ: 3.9 ± 32.9 QTc> 500: 8.6%	2.4%	2.4% due to QTc prolongation
Mercuro et *al* [89].	Single center, retrospective, observational cohort, USA	NR	HCQ + AZT: 53	Mean Δ: 23 (10–40) QTc> 500: 21% ΔQTc> 60: 13%	1 extreme QTc prolongation that developed TdP	1.1% due to QTc prolongation
			HCQ: 37	Mean Δ: 5.5 (−14-31) QTc> 500: 19% ΔQTc> 60: 3%	None	11.1% due to QTc prolongation
Chang et *al* [90].	Single center, prospective observational cohort study, USA	At least 1 dose IV 500	HCQ + AZT: 51 HCQ: 66	Mean Δ: 12.8 ± 29.3 Mean Δ: 3.9 ± 31.9	Atrial fibrillation: 12.8% Supraventricular tachycardia: 0.9%	None 1.5% due to QTc prolongation
Chorin et *al* [91].	Single center, retrospective, observational cohort, USA	500 OD. Duration NR	HQC + AZT: 84	QTc> 500: 11% ΔQTc> 60: 12%	None	NR
Lane et *al* [92].	Multinational, network cohort and self-controlled case study	NR	HCQ +AZT: 323,122 HCQ + AMX: 351,956	NR	*30-day cardiovascular mortality* CalHR: 2.19 (95% CI 1.22–3.94) *Chest pain/angina* CalHR: 1.15 (95% CI 1.05–1.26) *Heart failure* CalHR 1.22 (95% CI 1.02–1.45)	NR

(*Continued*)

Table 3. (Continued).

Ref.	Design and location	Treatment regimen (mg)	Number of patients	ΔQTc (ms)	Clinical outcome (arrhythmia, TdP)	Treatment discontinuation
Bessiere et *al* [87].	Single center, retrospective, observational cohort study	250 OD 5 days	HCQ + AZT: 18 HCQ: 22	QTc> 500: 33% QTc> 500: 5%	None	NR
Chorin et *al* [88].	Multicenter, observational study in Italy and USA	OR 500 OD 5 days	HCQ + AZT: 251	Mean Δ: 34 ± 30 QTc > 500: 20% Extreme QTc prolongation: 23%	NR	3.2% due to QTc prolongation

AMX: amoxicillin; AZT: azithromycin; BID: twice daily; CalHR: calibrated hazard ratio; CI: confidence interval; CLT: clarithromycin; HCQ: hydroxychloroquine; HR: hazard ratio; MCR: macrolides; NR: not reported; OD: once daily; TdP: Torsade de Pointes.
Hydroxychloroquine was administered orally. Azithromycin data on route administration was lacking except stating otherwise.
ΔQTc: the increment was reported either in milliseconds, number of patients (percentage) with increment in QTc, number of patients (percentage) with increment of QTc > 60 ms, number of patients (percentage) with QTc > 500 ms.

increased risk of cardiac event, heart failure, or arrhythmia with the use of azithromycin in this setting [85]. The underlying factors, patient demographics, and the indication for which antimicrobial was used may largely explain the increased risk in cardiovascular outcomes associated with antimicrobials, rather than directly because of the antimicrobials itself [86]. In influenza pneumonia, only two studies reported safety data showing that azithromycin were well tolerated [53,54]. The incidence and the severity of adverse events and the rate of treatment discontinuations were similar among studied groups.

In the context of COVID-19, the potential cardiotoxicity of azithromycin has been a concern. The main data assessing the cardiovascular risk of azithromycin in SARS-CoV-2 pneumonia have been summarized in Table 3.

In patients with mild disease, overall azithromycin and its combination with hydroxychloroquine were well tolerated, suggesting that toxicity may be associated with severity. Million et *al*. reported a 2.4% incidence of adverse events, mainly gastrointestinal with a very low rate of QTc interval prolongation [63]. None of the reasons for treatment discontinuation were cardiovascular. Guerin et *al*. reported no cardiovascular events [65]. In the study of Barbosa et *al*. the main adverse effect was diarrhea, but 12.9% of patients presented diarrhea before the onset of the treatment [66]. No cardiovascular adverse effects were recorded.

