Could azithromycin play a role in the treatment of COVID-19? A review

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Abstract

Azithromycin has shown antiviral and immunomodulatory actions that may be of interest in coronavirus disease-19 (COVID-19). The objective of this review was to summarize the potential usefulness of azithromycin in the COVID-19. Azithromycin has shown in vitro activity against SARS-CoV-2. The potential mechanisms of action include the impairment of virus binding and of membrane fusion, endocytosis, and lysosomal protease activation due to its lysosomotropic character. Among other immunomodulatory actions, azithromycin downregulates the production of proinflammatory cytokines, maintains epithelial cell integrity and may prevent lung fibrosis. These properties, which have been related to positive clinical outcomes in other settings as influenza pneumonia, may be beneficial throughout the course of COVID-19. However, scientific evidence is still scarce. Azithromycin has mostly been studied with hydroxychloroquine/chloroquine. In outpatients, this combination showed a reduction in time to clinical recovery or need for hospitalization without safety concerns. In hospitalized patients presented an increased risk of mortality and cardiovascular events. In the few studies that assessed the efficacy of azithromycin monotherapy, a reduction in the time to clinical recovery in outpatients and a trend towards a reduction in mortality in inpatients was observed. Data on critically ill patients are lacking. The quality of data was low, as most of the studies were observational and retrospective. Azithromycin may play a role in the treatment of COVID-19. Despite the paucity of data and associated limitations, azithromycin has shown promising results that deserve further study. The upcoming clinical trials will elucidate the role of this macrolide in COVID-19.

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1 - Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the coronavirus disease-19 (COVID-19)[1]. According to the WHO, this virus has been declared pandemic, and to date (7^{th}June), a total of 6,799,713 diagnosed cases and 397,388 deaths have been confirmed[2].

The treatment of choice for this new disease remains unknown, so it is urgent to find effective and safe treatments. Lopinavir/ritonavir, hydroxychloroquine/chloroquine with or without azithromycin, remdesivir, tocilizumab/sarilumab, nitazoxanide, ivermectin... have been employed. Excepting from remdesivir (that in a recent clinical trial was associated with improved clinical outcomes), high quality clinical data to support the evidence (or lack of) of the rest of the treatments are still lacking[3–6].

Azithromycin has been proposed as a potential therapy for SARS-CoV-2 given its antiviral, immunomodulatory and antibacterial activity[7,8]. However, its role in the treatment of COVID-19 remains unclear.

The objective of this review was to summarize the potential usefulness of azithromycin in the treatment of COVID-19.

2-SARS-CoV-2 and COVID-19.

SARS-CoV-2 enters the cell mainly via the human angiotensin converting enzyme 2 receptor (hACE2) through glycosylation[9]. In this process, SARS-CoV-2 is dependent upon plasmatic membrane components as gangliosides (specially GM-1), that act as attachment cofactors within lipid raft membrane platforms[9,10]. Dual recognition of both gangliosides and hACE2 by the spike protein is therefore needed[9,10]. For this purpose, viral protein displays two distinct domains: the receptor binding domain that binds to hACE2 receptor and the N-terminal domain, which binds to the ganglioside-rich domain in the membrane lipid raft[9,10].

Once this process has been performed, it subsequently penetrates through endocytosis[11]. Thereafter, lysosomal proteases such as cathepsins, transmembrane protease TMPRSS2 and furins must activate the fusion process by cleaving coronavirus surface spike proteins[12–14]. Without endocytosis and lysosome action the replication and infection of this virus are blocked[12].

In order to facilitate the therapeutic approach of COVID-19, a 3-stage classification system has been proposed[15]. The first stage is usually mild with non-specific symptoms and in this phase antiviral therapy may prevent progression of severity, minimize contagiousness, and reduce duration of symptoms. In the second stage patients may develop viral pneumonia 5-7 days after symptoms onset, needing in most cases hospitalization. The treatment consists of supportive measures and antiviral therapy. Although most patients are able to clear the infection in the lung, some will transition into the third and most severe stage, where the use of immunomodulatory agents may reduce systemic inflammation. In this phase, patients develop a dysfunctional immune response leading to a cytokine storm[16]. The development of such syndrome, characterized by an uncontrolled increase in the proinflammatory cytokines, has been associated with disease severity and prognosis[3,16]. This leads to multi-organ damage, including respiratory failure as a consequence of the development of lung fibrosis and acute respiratory distress syndrome (ARDS), which is the leading cause of mortality of this virus[16–18]. In the specific scenario of ARDS, cytokines may cause epithelial and capillary endothelial damage[19]. Recently, all these processes have shown to also induce endotheliitis, which may explain the systemic impaired microcirculatory function[20].

