



REVIEW ARTICLE

A critical analysis about the supposed role of azithromycin in the treatment of covid-19

Uma análise crítica sobre o suposto papel da azitromicina no tratamento da covid-19

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ABSTRACT

After over one year, the coronavirus disease 2019 (covid-19) has still affected millions of people. For this reason, global efforts to promote better treatment of covid-19 have been undertaken focused on the repurposing of existing medications. In Brazil, azithromycin, a broad-spectrum antibiotic, has been used in association with other drugs as an immunomodulatory, anti-inflammatory, and anti-viral agent, regardless of bacterial co-infection. Indeed, data from experimental studies have demonstrated the capacity of this drug in reducing the production of infection-induced pro-inflammatory cytokines, such as IL-8, IL-6, and TNF- α . However, observational studies revealed conflicting results regarding its effect, whereas well-conducted clinical trials have not shown a considerable effect of this agent on the improvement of clinical outcomes. This narrative review addressed the possible role of this antibiotic in the management of covid-19, based on data from clinical and preclinical studies.

PALAVRAS-CHAVE

Anti-infecciosos
 Azitromicina
 Covid-19
 SARS-CoV-2

RESUMO

Após mais de um ano, a doença coronavírus 2019 (covid-19) ainda afeta milhões de pessoas. Por esta razão, os esforços globais para promover um melhor tratamento para covid-19 têm sido realizados com foco no reaproveitamento de medicamentos existentes. No Brasil, a azitromicina, um antibiótico de amplo espectro, tem sido utilizada em associação com outras drogas como agente imunomodulador, antiinflamatório e antiviral, independentemente da coinfeção bacteriana. De fato, dados de estudos experimentais demonstraram a capacidade dessa droga em reduzir a produção de citocinas pró-inflamatórias induzidas por infecção, como IL-8, IL-6 e TNF- α . No entanto, estudos observacionais revelaram resultados conflitantes quanto ao seu efeito, ao passo que ensaios clínicos bem conduzidos não demonstraram um efeito considerável desse agente na melhora dos desfechos clínicos. Esta revisão narrativa teve como objetivo abordar o possível papel desse antibiótico no tratamento de covid-19, com base em dados de estudos clínicos e pré-clínicos.

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INTRODUCTION

The coronavirus disease 2019 (covid-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus first emerged in Wuhan, a central city in China¹. The genome of SARS-CoV-2 is a positive-sense single-stranded RNA, and its main structural component is the spike protein that plays a critical role in viral infection through its receptor-binding domain (RBD), which targets human respiratory epithelial cells².

At the end of December 2019, the covid-19 was no longer a local challenge and was declared a pandemic by the World Health Organization (WHO) on March 2020³. As of July 2021, over 500 thousand people lost their lives in Brazil, and the lack of containment measures associated with poor adherence to protection measures, such as mask-wearing and social distancing, has contributed to the uncontrolled spread of the virus, increasing cases and deaths^{4,5}.

Because of this critical scenario, therapeutic strategies were raised based on pathophysiological and clinical characteristics to improve the disease prognosis⁶. Once that the excessive immunological response with intense cytokine production, called "cytokine storm", is the main characteristic of covid-19 pathophysiology directly associated with an increased risk for mortality⁷, repurposing an agent that would act both reducing this excessive cytokine production and modulating the immune system could be extremely useful.

Azithromycin (AZM), a broad-spectrum antibiotic, has been widely used in the SARS-CoV-2 pandemic for this purpose in association with other drugs (such as ivermectin and hydroxychloroquine). Experimental studies have shown that AZM suppresses some pathogen-induced cytokine production, especially IL-1, IL-6, and TNF-alpha, and may exhibit antiviral effects in specific conditions^{8,9}. Nevertheless, current clinical assessments reported conflicting results regarding the use of AZM in patients with either suspected or confirmed covid-19¹⁰.

Therefore, given the growing use of this broad-spectrum antibiotic and considering the high probability of promoting bacterial resistance, we critically appraised the current literature regarding AZM use in the context of the pandemic. We addressed whether there is still a role for the use of AZM in covid-19.

