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Research Article

Azithromycin Treatment in COVID-19 is Not Associated with Clinical Course Shortening, But it Is Associated with a Reduction in Mortality. A Case Control-Study Paired By Sex, Age Group, Chronic Diseases and Severity

Jose Luis Turabian*

Specialist in Family and Community Medicine, Health Center Santa Maria de Benquerencia. Regional Health Service of Castilla la Mancha (SESCAM), Toledo, Spain.

ABSTRACT

Background: Azithromycin (AZT) has been proposed as a treatment for COVID-19 on the basis of its immunomodulatory actions.

Objective: To assess the association between exposure to AZT in patients with COVID-19, both admitted to the hospital and treated as outpatients, with respect to the duration of their clinical course and mortality.

Methodology: An observational and retrospective case control-study paired by sex, age group (> and = <65 years), presence or absence of chronic diseases, and severity (presence or absence of pneumonia) was conducted March 15, 2020 to March 15 , 2021, in a general medicine office in Toledo, Spain.

Results: 19 cases and 19 controls were included. 26% of the cases and 42% of the controls had a positive clinical result (improvement or clinical cure before 13 days of the onset of the disease) (Fisher exact test = 0.1051; Paired OR = 0.4545 (Confidence intervals at 95%: 0.1579, 1.308). In the 19 cases there were no deaths vs. 2 deaths in the control group (Fisher exact test = 0.0001; paired OR = 0.1053 (Confidence intervals at 95 % = 0.02452, 0.4519).

Conclusions: In the context of general medicine in Toledo (Spain), AZT did not shorten the duration of clinical symptoms, but it was shown to be a protective factor against mortality. Due to the limitations of the study design, the results should be considered with caution and the use of AZT in COVID-19 should be probably restricted, to date, to patients in whom there is an antimicrobial indication.

KEYWORDS

COVID-19; SARS-CoV-2; Disease & Medicine; Symptoms; General Practice; Epidemiology; Azithromycin.

Corresponding Author Information

Jose Luis Turabian
Health Center Santa Maria de Benquerencia Toledo, Spain.

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Introduction

Since December 2019, the coronavirus disease 2019 (COVID-19), due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1], has caused more than 117 million reported cases and more than 2.5 million of deaths to date [2]. In just over 12 months, numerous pharmacological and non-pharmacological interventions have been implemented to try to limit patient exposure, stop the spread and provide medical treatments to those infected [3,4]. But, the growing burden of coronavirus disease (COVID-19) has led to the massive use of other drugs with uncertain or unproven effects. It can be said that the pandemic has given a new impetus to the "medicine of despair" [5] in which therapies are used without scientific basis.

On the other hand, it must be taken into account that the strategies to develop a new therapy will require a lot of time and very extensive resources. Therefore, drug reuse has become an ideal strategy towards a smart, versatile and rapid way of possible COVID-19 treatments [6,7]. In any case, tools are needed to keep people alive, improve symptoms, and reduce the duration and severity of illness. One of the many challenges for clinical trials during a pandemic like COVID-19 is the need to provide reliable and clear answers quickly. Currently, high-risk patients with progressive symptomatic disease only have hospital treatment, and only in certain circumstances and still subject to scientific debate. Therapeutics that have proven beneficial for COVID-19 include an antiviral (remdesivir), immunosuppressives (dexamethasone, baracitinib), several outpatient monoclonal antibodies, and anticoagulants [8]. Thus, outpatient treatment is desperately needed to avoid hospitalization [9,10].

In this context, one of the proposed drugs is azithromycin (AZT), a macrolide antibiotic, widely used in hospital and primary care, with high tissue permeability and cell adhesion. AZT kills bacteria by reversibly binding to the 50S subunit of the bacterial ribosome and inhibiting protein synthesis [11]. In addition to its antibacterial activity, AZT also plays an anti-inflammatory role by inhibiting the secretion of pro-inflammatory cytokines, including interleukin-8 (IL-8). IL-8 is one of the first chemokines to activate neutrophils secreted by lipopolysaccharide-stimulated monocytes [11]. AZT can also inhibit airway mucus hypersecretion [12]. Long-term treatment with low doses of AZT is associated with down-regulation of genes that regulate antigen presentation, T-cell and interferon responses, and multiple inflammatory pathways in the airways and blood of patients with neutrophilic COPD [11].