Azithromycin presents antiviral and immunomodulatory properties that could be of interest in all these stages, although specific data are lacking.

3-Azithromycin.

3.1- Pharmacology

Azithromycin is an antibiotic that belongs to the macrolide family used in a wide variety of bacterial diseases (respiratory tract infections, sexually transmitted diseases, etc.)[7]. Its antibacterial mechanism of action consists of the inhibition of the protein synthesis by interfering with the assembly of the 50S ribosomal subunit[7].

Azithromycin can be given either 500 mg once daily (OD) for 3-5 days or 500 mg on day 1 followed by 250 mg OD on days 2-5[21].

Although a 37 % of oral bioavailability has been described, the extensive tissue accumulation offsets its sub-optimal absorption[7]. Its plasma protein binding is 30 %, with a large volume of distribution of 23-30 L/kg, mainly due to the confinement in intracellular compartments[7,22]. Azithromycin accumulates in epithelial cells, fibroblasts, lymphocytes and alveolar macrophages where, compared to serum, 400 to 1,000-fold higher concentrations can be achieved[7]. This accumulation is due to its dibasic nature (pK_{a1}8.1;pK_{a2} 8.8), which in acidic environments as intracellular lysosomes causes the protonation and trapping into the cells[23]. The ability to bind to negatively charged phospholipids in its protonated form further increases this accumulation[23]. The chemotactic drug delivery increases local drug concentrations, as blood phagocytes and other cells that migrate into infected and inflamed tissues release accumulated azithromycin[7,23]. As a consequence, azithromycin presents a long half-life of 68-79h.[23]

All these properties explain its excellent lung tissue penetration and sustained drug concentrations[7,21,23]. Following 500 mg OD for three days, a C_{max} of 0.72-0.83 μg/mL in bronchial washing and 8.93-9.13 μg/mL in lung tissue, compared to 0.18 μg/mL in plasma, was found[21,24]. 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[22,25]. Finally, azithromycin is mainly excreted unchanged in feces[7].

3.2- Safety data

Azithromycin is considered to be safe, with a low risk for severe adverse effects[26]. The most frequently reported azithromycin's adverse events were gastrointestinal (diarrhea, nausea, and abdominal pain), central and peripheral nervous system (headache or dizziness), hepatotoxicity and the development of antibacterial resistance[7]. Its use, as occurs with other macrolides, has been related to QTc interval prolongation, torsade de Pointes (TdP), ventricular tachycardia and sudden cardiac death[26]. A study showing an increased risk of cardiovascular death prompted the FDA to introduce a black box warning[7]. 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])[27]. In other systematic review and meta-analysis, macrolide use was not associated neither with an increased 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])[26]. The proarrhythmic mechanism of azithromycin is thought to be due to intracellular sodium overload[28].

3.3 – Antiviral in vitro and animal data

Azithromycin has shown *in vitro* activity against a wide variety of viruses (zika, Ebola, rhinovirus, enterovirus, influenza)[29–31]. The 50 % effective concentration (EC_{50}) ranged between 1.23-6.59 μM depending on the virus, the cell analyzed and the multiplicity of infection (MOI)[24,30–32]. In infections caused by zika and rhinovirus azithromycin upregulated virus-induced Type I and III interferon responses reducing viral replication, suggesting that rather than its antiviral activity its immunomodulatory actions may be involved[31–35]. In mice with influenza A(H1N1) pre-treatment with azithromycin was associated with the blocking of internalization into host cells, leading to a reduction in viral load[36].

3.4-Immunomodulatory in vitro and animal data

Azithromycin exerts its immunomodulatory effects on different points in the inflammatory cascade, modulating cell functions and cell signaling processes[7,37,38].

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 [37,39,40]. They can also decrease mucus hypersecretion in vitro and in vivo, even when not produced by bacteria, which may improve mucociliary clearance [37,41,42]. Azithromycin use directly relaxed pre-contracted airway smooth muscle cells [7].