In hospitalized patients, the use of hydroxychloroquine and azithromycin has been related to a higher incidence of cardiac adverse events. This combination has been associated with a higher risk of QTc prolongation, ventricular arrhythmia, TdP (with an incidence of 0.4%), atrial fibrillation, atrioventricular block, or cardiac arrest [68,71,80,89,90]. These abnormal findings appear to be developed at day 3–4 of the treatment [80,90,91]. Rosenberg et *al*. showed that patients treated with hydroxychloroquine alone presented a higher risk of cardiac arrest (aOR 2.97 [95% CI 1.56–5.64]) than those treated with azithromycin [71]. This difference among the two treatments was maintained even in patients without mechanical ventilation (aOR 3.01 [95% CI 1.07–8.51]), excluding other factors for adverse events as severity. Moreover, azithromycin monotherapy did not increase the risk of cardiovascular adverse events compared to the standard of care group. Therefore, this study concluded that the main driver of cardiac toxicity of the combination could be the hydroxychloroquine [71]. In another study, the addition of azithromycin to hydroxychloroquine increased the risk of 30-day cardiovascular mortality [92]. However, another 12 outcomes were analyzed without finding significant differences in any of them and, when accounting by the standard Bonferroni correction of multiple comparison, only chest pain/angina remained statistically significant [67]. These studies were not without limitations, as patients in the treatment groups were sicker, which may have affected safety outcomes despite adjusting for confounding variables. The rate of treatment discontinuation was not systematically reported, and treatment regimens were different across the studies. In the only randomized controlled clinical trial published to date in hospitalized patients with COVID-19, the use of hydroxychloroquine and azithromycin or hydroxychloroquine alone was associated with a higher

risk of adverse events, QTc prolongation, or arrhythmia than azithromycin alone or standard of care [73].

Other factors may also play a role in the development of these adverse events. The use of loop diuretic drugs, baseline QTc ≥450 ms, more than 2 systemic inflammatory response syndrome criteria, and intensive care status at the time of test were associated with a higher risk of developing QTc ≥500 ms [89]. The use of other medications that prolong the QTc, electrolyte disturbances, female gender, older age, personal or family history of QT interval prolongation and other diagnoses as chronic renal failure, cardiac heart failure, structural heart disease, genetic polymorphisms, and congenital long QT syndrome are other potential risk factors [80,93].

Some algorithms have been proposed to try to minimize the associated risks [93]. A careful revision of the history of the patient to detect any diseases with an increased risk of QTc prolongation, together with the assessment of potential electrolyte disturbances and the presence of other QTc-prolonging medications and their interactions is advised before initiating the treatment [93]. An electrocardiogram and electrolyte monitoring are recommended during the first days of therapy to detect any potential alterations [93].

4.7. Contraindications, precautions, and monitoring

The drug is contraindicated in patients with hypersensitivity to azithromycin, erythromycin, any macrolide or ketolide drug and with a history of cholestatic jaundice or hepatic dysfunction associated with prior use of azithromycin [94]. According to the information available in the data sheet, caution is warranted in patients with hepatotoxicity, infantile hypertrophic pyloric stenosis, *Clostridoides difficile* associated-diarrhea, myasthenia gravis (this antibiotic may exacerbate muscle weakness) and patients with the previous prolongation of QTc interval or TdP [94].

5. Conclusion

Azithromycin presents antiviral and immunomodulatory properties that could be of interest in the treatment of COVID-19. The use of this macrolide has been associated with improvements in clinical outcomes in other viral infections and CAP. Azithromycin has shown *in vitro* activity against SARS-CoV-2 and may act in different points of viral cycle. Furthermore, given that immunomodulation has improved clinical outcomes in severe COVID-19, its ability to downregulate cytokine production, maintain epithelial integrity, and prevent lung fibrosis could play a role in the hyperinflammatory stage of COVID-19.

However, despite azithromycin being a promising therapy, there is a paucity of data of its use in COVID-19. This macrolide has mostly been administered with hydroxychloroquine, which has been shown not to provide benefits in the treatment of SARS-CoV-2 pneumonia.

Azithromycin presents a well-known safety profile. In the context of COVID-19, however, safety concerns have been raised due to its potential cardiotoxicity, especially when combined with hydroxychloroquine. Yet, recent evidence suggests that this toxicity may be attributable to hydroxychloroquine. Upcoming clinical trials will confirm the promising role of this drug in the treatment of COVID-19, including the effect of its combination with other drugs as corticosteroids or remdesivir/favipravir and the optimal stage where its use will be justified to a maximum.