METHODS

A literature search was performed on PubMed with the following search terms: "azithromycin AND covid-19" and "azithromycin AND SARS-CoV-2". We selected the most up-to-date evidence regarding both *in vitro* data (preclinical studies) and clinical assessments (through epidemiological studies).

This article describes an overview of experimental data that could justify AZM use in covid-19, followed by summarized data from the observational and clinical studies.

KEY ASPECTS OF COVID-19 PATHOPHYSIOLOGY

When the SARS-CoV-2 enters the human cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor through the interaction of its spike protein, several mechanisms are associated with the covid-19 pathogenesis. For instance, direct virus-mediated endothelial cell damage and dysregulation of the renin-angiotensin-aldosterone system are markers of clinical severity and may occur with microcirculation dysfunctions, including thromboinflammation⁷. Surrounding this complex pathological process, the dysregulation of the immune response, followed by a state of hyper-inflammation with strong release of pro-inflammatory cytokines, is the main characteristic of patients with the disease.

Viral entry stimulates an exuberant innate immune response to recruit monocytes and other cells: RNA viruses like SARS-CoV-2 can be recognized by innate immune cells that express pattern recognition receptors (PRRs). This recognition leads to the production of type I interferons (IFNs), a cytokine essential to block viral replication¹¹. However, SARS coronavirus expresses proteins that inhibit type I IFN production, impairing the antiviral response and improving rapid viral replication¹². Because of this type I IFN response inhibition associated with chemokines with potent ability to recruit monocytes, there is an excessive infiltration of monocyte/macrophages and neutrophils into the infected tissue, which will produce high levels of pro-inflammatory cytokines¹³. Consequently, the inflammatory profile observed in patients with covid-19 includes increased macrophage-derived cytokines with high levels of IL-6, IL-8, and tumor necrosis factor (TNF), which amplify the state of hyper-inflammation by recruitment of other innate immune cells with subsequent more cytokine production, culminating in the cytokine storm^{14,15}.

Circulating cytokines and viral particles activate endothelial cells, which also recruit neutrophils to elicit the formation of neutrophil extracellular traps (NETs). NETs may act activating both extrinsic and intrinsic coagulation pathways, promoting blood coagulation abnormalities¹⁶. This cytokine overproduction associated with microvascular dysfunction is correlated with the severity of the disease, reinforcing the idea of covid-19 as a microvascular disease¹⁷. Although NET formation plays a role in responses to extracellular pathogens, it also occurs in viral infections, predicting poor clinical outcome¹⁸. Moreover, it is directly related to acute respiratory distress syndrome due to the production of reactive oxygen species and leukotrienes that lead to acute lung injury¹⁹.

The pathophysiology also involves an inflammatory-induced impairment of lymphopoiesis with profound T cell lymphopenia, probably due to virus-induced direct cytopathic effects and enhanced T-cell apoptosis by cytokine storm^{13,20}. This global T-cell lymphopenia has been detected in infections with other coronaviruses and is more pronounced in the CD8+ T cell compartment, especially in patients with severe disease¹⁴. Consequently, in most patients with covid-19,

there is a critical reduction in the total number of CD4+T cells and CD8+T, contributing to the higher risk of severe disease and increased length of hospitalization²¹.

Thus, the covid-19-associated hyperinflammation appears to be an essential factor leading patients in the course of coronavirus disease to multiple organ failure and even death.

AZITHROMYCIN AS A BROAD-SPECTRUM AGENT

Once that the underlying pathophysiology of covid-19 involves an intense inflammatory response with substantial activation of monocytes and neutrophils, there is a concept that an immunomodulatory and anti-inflammatory agent may be effective against SARS-CoV-2 immune response²².

In this scenario, macrolides represent the most common agent in adjuvant covid-19 therapy. They are an anti-bacterial class that inhibits protein synthesis through irreversible binding to a location in the 50S ribosomal subunit. Because of this, they have a bacteriostatic effect, are effective against various Gram-positive and atypical bacterial species associated with respiratory infections and have additional effects on host-defense reactions and chronic human diseases²³.