This macrolide can decrease the hypersecretion of pro-inflammatory cytokines and chemokines by acting in many inflammatory cells as monocytes, macrophages and fibroblasts [7]. Its use has been related with a reduction of IL-1β, IL-4, IL-5, IL-6, IL-8, IL-12, IFN-γ, IP-10, TNF-α, and GM-CSF [7,37,38,43]. In alveolar macrophages, azithromycin attenuated Th-1 cell responses, shifting polarization of alveolar macrophages to their alternative activated anti-inflammatory M2 phenotype [7]. It also increased phagocytosis of apoptotic bronchial epithelial cells by macrophages [44]. In fibroblasts, macrolides have demonstrated to inhibit fibroblast proliferation, collagen production and to decrease transforming growth factor (TGF-β) levels [45]. In lymphocytes, azithromycin has shown to suppress CD4+ T-cell activation [46] All these findings have been demonstrated in vitro. On the contrary, azithromycin can increase the release of IL-10, an anti-inflammatory cytokine related to the reparation of the inflamed tissues [7,37,43,45].

In animal models, the treatment with azithromycin reduced mortality in pneumococcal pneumonia, viral bronchiolitis and polymicrobial sepsis in mice[47–49]. 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, 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[47,49].

In the late fibroproliferative-fibrotic phase of ARDS, azithromycin may suppress lung fibrosis[19]. In a murine model of acute lung injury caused by bleomycin, it 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].

3.5- Antiviral and immunomodulatory clinical efficacy

Macrolides have shown their clinical efficacy in a wide variety of respiratory viral infections[52]. Specifically, azithromycin has been studied in influenza and Middle East Respiratory Syndrome coronavirus (MERS-CoV) infections[53–57]. The clinical studies of azithromycin in viral infections were 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 [57]. A trend towards a faster symptom resolution was noticed, with no differences in viral load change or culture negativity among groups [57]. The mean time from symptom onset to randomization was 2 days. Kakeya et*al.* showed that the treatment with azithromycin and oseltamivir, if initiated within 48h of the onset of symptoms, in patients with mild influenza A pneumonia was associated with a significant faster resolution of fever and sore throat, without differences in the expression levels of cytokines and chemokines [56]. The low baseline values of these substances, however, may have affected the outcomes [56]. These studies are not without 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 measures 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 [53]. 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[37,38]. In addition, the dose and duration of macrolide therapy was not described, and the treatment was initiated late in the disease (5 days).

Recently, in hospitalized patients presenting after 48h of symptoms onset with influenza A pneumonia, the addition of azithromycin (initiated 6-8h after diagnosis) significantly improved meaningful clinical outcomes as length of stay or the need for respiratory support during hospitalization[55]. In patients with age [?]50 years, furthermore, a significant reduction in vasopressor use was noticed[55]. 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.

A group of experts recommended the use of macrolides in combination with antivirals for the treatment of H1N1 influenza severe disease to reduce the systemic inflammatory response[58].

In MERS-CoV infection, macrolides were not associated with a significant benefit[54]. Again, it was a secondary analysis of an observational study, macrolides were grouped, and the dose and duration of the treatment were not reported. In addition, the sum of the patients treated with macrolides (147) was higher than stated (136) and no data concerning the time from symptom onset to treatment initiation was shown.

Only two studies reported safety data showing that azithromycin was well tolerated[56,57]. The incidence and the severity of adverse events and the rate of treatment discontinuations was similar among studied groups.

Beyond its antibacterial and potential antiviral activity, the immunomodulatory action of azithromycin may provide further clinical benefits[7]. In chronic diseases as asthma, chronic obstructive pulmonary disease,

bronchiectasis, diffuse panbronchiolitis or*Pseudomonas aeruginosa* colonization, azithromycin use was associated with positive clinical outcomes and reduced risk of exacerbations[7,59,60].

In the treatment of community-acquired pneumonia (CAP) its use is recommended in combination with beta-lactams, including in those admitted to the intensive care unit (ICU)[61]. For patients with bacteremic pneumococcal pneumonia, not adding a macrolide to a beta-lactam-based initial antibiotic regimen was an independent predictor of in-hospital mortality[62]. In a systematic review and meta-analysis that included 10,000 critically ill patients with CAP, macrolide use was associated with a significant reduction in mortality[42]. The immunomodulatory properties of macrolides may account for this difference, given that these benefits were even demonstrated in infections produced by macrolide-resistant strains[42,48].