In other words, it is gradually becoming accepted that macrolides have both antibacterial activity and immunomodulatory effects, and it tends to be recognized that AZT plays a role in the treatment of chronic airway diseases by decreasing mucus hypersecretion and exerting an anti-inflammatory function [11]. Thus, AZT and other macrolides have been suggested as a treatment for COVID-19 due to their supposed role in the prevention of bacterial superinfection and their immunomodulatory and anti-inflammatory effects [13].

However, on the other side of the scale, the prescription of possibly ineffective drugs or those without proven efficacy (such as chloroquine, hydroxychloroquine, ivermectin, doxycycline, etc.) has been encouraged, as well as AZT itself at the present time [14]. Thus, it has been reported that macrolides in the treatment of patients with COVID-19 did not show beneficial effects compared to standard treatment. However, the evidence for all results is inconclusive. On the other hand, clinical studies evaluating the use of macrolides in the treatment of adult or pediatric patients with different respiratory infections, such as influenza or respiratory syncytial virus, have shown conflicting results. Despite these results, macrolides have been empirically prescribed in patients with pneumonia caused by novel coronaviruses such as SARS and MERS and, more recently, SARS-CoV-2 [15]. This is so, although it is recognized that routine antibiotics should be avoided in the absence of a strong suspicion of a secondary bacterial infection [16].

In this scenario, a small study of cases exposed to AZT and controls not exposed to AZT is presented, paired by the main confounding variables regarding the clinical results (improvement or cure) and mortality of patients with COVID-19, in a general medicine office, in Toledo, Spain.

Material and Methods

An observational and retrospective case control-study, paired by sex, age group (> and = <65 years), presence or absence of chronic diseases and severity (presence or absence of pneumonia), was conducted from March 15, 2020 to March 15, 2021, in a general medicine office in Toledo, Spain. The outcome of interest was clinical improvement or cure and mortality. In this sense, the collected result (positive or clinical improvement; negative or no clinical improvement) was compared by calculating the odds ratio (OR). The interpretation of the OR was as follows: When the result of the OR calculation was equal to one (null value) the disease is not associated with the exposure; When the result is greater than 1.0, the interest result is positively associated with the exposure. When the OR is less than 1.0 the association is inverse, that is, that the status of being a case has a lower probability of having been exposed to the risk factor, in which case, it can be assumed that it is a protective factor [17]. To calculate the OR, the Open Source Epidemiologic Statistics for Public Health program [18] was used.

Study location

The study was conducted at a general medicine office in the Santa Maria de Benquerencia Health Center, Toledo, Spain, which has a list of 2,000 patients> = 14 years of age (In Spain, general practitioners (GPs) care for people > = 14 years of age, with exceptions requested by the child's family and accepted by the GP). The study collected data from the patients' medical records for 12 months, from March 15, 2020 to March 15, 2021.

Diagnosis of COVID-19

The diagnosis was confirmed with reverse transcriptase-polymerase chain reaction (PCR) test for SARS-CoV-2 oropharyngeal.

Information on COVID-19 patients was obtained from the registry systems used by GP in the consultation. A confirmed case with active infection was considered to be any person with a clinical picture of sudden onset acute respiratory infection of any severity that occurs, among others, with fever, cough or feeling of shortness of breath; Other symptoms such as odynophagia, anosmia, ageusia, muscle pain, diarrhea, chest pain or headache, among others, were also considered symptoms of suspected SARS-CoV-2 infection according to clinical criteria; and the existence of a positive PCR test. People with symptoms compatible with COVID-19 who had already had a PCR-confirmed SARS-CoV-2 infection in the previous 90 days were not considered suspect cases again [19].

Collected variables

The following variables were collected: age, sex, symptoms, chronic diseases (defined as "any alteration or deviation from normal that has one or more of the following characteristics: is permanent, leaves residual impairment, is caused by a non-reversible pathological alteration , requires special training of the patient for rehabilitation, and / or can be expected to require a long period of control, observation or treatment" [20], and severity of the disease (mild cases: clinical symptoms are mild and no manifestation of pneumonia can be found on images; moderate-severe cases: with symptoms such as fever and respiratory tract symptoms, etc., and the manifestation of pneumonia can be seen on the imaging tests [21].

Cases

They were defined as COVID-19 patients who received AZT treatment (usual care with AZT at standard doses of 500 mg / day for 3 days, 6 days, or up to 9 days).