Azithromycin is the widely used agent of this class, primarily because of its good oral availability with a better effect profile and due to its lack of inhibition of CYP3A4²⁴. Results of several investigations indicate that, beyond its bacteriostatic effect, the immunomodulatory effects of AZM and other macrolide antibiotics have been associated with immunomodulatory and anti-inflammatory activities, mainly acting by decreasing the pro-inflammatory cytokine release and attenuating the lipopolysaccharide-induced release of IL-8 and GM-CSF^{25,26}.

Azithromycin and other macrolides (e.g., clarithromycin and erythromycin) promote the resolution of experimental inflammation by downregulating prolonged inflammatory response and attenuating excessive cytokine production in several infections, reducing the production of reactive oxygen species, and inhibiting neutrophil activation and mobilization. Evidence from *in vitro* data has shown that in the acute phase of the inflammatory response, azithromycin may suppress some pro-inflammatory cytokines, especially IL-8, IL-6, and TNF-alpha, by modulating the functions of dendritic cells²⁷. Furthermore, AZM blocks neutrophil recruitment to the lung through a reduction in pro-inflammatory cytokine expression and inhibition of neutrophil migration via extracellular signal-regulated kinase-1 and -2 signal transduction pathway²⁸. In experimental models of bronchial epithelial cells infected with rhinoviruses, AZM stimulated interferon and interferon-stimulated gene expression, reducing rhinovirus proliferation²⁹.

As an immunomodulatory agent, AZM may act through interaction with structural cells, including leukocytes (such as macrophages, neutrophils, mononuclear leukocytes or monocytes, T cells, and dendritic cells), inhibiting the granulocyte differentiation, suppressing cell proliferation and CD4+ T cell cytokine secretion, as well as may inhibit many

immune cells including eosinophils and basophils³⁰. Due to its high accumulation in cells, particularly phagocytes, AZM is delivered in high concentrations to sites of infection, contributing to the resolution of acute infections. Also, AZM works in neutrophils downregulating the chemokine production (markedly IL-8), followed by attenuation of neutrophil oxidative burst responses and down-regulation of myeloperoxidase (MPO) production, and increasing neutrophil apoptosis³¹. These effects may be helpful in the resolution of acute infections and reduction of exacerbations in chronic airway diseases.

Nevertheless, we highlight that macrolides require much higher doses when compared with corticosteroid immunomodulatory and anti-inflammatory effects, which might increase their well-known adverse effects, especially the potential effect of cardiac QT prolongation³². Moreover, macrolide-induced enhancement in the immune response is restricted to short-term administration, whereas the long-term administration can result in immunosuppression³³. Hence, we considered that its risk-benefit must be carefully considered in all situations.

Also, we found two recent experimental studies that demonstrated the ability of AZM to suppress SARS-CoV-2 replication in cultured cells: the first study, conducted by Andreani et al.³⁴ (2020), used a combination of hydroxychloroquine with azithromycin and found a synergistic effect *in vitro*. However, the drug concentrations for viral inhibition used *in vitro* are difficult to be achieved clinically. In the second study, Du et al.³⁵ (2021) found that AZM could also markedly block the entry of SARS-CoV-2 in human embryonic kidney cells expressing ACE2 using a pseudotyped virus model but showed that a relatively low dose of AZM does not inhibit the SARS-CoV-2 internalization. We considered that more comprehensive experimental research is needed to clarify the anti-SARS-CoV-2 effect of AZM.

CLINICAL ASSESSMENTS ON THE USE OF AZITHROMYCIN AGAINST COVID-19

Observational studies

Because of its anti-inflammatory and immunomodulatory properties, azithromycin would be clinically meaningful in managing covid-19 disease. Several observational studies have been investigating different aspects of its use. Here, we will describe only its effects on clinical status (such as mortality, length of stay, intensive care unit (ICU) admission), as we summarized in Table 1.

In most patients with suspected or confirmed covid-19, azithromycin alone or associated with other drugs showed some encouraging results. For instance, a multicenter study by Arshad et al. (2020)³⁶ revealed that both azithromycin alone and hydroxychloroquine (HCQ) plus azithromycin (HCQ-AZM) were associated with a reduction in covid-19-related mortality among patients with a median age of 64 years old. Despite its large sample size, the study did not provide sufficient information about the duration of symptoms before hospitalization, affecting the study's internal validity

Table 1 – Summarized data from observational studies surrounding azithromycin use in the covid-19 treatment.