In a secondary analysis of a multicenter, randomized controlled clinical trial, 235 patients were included with acute lung injury[63]. 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]). Acute lung injury was mainly due to pneumonia and macrolides were started within 60 h of diagnosis with a median duration of 4 days (dose unknown). 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[19]. 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]). The main causes of ARDS were pneumonia and sepsis. Azithromycin was initiated within 24h of diagnosis and used for 5 days (dose unknown).

4-Azithromycin and SARS-CoV-2

4.1 – In vitro data

In Vero E6 cells with a 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[64]. On the contrary, in another study performed in Vero E6 cells with a MOI of 0.25 azithromycin alone did not show any antiviral activity[65]. However, the combination of hydroxychloroquine at 5 μM with azithromycin at 5 and 10 μM was found to be synergistic and significantly inhibited viral replication[65]. The different MOI among the two studies may have accounted for these differences. Anyway, caution is advised when interpreting these results given the different MOI, cell lines, incubation times and analytical methods[24].

Azithromycin used at conventional doses could achieve therapeutic concentrations in the lung to be effective against SARS-CoV-2[24,65]. Based on previous 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[24,65]. In the study of Andreani et *al.* authors concluded that the observed synergy was achieved at concentrations achieved *in vivo* in the lungs[65].

Other macrolides have also demonstrated *in vitro* antiviral activity against SARS-CoV-2, as bafilomycin A decreased the entry of pseudovirions by 99 % compared to the control group[11].

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

Azithromycin could act in 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 [12,45]. 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 [45]. Authors postulated that this increase in pH in TGN may alter glycosylation of hACE2 and other proteins [45]. Using molecular dynamic simulations, another direct antiviral mechanism of this macrolide was theorized [9]. 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 that are involved in SARS-CoV-2 pathogenesis [9]. Ulrich et *al* . concluded that azithromycin may have antiviral activity against SARS-CoV-2 by interfering in the spike protein/CD147 interaction or CD147 expression[66].

Endocytosis and fusion process activation by lysosomes are essential for SARS-CoV-2 entry and infection [12]. The increase in the lysosomal pH by azithromycin may alter the endocytosis process [7,67,68]. Furthermore, the impairment of lysosomal proteases such as cathepsins and furin may also play a role [7,45,67,69]. Poschet et al found that 100 μM of azithromycin could normalize the excessive processing and activation of furin [45]. 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 furin by azithromycin could prevent the entry of SARS-CoV-2 into human epithelial cells [13,70].

4.2 – Clinical data

All available evidence on the use of azithromycin in the treatment of COVID-19 was summarized in Table 2.

In March, Gautret et *al.* showed that the early treatment either with hydroxychloroquine presented superior virological clearance compared to standard of care[8]. Moreover, the addition of azithromycin further improved the activity of hydroxychloroquine alone. However, only 6 patients were treated with hydroxychloroquine and azithromycin.

Based on this study and *in vitro* data showing synergic activity, some hospitals started to spread the use of this combination. Nevertheless, the International Society of Antimicrobial Chemotherapy raised concerns as they believed that did not meet the society's expected standard[71].

These authors subsequently expanded the number of included patients evaluating this combination[72,73]. They included those admitted to the infectious disease ward or treated in day-care hospital, so 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[74]. In all these studies, unfortunately, a control group was lacking.

Mahevas et *al.* assessed the efficacy of hydroxychloroquine in 173 hospitalized patients showing no effect in any outcomes[75]. 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 [76]. 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[77]. 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 was not shown). In the multivariate analysis hydroxychloroquine or azithromycin use was not associated with the composite primary endpoint.

Rosenberg et *al.* showed a trend towards reduced mortality in the azithromycin alone group, after adjusting for multiple factors[78]. 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.

Another pre-print study showed potential benefits of azithromycin alone, but unfortunately was withdrawn.

Recently, Guérin et *al.* assessed the time to clinical recovery of azithromycin and its combination with hydroxychloroquine compared to standard of care in outpatients[79]. Both treatments accelerated recovery both in the global cohort and after adjusting in a case-control analysis. No significant differences were found when azithromycin monotherapy and combination therapy were compared (P=0.26).

Barbosa et *al* . evaluated the combination therapy in the need for hospitalization in outpatients[80]. 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 (1.17 % vs. 3.2 %, P<0.001).

To date, 36 clinical trials are recruiting patients to evaluate azithromycin in a wide variety of scenarios (outpatients, combined with hydroxychloroquine or other drugs, ICU...).