6. Expert opinion

As with other therapies, the use of azithromycin in the treatment of COVID-19 is a matter of debate. This drug presents promising pharmacokinetic and pharmacological characteristics that could be useful in the treatment of SARS-CoV-2 infection.

From a pharmacokinetic point of view, azithromycin's ability to concentrate in the lung and its chemotactic drug delivery allows the achievement of therapeutic and sustained concentrations [38,40]. The optimal dose of azithromycin in the treatment of viral infections or COVID-19 remains unknown. According to the IDSA guidelines and RECOVERY trial, in severe patients, 500 mg OD should be employed. However, taking into account the pharmacokinetic properties together with the fact that immunomodulatory actions could be obtained with lower doses leads to the hypothesis that the advantages obtained with this macrolide could be achieved with lower and safer doses [40]. Future clinical trials will determine the optimal dose of this drug in this setting.

Azithromycin could act at different points in the viral cycle, including the binding or the activation of the fusion process by lysosomal proteases. This macrolide presents *in vitro* activity against SARS-CoV-2 with an EC_{50} of 2.12 μM. Beyond its *in vitro* activity, azithromycin has shown important immunomodulatory activity *in vitro* and in *vivo*, acting on different points of the inflammatory cascade reducing the production of cytokine production, among other actions.

These properties, together with the clinical efficacy seen in influenza pneumonia or CAP, allowed the consideration of this drug as a potential therapy for the treatment of SARS-CoV-2 pneumonia. However, the available data do not allow us to recommend its widespread use due to important methodological limitations, mainly due to their retrospective observational nature. Furthermore, it has mainly been studied with hydroxychloroquine, which makes the analysis of the effect of azithromycin difficult, especially considering the latest negative data on its efficacy [68,70,73]. In addition, this drug has not been adequately studied in a subgroup of patients (outpatients, critically ill) where it may offer the greatest clinical benefits.

In the early phase of COVID-19 azithromycin alone or combined with hydroxychloroquine could reduce the need for hospitalization or time to clinical recovery [65,66]. The potential implications of these outcomes are huge, considering the overload of patients to which hospitals have been subjected. In addition, due to its long half-life with sustained concentrations, whether the early administration in the disease could have positive effects in later stages, when the main problem relies on the host immune response, is a plausible hypothesis that should be further investigated.

In the second stage of COVID-19, the combination therapy does not seem to confer any benefit [68,71,73]. However, azithromycin may provide additional benefits without safety concerns if given without hydroxychloroquine. Two studies analyzed the effect of this macrolide administered in monotherapy. Geleris et *al*. did not find any clinical benefit [70]. However, they did not demonstrate any clinical benefit either with remdesivir, which reduced the time to clinical recovery in a randomized controlled trial [8]. This fact raises concerns about the conclusions of this study, given that both azithromycin and remdesivir were assessed as potential covariates without showing specific data of patients that received them [70]. In the study of Rosenberg et *al*. reporting data on the sickest patients to date, this macrolide was associated with a trend toward reduction in mortality, although it was a retrospective observational study [71].

The third and more severe stage of COVID-19 is characterized by the development of hyperinflammation and cytokine storm, so immunomodulatory therapies have been proposed [1,5,,7]. Of interest, unlike in other viral diseases, immunomodulation with corticosteroids or tocilizumab has proven to be a benefit in the treatment of COVID-19, so in this setting, azithromycin may play a role [5,]. This drug has provided important clinical benefits in other severe respiratory infections. In critically ill patients with CAP, its use has been associated with a mortality reduction, even in macrolide-resistant strains. In influenza pneumonia, azithromycin treatment was associated with a reduction in the length of mechanical ventilation or length of stay when given early in the disease [53,56]. A group of experts recommended its use in combination with antivirals for the treatment of H1N1 influenza severe disease to reduce the systemic inflammatory response [95].

Furthermore, the use of azithromycin in severe lung injury and ARDS when initiated early in the disease has been associated with a reduction in the time to successful ventilation discontinuation and mortality, which given the similarities in cytokine profiles could be applicable to COVID-19 [14,59]. In addition, its potential antifibrotic activity may be useful in ARDS or in patients who develop lung fibrosis. Recent evidence has demonstrated that COVID-19 can cause microvascular damage with endotheliitis, suggesting that therapies that stabilize the endothelial cells may be of interest [96–98]. Azithromycin may be useful since it has shown to stabilize and maintain the integrity of the epithelial cells [9].