Author	No. of patients and treatment	AZM dose indicated	Study type	Enrolled patients	Results
Arshad et al. (2020)	Total (n = 2541): Neither drug (n = 409), HCQ alone (n = 1202), AZM alone (n=147), HCQ + AZM (n = 783).	AZM 500 mg once daily on day 1 followed by 250 mg once daily for the next 4 days.	Multicenter retrospective observational study.	Consecutive patients hospitalized with a covid-related admission in the health system from March 10, 2020 to May 2, 2020.	Treatment with hydroxychloroquine alone and in combination with azithromycin was associated with reduction in COVID-19 associated mortality.
Gautret et al. (2020)	Total (n = 80) received a combination of hydroxychloroquine and azithromycin.	AZM 500 mg on day 1 followed by 250 mg per day for the next four days.	Uncontrolled, non-comparative, observational study in a cohort.	80 relatively mildly infected patients.	All patients improved clinically except one 86-year-old patient who died, and one 74-year-old patient still in intensive care.
Rosenberg et al. (2020)	Total (n = 1438) HCQ+AZM (n = 735) Hydroxychloroquine alone (n = 271) Azithromycin alone (n = 211) Neither drug (n = 221).	Uninformed	Retrospective cohort study.	Random sample of all admitted patients with laboratory-confirmed covid-19 in 25 hospitals.	Treatment with azithromycin was not associated with significantly lower in-hospital mortality.
Lauriola et al. (2020)	Total (n = 377) HCQ + AZM (n = 297) Hydroxychloroquine alone (n = 17) Neither drug (n = 63)	AZM 500mg for 10 days.	Retrospective single-center cohort study.	Patients admitted for pneumonia related to covid-19.	The study found a lower mortality rate in patients with covid-19 treated with a combination of HCQ and azithromycin.
Blanco et al. (2021)	Total (n = 84) Azithromycin (n = 25)	AZM 500mg for 3 days if there is rapid improvement, and for 6 days if the duration of symptoms is prolonged. If recurrence of symptoms, Azithromycin 500 mg/24h was prescribed for 3 more days.	Retrospective observational study.	Elderly patients diagnosed with covid-19, living in two nursing homes in a rural area of Toledo.	Early treatment of symptomatic covid-19 patients with antihistamines and azithromycin improved outcomes reducing fatality rate, hospital admissions and ICU admissions in the elderly population, regardless of patient's age and risk factors.
Ip et al. (2020)	Total (n=2512) At least one dose of hydroxychloroquine (n = 1,914) Hydroxychloroquine with azithromycin (n = 1473)	Most patients received 800 mg on day 1, and 400 mg on day 2-5 (Hydroxychloroquine with Azithromycin).	Retrospective observational cohort study.	Patients were hospitalized at a 13-hospital network spanning New Jersey USA between March 1, 2020 and April 22, 2020 with positive polymerase chain reaction results for SARS-CoV-2.	Hydroxychloroquine, either alone or in combination with azithromycin, was not associated with a survival benefit among hospitalized covid-19 patients.

Table 1 – Summarized data from observational studies surrounding azithromycin use in the covid-19 treatment (cont.).