5.2- Safety data

In the context of COVID-19, the potential cardiotoxicity of azithromycin has been a concern. Hydroxychloroquine is known to prolong the QTc interval, and the combination of these drugs has been associated with an increased risk of adverse events[78].

Several reports have shown the higher risk of QTc prolongation with the use of hydroxychloroquine alone or in combination with macrolides[28,76,81–85]. Furthermore, these treatments have been related to a higher risk of developing cardiac arrest or ventricular arrythmia. An incidence of 0.4 % in the development of TdP was described with the use of combination therapy[85]. This abnormal findings appear to be developed at day 3-4 of the treatment[28,75,82]. The main data concerning the cardiovascular risk of the treatment with azithromycin (alone or in combination) were detailed in Table 3.

In patients with mild disease, overall azithromycin and its combination with hydroxychloroquine were well tolerated. Million et *al* . reported a 2.4 % incidence of adverse events, mainly gastrointestinal with a very low rate of QTc interval prolongation[73]. None of the reasons for treatment discontinuation in 3 (0.28 %) patients were cardiovascular. Guerin et *al* . reported no cardiovascular events[79]. 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.[80] No cardiovascular adverse effects were recorded.

Rosenberg et *al.* reported a higher risk of cardiac arrest and arrhythmia with the use of hydroxychloroquine alone or in combination[78]. 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. 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. Azithromycin alone did not increase the risk of cardiovascular adverse events compared to standard of care group. This was also shown in another study where the addition of azithromycin to hydroxychloroquine increased the risk of 30-day cardiovascular mortality[86]. In this study, however, in the other analyzed outcomes no differences were found and, in addition, when accounting by the standard Bonferroni correction of multiple comparison, only chest pain/angina remained statistically significant[87].

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 time of test were associated with a higher risk of developing QTc [?] 500 ms[81]. 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[28,88].

Some algorithms have been proposed to try to minimize the associated risks[88]. 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[88]. An electrocardiogram and electrolyte monitoring are recommended during the first days of therapy to detect any potential alterations[88].

Discussion

Azithromycin presents numerous characteristics that could confer a clinical benefit in the treatment of COVID-19. Its potential antiviral, immunomodulatory and antibacterial properties could be of interest in all the three proposed stages of COVID-19. However, despite all these promising benefits, the use of azithromycin in the treatment of SARS-CoV-2 pneumonia remains unclear.

Unfortunately, studies carried out on the potential value of azithromycin have been mostly accompanied by its prescription in association with hydroxychloroquine/chloroquine. This constitutes a great limitation and hampers the assessment of the potential benefits of the macrolide, especially given the recent negative benefit-risk balance of hydroxychloroquine and chloroquine.

In other viral infections as influenza, although the available evidence is of low quality, azithromycin has proven to be useful when given early in the disease. In the early stage of COVID-19, where the use of antivirals might be indicated, azithromycin could reduce the number of complications, including the need for hospitalization. However, most studies have focused on advanced forms of the disease, so outpatients have been misrepresented.

Gautret et *al* . assessed the early treatment with hydroxychloroquine and azithromycin in patients with mild disease (mostly asymptomatic or with upper respiratory tract infections)[8]. This was the first study assessing the efficacy of this combination. Although a higher virological clearance was observed, this assumption should be taken with caution given the limitations. This was a non-randomized open-label clinical trial that only included 36 patients. Only 6 patients were treated with combination therapy, without adequate controls. 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. Patients in the treatment group had higher viral loads, so a likely benefit was easier to demonstrate[5]. Finally, baseline clinical data were lacking, and no clinical outcomes or safety data were reported. Thereafter, Gautret et *al* . and Million et *al* . showed positive results without significant safety issues when given this combination in a higher number of mild patients at day 5-6 of the symptom onset[72,73]. Again, the lack of control group, however, prevents the attribution of any benefits to this therapy.

A recent review concluded that this combination should be used in symptomatic high-risk outpatients, mainly based on the study performed by Barbosa et *al,* where although treated patients were sicker presented a reduction in the need of hospitalization[87]. However, this is a pre-print study open-label study and was performed by a telemedicine healthcare team, so it may not be applicable to other settings. The study of Guerin et *al* . seems to confirm the potential benefit of this strategy[79]. Interestingly, this study showed that azithromycin alone presented similar outcomes when compared to combination therapy[79]. Again, it was a pre-print study with a 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 and 1 in the azithromycin alone group in day 40. The lack of data concerning the viral load is another limitation that prevents the evaluation of the potential antiviral activity of azithromycin. Azithromycin was well tolerated in these studies with no associated cardiovascular events, suggesting that

these toxicities may be more evident in sicker patients[72,73,79,80,87].