Despite all these potential benefits in critically ill patients, these patients have been misrepresented. In all but one of the previous studies [71], patients admitted to the ICU at the time of treatment initiation were excluded. This is important since, at least in CAP, the beneficial immunomodulatory protective effect seems to be more evident in the most severe patients [55]. Unfortunately, the potential usefulness in COVID-19-induced lung injury, ARDS, or fibrosis remains unknown.

Considering the available evidence with other drugs, it is essential to determine, if any, the role of azithromycin in combination with other drugs. Concerning the antivirals, remdesivir and favipiravir have been associated with positive outcomes in COVID-19 [2,8]. Other drugs are known to reduce the cytokine production as hydroxychloroquine, minocycline, corticosteroids, or tocilizumab [2,4]. The use of hydroxychloroquine was not associated with any clinical benefit in a recent clinical trial [73]. Minocycline presents interesting advantages in terms of potential efficacy in patients with ARDS and myocardial injury, but clinical data are still scarce [4]. With the current available evidence, only corticosteroids have demonstrated a mortality benefit in randomized-controlled trials, and, according to guidelines, should be the standard of care in patients requiring mechanical ventilation or oxygen support [76]. Tocilizumab has shown clinical benefits (reduction in the risk of invasive mechanical ventilation or death), with a higher risk of superinfection [5,6]. However, this drug has only been studied in retrospective cohort studies. Unfortunately, data on the combination of azithromycin with these drugs are lacking. The potential benefit/harm of azithromycin in combination with these drugs is of utmost importance and should be addressed in clinical trials. This macrolide should not be used over any of the drugs currently employed in COVID-19 as corticosteroids or remdesivir/favipiravir until evidence of its role in the treatment of COVID-19 is established.

The stage of the disease where azithromycin provides the greatest advantages in COVID-19 remains unknown. As has been mentioned, this drug could be useful in the different stages of COVID-19. However, the knowledge of the type of patient where the use of azithromycin will be justified to the maximum is important since the risk of adverse events is different depending on the severity. In this regard, after the results on the use of dexamethasone, hydroxychloroquine, and lopinavir/ritonavir, the outcomes of the RECOVERY trial on the use of azithromycin in hospitalized patients are eagerly awaited. Other clinical trials should assess the potential benefits of this drug in other stages as in outpatients or critically ill patients.

From a safety perspective, some concerns have been raised due to its potential cardiotoxicity. However, recent meta-analyses have shown that its use did not increase the risk of cardiac adverse events.

In COVID-19 outpatients treated with azithromycin, no safety concerns were noticed. In the second stage of COVID-19, the combination of hydroxychloroquine and azithromycin was associated with an unacceptable higher risk of cardiac toxicity and arrhythmias. These adverse events, however, were observed in severe patients in treatment with hydroxychloroquine, so other confounding factors may have been present. In the only randomized-controlled clinical trial performed to date in hospitalized patients, azithromycin alone was not associated with a higher risk of adverse events, unlike hydroxychloroquine and its combination with azithromycin [73]. In fact, hydroxychloroquine is thought to be the main driver of cardiac toxicity and not azithromycin by itself, which is in line with previous evidence where this macrolide was not associated with an increased risk of cardiac adverse events. In the context of COVID-19, according to recent data showing the lack of benefit of hydroxychloroquine and due to safety concerns of the combination, it is unlikely that azithromycin will be given with hydroxychloroquine. We believe that these data allow azithromycin to continue to be studied in COVID-19 from a safety perspective. Nevertheless, a careful risk-benefit consideration is warranted, as well as a strict monitoring and follow-up of potential adverse events. Oral route should be

preferred due to a lower risk of cardiac toxicity when possible [98]. Until these data are available azithromycin should not be used outside of the context of clinical trials. The studies considered in this review have mainly analyzed outcomes as mortality or need for intubation. However, the analysis of other outcomes such as time to clinical recovery, number of secondary bacterial superinfections, length of stay, or mechanical ventilation were not analyzed and could offer another vision of the treatment. In addition, to assess the potential effectiveness of this macrolide, its impact on other analytical parameters as proinflammatory cytokine production should be considered. The upcoming clinical trials will elucidate the role of this macrolide in the treatment of COVID-19.

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