Author	No. of patients and treatment	AZM dose indicated	Study type	Enrolled patients	Results
Basaran et al. (2020)	Total (n = 174) Hydroxychloroquine alone (n = 23) HCQ+AZM (n = 113) Favipiravir (n = 32).	Uninformed	Prospective, observational, single-center study.	174 consecutive probable/confirmed covid-19 hospitalized patients.	15 patients (8.5%) were transferred to the ICU. Four patients died (2.2%).
Dubernet et al. (2020)	Total (n = 164) HCQ+AZM (n = 164)	500 mg on the first day, followed by 250 mg daily for the next 4 days	Retrospective observational study.	Patients infected with covid-19 admitted to Félix Guyon University Hospital.	Hydroxychloroquine/azithromycin treatment was associated with a lower ICU admission rate ($p = 0.008$).
Rodríguez-Molinero et al. (2020)	Total (n = 418) Azithromycin (n = 239)	500 mg on the first day (oral or intravenous), followed by 250 mg daily, until completing 5 days of treatment.	Observational study on a cohort.	Patients admitted to three regional hospitals in Catalonia, Spain.	There was not a clinical benefit associated with the use of azithromycin, in terms of lung function 48 hours after treatment or length of hospital stay.
Satlin et al. (2020)	Total (n = 153) Ceftriaxone with hydroxychloroquine (n = 47) HCQ+AZM (n = 27) Doxycycline with hydroxychloroquine (n = 24)	Uninformed	Retrospective cohort study.	Hospitalized patients with covid-19 who received ≥ 1 dose of HCQ at two New York City hospitals.	Co-administration of azithromycin was not associated with improved outcomes.

HCQ, hydroxychloroquine; AZM, azithromycin; ICU, intensive care unit.

Table 2 – Summarized data from clinical trials testing azithromycin against covid-19.

Author	No. of patients and treatments	AZM dose indicated	Study type	Enrolled patients	Results
Gautret et al. (2020)	Total (n = 36), Hydroxychloroquine treated patients (n = 20) Control patients (n = 16) Six patients received azithromycin	Azithromycin 500 mg on day 1 followed by 250 mg per day for the next four days.	Controlled trial	Hospitalized patients with confirmed COVID-19	At day 6, 100% of patients treated with a combination of hydroxychloroquine and azithromycin were virologically cured compared to 57.1% of patients treated with hydroxychloroquine only, and 12.5% in the control group ($p < 0.001$)

Table 2 – Summarized data from clinical trials testing azithromycin against covid-19 (cont.).

Author	No. of patients and treatments	AZM dose indicated	Study type	Enrolled patients	Results
Cavalcanti et al. (2020)	Total of 667 patients underwent randomization. Hydroxychloroquine plus Azithromycin (n = 217) Hydroxychloroquine (n = 221) Control (n = 227)	Azithromycin 500 mg once daily for 7 days.	Multicenter, randomized, open-label, three-group, controlled trial.	Hospitalized patients with suspected or confirmed Covid-19 who were receiving either no supplemental oxygen or a maximum of 4 liters per minute of supplemental oxygen.	As compared with standard care, the proportional odds of having a higher score on the seven-point ordinal scale at 15 days was not affected by either hydroxychloroquine alone (odds ratio, 1.21; 95% CI, 0.69 to 2.11; p = 1.00) or hydroxychloroquine plus azithromycin (odds ratio, 0.99; 95% CI, 0.57 to 1.73; p = 1.00)
Sukhavati et al. (2020)	All patients (n = 111) Case group (azithromycin, hydroxychloroquine (HCQ) and lopinavir/ritonavir (LPV/r) (n = 56) Control group (hydroxychloroquine (HCQ) and lopinavir/ritonavir (LPV/r) (n = 55)	Azithromycin 500mg daily.	Open randomized controlled trial	202 patients with compelling clinical symptoms for a diagnosis of COVID-19 with a positive RT-PCR test and significant findings compatible with radiographic imaging of COVID-19 pulmonary involvement.	The mortality rate between the two groups wasn't significant in patients who received AZM in addition to HCQ and LPV/r had a better general condition.
Abbas et al. (2020)	All patients (n = 161)	Azithromycin 500mg on the first day, then 250 mg daily for 5 days to covid-19 patients without pneumonia; Azithromycin 500 mg on the first day, then 250 mg daily for 14 days to covid-19 patients with pneumonia in the ward; Azithromycin 500 mg on the first day, then 250 mg daily for 14 days to covid-19 Patients with pneumonia in the ICU.	Prospective study used a pre- and post-intervention design without a comparison group.	Covid-19 patients without pneumonia; Covid-19 patients with pneumonia in the ward; Covid-19 patients with pneumonia in the ICU.	23.6% of the patients were admitted to the respiratory care unit (RCU); 84.5% recovered and were discharged without symptoms after testing negative with RT-PCR 6.8% patients died during the study period.