The use of azithromycin in the first stage of COVID-19 has been therefore poorly studied, with a low number of patients included and studies with many flaws. However, the available evidence suggests a potential benefit of its use alone or in combination that requires further study.

Based on the first study of Gautret et *al* ., combination therapy began to be used in the second stage of COVID-19[8]. After adjusting for confounding factors, this combination was associated with an increased risk of mortality and adverse events[78]. Other studies also reported a lack of benefit of the treatment with hydroxychloroquine[75,77]. Again, these findings must be interpreted with caution given the many limitations of the included studies. Some of them presented low sample sizes so were underpowered. None of the studies was a placebo controlled randomized clinical trial (all were observational studies), so they 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[89]. Among confounding factors, the use of other therapies such as antivirals, immunomodulators (specially glucocorticoids) and anticoagulation therapy were not either described or adequately controlled. This is of upmost importance given recent evidence showing clinical benefits with the use of remdesivir, corticoids or anticoagulation therapy[4,90,91]. 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.

All these studies have evaluated robust and objective clinical outcomes as in-hospital mortality or need for intubation. However, other outcomes such as time to clinical recovery, time to symptom resolution or length of stay were not analyzed and could offer another vision of the treatment.

The time from the onset of symptoms until the initiation of treatment is another important issue. Only one study reported these data and treatments were initiated late (8 days)[75]. This could have underestimated combination treatment efficacy as was not initiated when it should be more active. One might think that by this time azithromycin should show clinical benefits since there might already be some hyperinflammation. However, patients included in these studies presented mild disease with a low incidence of comorbidities, which prevents demonstrating the potential benefit of azithromycin in this setting.

Three studies analyzed the effect of this macrolide alone. Geleris et *al* . did not find any clinical benefit with the use of this macrolide[77]. However, they did not demonstrate any clinical benefit either with remdesivir, which has recently shown significant clinical benefits in a randomized controlled trial[4]. 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[77]. In the study of Rosenberg et *al* . reporting data on the sickest patients to date, this macrolide was associated with a trend towards reduction in mortality[78]. Another pre-print study showing potential benefits with azithromycin alone was withdrawn. As commented when evaluating the outcomes with combination therapy, caution is advised given the multiple limitations. As observational studies, other unmeasured confounding factors may have been present[78].

In the second phase of COVID-19, the combination therapy, after adjusting for confounding factors, has been associated with an unacceptable risk of cardiovascular toxicity and arrhythmias. However, 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.

Azithromycin, without concomitant hydroxychloroquine/chloroquine treatment, does not seem to confer the same risk of adverse events[27]. This may suggest that the main driver of toxicity in this setting is the use of other drugs and not azithromycin by itself. If this macrolide is considered, when possible oral route should be preferred due to lower peak levels that have been associated to a lower risk of cardiac toxicity[92].

Despite all the limitations, the treatment with hydroxychloroquine and azithromycin does not seem to offer

any benefit in the second stage of COVID-19 and, on the contrary, has been associated with an increased risk of adverse effects and mortality. Therefore, until more data are available, its use should not be recommended outside from clinical trials. Azithromycin alone in addition to standard of care may provide additional benefits without safety concerns that need to be validated in clinical trials.

The third and more severe stage of COVID-19 is characterized by the development of hyperinflammation and cytokine storm. In this setting, other immunomodulatory therapies as corticosteroids or anti-IL6 have been proposed[18,90]. Azithromycin's immunomodulatory effects may therefore play a role, given its ability to reduce cytokine expression among other properties. In other diseases as CAP, the immunomodulatory activity of azithromycin observed *in vitro* and in animal models has been demonstrated in high-quality clinical studies without safety issues. Furthermore, the potential benefits of azithromycin in severe lung injury and ARDS when initiated early in the disease have been demonstrated[19,63]. These benefits may be translated into patients with COVID-19, as a recent pre-print 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[93]. 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[20]. Azithromycin may be useful since it has shown to stabilize and maintain the epithelial cells integrity[7].

In spite of all these potential benefits in critically ill patients, these patients have also been misrepresented. In all but one of the previous studies, 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[53]. Unfortunately, the its potential usefulness in COVID-19 induced lung injury, ARDS or fibrosis remains unknown.