Table 2 – Summarized data from clinical trials testing azithromycin against covid-19 (cont.).

Author	No. of patients and treatments	AZM dose indicated	Study type	Enrolled patients	Results
Furtado et al. (2020)	All patients (n = 447) Case group azithromycin (n = 214) Control group (n = 183)	Azithromycin 500 mg once daily plus standard of care for 10 days. Control group received standard care without macrolides.	Open label, randomized clinical trial multicenter	Patients admitted to hospital with suspected or confirmed covid-19 with fewer than 14 days since symptom onset plus severity criteria: use of oxygen supplementation of more than 4 L/min flow; use of high-flow nasal cannula; use of non-invasive positive-pressure ventilation; or use of mechanical ventilation.	Death at 29 days: Case group n = 90 (42%) Control group n = 73 (40%) CI 1.08 (0.79 to 1.47) p value = 0.63
RECOVERY (2021)	Total (n = 7,763) Allocated to Azithromycin (n = 2,582) Allocated to usual care alone (n = 5,181)	Azithromycin 500 mg by mouth, nasogastric tube, or intravenous injection once a day for 10 days or until discharge, if sooner.	Randomized, controlled, open-label, adaptive platform trial.	Patients admitted to hospital had clinically suspected or laboratory confirmed SARS-CoV-2 infection and no medical history that might put the patient at substantial risk.	561 (22%) patients allocated to azithromycin died within 28 days. 1162 (22%) patients allocated to usual care died within 28 days (rate ratio 0.97, 95% CI 0.87-1.07; p=0.50). Azithromycin did not improve survival or other prespecified clinical outcomes.
PRINCIPLE Trial (2021)	Total (n = 2,265) Allocated to Azithromycin plus usual care (n = 540), usual care alone (n = 875), and other treatments (n = 850)	Azithromycin 500 mg daily for three days.	Open-label, multi-arm, adaptive platform randomized trial.	People in the community aged 65 years and older, or 50 years and older with comorbidities, and with ongoing symptoms from PCR-confirmed or suspected covid-19.	There was little evidence of a meaningful benefit in the azithromycin plus usual care group in time to first reported recovery versus usual care alone (hazard ratio 1.08, 95% Bayesian credibility interval [BCI] 0.95 to 1.23). The absolute benefit for hospitalization in percentage was 0.3% (95% BCI -1.7 to 2.2).

HCQ, hydroxychloroquine; AZM, azithromycin; ICU, intensive care unit.

once the disparity between participants' clinical status may lead to different outcomes.

Two other cohorts conducted by Gautret et al.³⁷ (2020) and Lauriola et al.³⁸ (2020) observed improved clinical status and a lower mortality rate, respectively. However, these studies had several limitations, such as small sample size, lack of randomization, and low prevalence of comorbidities, which does not allow for correcting the confounding factors completely. Furthermore, Gautret's primary endpoint was essentially a surrogate outcome (characterized by a decrease in viral load). Considering that surrogate outcomes do not necessarily affect clinical outcomes, we considered that the study results were not associated with practical relevance.

Blanco et al.³⁹ (2021) conducted another study with positive results. They evaluated the early treatment of symptomatic covid-19 patients with AZM associated with antihistamines and found that it could prevent severe disease progression in elderly patients. However, the small sample population evaluated and the absence of correction to confounding factors may have overestimated the results. Moreover, despite the elderly patients, their clinical symptoms were primarily mild, which is an important indicator of better prognosis.

Similar limitations were shown Basaran et al.⁴⁰ (2021), a single-center observational study with a small number of patients (174) that observed a low mortality rate in overall patients who received HCQ-AZM. However, critically ill patients at the time of admission were excluded, and the authors did not perform a risk factor analysis for disease progression or outcomes, nor a comparison between different treatment regimens. Likewise, Dubernet et al.⁴¹ (2020) found a lower ICU admission rate among 164 enrolled patients, of which only 36 received HCQ-AZM treatment ($p = 0.008$), although a multivariate analysis was not performed. These findings align with other studies with positive results, but the small number of participants and the absence of an adequate statistical analysis made it impossible to distinguish between real effects and confounding factors.

Rosenberg et al.⁴² (2020) found an opposite result: treatment with AZM was not associated with significantly lower in-hospital mortality. In addition, Ip et al.⁴³ (2020) found no significant differences in associated mortality for patients receiving HCQ-AZM compared to those receiving neither drug. Rodríguez-Molinero et al.⁴⁴ (2020) did not find a clinical benefit associated with the use of AZM in terms of lung function or length of hospital stay, and, in contrast, AZM was associated with a longer time to discharge. Although the small sample size, Satlin et al.⁴⁵ (2020) did not find that co-administration of AZM was associated with improved outcomes in patients receiving HCQ (importantly, there was not a control group).

We know that all observational studies have limitations, mainly because none of them permit causality inference. This limitation is aggravated when the study does not randomize the participants and does not include a significant sample size, allowing adequate generalization of the results. Therefore, we highlight the importance of randomized, placebo-controlled clinical trials for assessing the efficacy of repositioning AZM for covid-19 treatment.

Clinical trials

We identified seven clinical trials with available data regarding the use of azithromycin associated or not with other medications for covid-19. In most studies, azithromycin was tested in association with hydroxychloroquine (HCQ-AZM). All summarized data are provided in Table 2.

One study by Gautret et al.⁴⁶ (2020) showed an intense decrease in viral load at 6-day of follow-up in 100% of patients included in the HCQ-AZM group compared with 57.1% of patients treated with HCQ alone and 12.5% in the control group. Nevertheless, a patient in the HCQ-AZM arm who had a negative PCR test at 6-day tested positive again at 8-day after inclusion in the survey. The specific finding (decrease in viral load) was not associated with clinical improvement. Moreover, the study had two main limitations: a small sample size (36 patients, of which only six were assigned to receive HCQ-AZM), a limited follow-up, and safety data not sufficiently reported. Likewise, another trial conducted by Abbas et al.⁴⁷ (2020), involving 161 patients, demonstrated that HCQ-AZM was useful to improve time to resolution of symptoms. However, it was a single-center study conducted without a control group. These limitations may raise systematic errors, making the findings neither feasible nor generalizable.

In contrast, Cavalcanti et al.⁴⁸ (2020) enrolled 217 hospitalized patients to receive HCQ (400 mg twice daily) plus AZM (500 mg once daily for seven days) and showed that the improvement of clinical status at 15-day of follow-up was not significantly different from the standard treatment group. A small trial conducted by Sekhavati et al.⁴⁹ (2020) enrolled 111 patients with symptoms of covid-19 to receive a control treatment (composed by oral Lopinavir/Ritonavir (400/100 mg twice daily) with oral HCQ (400 mg daily for 5 days), or control treatment plus AZM (500 mg daily for 5 days). After the follow-up, there were no significant differences in the primary outcome characterized by the mortality rate in the AZM group.

In a study by Furtado et al.⁵⁰ (2020), the patients who received AZM alone had worse outcomes in terms of clinical status at 15-day compared to the standard treatment group. The RECOVERY Trial⁵¹ (2021), the largest trial with over seven thousand patients assigned, has not shown a significant difference between the AZM group and standard care in the primary outcome characterized by 28-day mortality. Finally, the PRINCIPLE Trial⁵² (2021), a multi-arm, primary care, open-label trial, with over two thousand participants from a community who had an increased risk of complications, randomly assigned people to usual care plus azithromycin 500 mg daily for three days. Their findings did not support the routine use of azithromycin for reducing the time to recovery or risk of hospitalization.

CONCLUSION

Our analysis demonstrated that AZM exhibited some experimental activity against several infections, and they may act attenuating the infection-induced cytokine storm in preclinical assessments. However, this

antibiotic has not shown expressive improvement in clinical outcomes, especially as demonstrated by clinical trials with solid methodological procedures.

This discrepancy between preclinical data, generated in well-controlled conditions, and clinical studies conducted in real-world settings, demonstrates the complexities of the immune response to several viral infections, especially against SARS-CoV-2 infection. For this reason, we considered that azithromycin use in covid-19 treatment, without a proven bacterial co-

infection, is not based in sufficient evidence.

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