Concerning its bacterial activity, a recent meta-analysis showed that 7 % (14 % if admitted to ICU) presented bacterial co-infections, which was lower than with other viruses like influenza[94].*Mycoplasma pneumoniae* was found in 42 % of confirmed co-infections, although they were diagnosed serologically through the detection of IgM, which may have overestimated the rate of infections[94]. Unlike in influenza, where this macrolide reduced the rate of bacterial superinfections, the potential antibacterial benefit of azithromycin in the setting of COVID-19 has not been studied[55].

Azithromycin has demonstrated clinical benefits in other settings due to its antiviral and immunomodulatory action. However, in the treatment of COVID-19 it has been poorly studied, mainly in combination with hydroxychloroquine. Moreover, it has been studied in a very specific subgroup of patients, with other subgroups where it may offer the greatest clinical benefits being misrepresented. Although the paucity of data and associated limitations, azithromycin has shown promising results that deserve further study and may play a role in the treatment of COVID-19. The upcoming clinical trials will show whether this macrolide, alone or in combination, may be useful and which patients benefit most from it in the treatment of COVID-19.

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Ref.	Design and location	Severity, % or mean value	Virus	Treatment regimen (mg)	Number of patients	Main results
Lee et *al.*[57]	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.* [56]	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.*[53]	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)

Ref.	Design and location	Severity, % or mean value	Virus	Treatment regimen (mg)	Number of patients	Main results
Ishaqui et *al.* [55]	Multicenter, retrospective observational cohort study, Saudi Arabia	Lymphocytes: 240 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, 0.1-0.81) 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.*[54]	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)

Table 1: Clinical efficacy of azithromycin in viral infections.

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

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.*[8]	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		*PCR negative day 6 (P=0.001)*
						HCQ: 20	57.1 %
						HCQ + AZT: 6	100 %
						SOC: 16	12.5 %
Gautret et *al.*[72]	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.*[73]	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.*[74]	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
Mahevas et *al.*[75]	Multicenter, retrospective, propensity-score matched observational study, France	HBP: 51 Diabetes: 9 Obesity: 26	>50 % extend on CT: 33	7	500 day 1, 250 OD days 2-5		*21-day mortality % and HR:*
						HCQ: 84 *HCQ + AZT: 15*	HCQ: 11 %, 1.2 (95 % CI 0.4-3.3)
						Control: 89 *AZT: 26 (29.2 %)*	Control: 9 %. Reference

Ref.	Design and location	Comorbidities, %	Severity, %	Days from symptoms onset	Treatment regimen (mg)	Number of patients	Main results
Magagnoli et *al.*[76]	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		*In-hospital mortality % and aHR:*
						HCQ: 198	19.2, 1.83 (95 % CI 1.16-2.89)
						HCQ + AZT: 214	22.9, 1.31 (95 % CI 0.80-2.15)
						No HCQ: 395 *AZT: 91 (23.0 %)*	9.4 %. Reference
Geleris et *al.*[77]	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 (59.9 %)*	*Time to intubation or death HR:* HCQ: 1.04 (95 % CI 0.82-1.32) AZT: 1.03 (95 % CI 0.81-1.31)
						No HCQ: 274 *AZT: 102 (37.2 %)*	
Rosenberg et *al.*[78]	Multicenter, retrospective, observational study, USA	HBP: 57 Diabetes: 7 Obesity: 43	ICU: 12.8 Mechanical ventilation: 9.5	NR	500 OD. Unknown duration		*In-hospital mortality aHR:*
						HCQ + AZT: 735	1.35 (95% CI 0.76-2.40)
						HCQ: 271	1.08 (95 % CI 0.63-1.85)
						AZT: 211	0.56 (95 % CI 0.26-1.21)
						SOC: 221	Reference

Ref.	Design and location	Comorbidities, %	Severity, %	Days from symptoms onset	Treatment regimen (mg)	Number of patients	Main results
Guerin et *al.*[79]	Retrospective, observational study, France	HBP: 12.8 Diabetes: 3.4 Obesity: 13.6	Outpatients	1 (41 %) Within 15 (57.9)	500 day 1, 250 OD days 2-5		*Time to clinical recovery, median (range):*
						HCQ + AZT: 20	7 (2-40)
						AZT: 34	7 (3-48)
						SOC: 34	27 (6-48)
Barbosa et al.[80]	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		*Need for hospitalization*
						HCQ+AZT: 412	1.9 %
						SOC: 224	5.4 %

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

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)	Clinical outcome (arrhythmia, TdP)	Treatment discontinuation
Saleh et al.[28]	Multicenter, prospective, observational study, USA	OR/IV 500 OD 5 days			*Ventricular arrhythmia*	*Ventricular arrhythmia*	4.2 % due to QTc prolongation
			HCQ + AZT: 119	Mean Δ: 27.5 ± 44.3 QTc> 500: 9.2 %	5.0 %	5.0 %	
			HCQ: 82	Mean Δ: 3.9 ± 32.9 QTc> 500: 8.6 %	2.4 %	2.4 %	2.4 % due to QTc prolongation

Ref.	Design and location	Treatment regimen (mg)	Number of patients	ΔQTc (ms)	Clinical outcome (arrhythmia, TdP)	Clinical outcome (arrhythmia, TdP)	Treatment discontinuation
Million et *al.*[73]	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	None	3 (abdominal pain, urticaria, erythematous and bullous rash)
Mahevas et *al.*[75]	Multicenter, retrospective, propensity-score matched observational study in France	500 day 1, 250 OD day 2-5	HCQ: 84	ΔQTc > 60: 8.3 %	1.2 % atrioventricular block	1.2 % atrioventricular block	8 (10 %)
			HCQ + AZT: 15	None	None	None	None
Rosenberg et *al.*[78]	Multicenter, retrospective, observational cohort study, USA	OR/IV 500 OD. Duration NR			*Cardiac arrest:*	*Arrhythmia*	NR
			HCQ + AZT: 735	81 (11.0 %)	15.5 %	20.4 %	
			HCQ: 271	39 (14.4 %)	13.7 %	16.2 %	
			AZT: 211	15 (7.1 %)	6.2 %	10.9 %	
			SOC: 221	13 (5.9 %)	6.8 %	10.4 %	
Guerin et *al.*[79]	Retrospective, observational study, France	500 day 1, 250 OD days 2-5	HCQ + AZT: 20	None	None	None	None
			AZT: 34				
			SOC: 34				

Ref.	Design and location	Treatment regimen (mg)	Number of patients	ΔQTc (ms)	Clinical outcome (arrhythmia, TdP)	Clinical outcome (arrhythmia, TdP)	Treatment discontinuation
Barbosa et *al.*[80]	Open label, controlled non-randomized trial, Brasil	500 OD 5 days	HCQ+AZT: 412	None	None	None	None
			SOC: 224	None			
Mercuro et *al.*[81]	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 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	None	11.1 % due to QTc prolongation
Chorin et *al.*[82]	Single center, retrospective, observational cohort, USA	500 OD. Duration NR	HQC + AZT: 84	QTc> 500: 11 % ΔQTc> 60: 12 %	None	None	NR
Bessiere et *al.*[83]	Single center, retrospective, observational cohort study	250 OD 5 days	HCQ + AZT: 18	QTc> 500: 33 %	None	None	NR
			HCQ: 22	QTc> 500: 5 %			
Chang et *al.*[84]	Single center, prospective observational cohort study, USA	At least 1 dose IV 500	HCQ + AZT: 51	Mean Δ: 12.8 ± 29.3	Atrial fibrillation: 12.8 % Supraventricular tachycardia: 0.9 %	Atrial fibrillation: 12.8 % Supraventricular tachycardia: 0.9 %	None
			HCQ: 66	Mean Δ: 3.9 ± 31.9			1.5 % due to QTc prolongation

Ref.	Design and location	Treatment regimen (mg)	Number of patients	ΔQTc (ms)	Clinical outcome (arrhythmia, TdP)	Clinical outcome (arrhythmia, TdP)	Treatment discontinuation
Chorin et *al.*[85]	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	NR	3.2 % due to QTc prolongation
Lane et *al.*[86]	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)	*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

TdP: Torsade de Pointes; HCQ: hydroxychloroquine; AZT: azithromycin; BID: twice daily; OD: once daily; NR: not reported; MCR: macrolides; CLT: clarithromycin; HR: hazard ratio; CI: confidence interval; AMX: amoxicillin; CalHR: calibrated hazard ratio

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.

Figure legends:

1. *SARS-CoV-2 binding:* the increase in the pH of Trans-Golgi network may alter hACE2 glycosilation. 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. *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).
4. *Lymphocytes:* suppression of CD4+ T-cell activation.

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.

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