Controls and randomization with matching

Controls were defined as COVID-19 patients who received usual standard care without AZT. Each case was randomly matched with a control: another patient of the same sex (male, female), age group (under and over 65 years of age), presence or absence of chronic diseases, and severity (presence or absence of pneumonia). The choice of controls was made based on the list of patients diagnosed with COVID-19 in the consultation under study, during the period from March 15, 2020 to March 15, 2021; For each case, the list of patients diagnosed with COVID was reviewed in chronological order until one found one that presented the same characteristics of sex, age group, chronic diseases and severity, as the case, and was assigned as its control. The procedure was repeated until all cases were matched.

Definition of result

Patient-reported outcomes - self-assessments of patient health status - are central to COVID-19 response, recovery, and resilience [22]. Based on this criterion, the result was the duration of the disease assessed by the number of days of duration from the onset to the disappearance of symptoms, reported and assessed by the patient. The result was quantitatively defined on the basis of the data found in a previous study in the same population of the general medicine consultation object of the study, of the mean duration of the symptoms, which turned out to be 13 days [23]. Thus, the clinical outcome was defined as follows: the presence of clinical cure or improvement within 13 days from the onset of the disease was defined as a positive result; the absence of improvement or cure before 13 days from the onset of the disease or death, was defined as a negative result. In addition, mortality was assessed: the presence of death or the survival of the patient.

Statistic analysis

The bivariate comparisons were performed using Fisher Exact Test for paired data [18].

Results

19 cases and 19 controls were included. 26% of the cases and 42% of the controls had a positive clinical result (improvement or clinical cure before 13 days of the onset of the disease) (Fisher exact test = 0.1051; Paired OR = 0.4545 (Confidence intervals at 95%: 0.1579, 1.308) (Table 1). Given that the confidence intervals of the OR include the value one (null value), we can say that the clinical improvement of the disease is not associated with the exposure. In the 19 cases there were no deaths vs. 2 exitus in the control group (Fisher exact test = 0.0001106. Significant at p <.05; paired OR = 0.1053 (95% confidence intervals = 0.02452, 0.4519) (Table 2); that is, since the OR is less than 1.0 we can say that the association is inverse, so it can be assumed that the treatment with AZT is a protective factor of the mortality.

Results	Controls (Not Exposed to AZT) N=19	Cases (Exposed to AZT) N=19	Statistical Significance and Or
POSITIVE (Improvement or healing in <13 days of evolution)	8 (42)	5 (26)	Fisher exact test= 0.1051 NS.
NEGATIVE (No improvement or cure in <13 days of evolution, or death)	11 (58)	14 (74)	Paired OR= 0.4545 (Intervalos de confianza al 95%: 0.1579, 1.308)

Table 1: Comparison between cases and controls about clinical result of improvement before 13 days of evolution.
( ): Denotes percentages
NS: Not significant at p < .05.

Results	Controls (Not Exposed to AZT) N=19	Cases (Exposed to AZT) N=19	Statistical Significance and Or
Positive (No Exitus)	17 (89)	19 (100)	Fisher exact test= 0.0001106. Significant at p < .05.
Negative (Exitus)	2 (11)	0	Paired OR = 0.1053 (Intervalos de confianza al 95%= 0.02452, 0.4519)

Table 2: Comparison between cases and controls about survival.
( ): Denotes percentages
NS: Not significant at p < .05.

Discussion

Multiple drugs have been proposed as possible treatments for patients with moderate to severe COVID-19. The severity of some cases of COVID-19, added to the lack of treatment, has led to the reuse of different drugs. AZT is one of them. The use of AZT was proposed at the beginning of the pandemic due to its immunomodulatory, anti-inflammatory and antiviral effects shown in other viral diseases [11,13]. Studies, initially of an anecdotal nature, have been reported in a population similar to our study, with good results with the use of AZT added to other drugs [24]. Our study also has these limitations and mixed results, but it refers to a population from the same geographic area, and with different methodology, what could support the hypothesis of some benefit from the use of AZT.

Proposed mechanisms of the antiviral, anti-inflammatory, and respiratory system actions of AZT

AZT is an antibiotic that tends to be prescribed empirically in COVID-19. It has undergone some experimental treatments for coronavirus patients [25,26]. As well as for severe respiratory syncytial virus infection in young children, since it could reduce hospital stay and endotracheal markers of viral replication [27].

AZT is a classic antibiotic in the treatment of respiratory infections of bacterial origin. However, its use in COVID-19 is being analysed due to the high risk of associated bacterial infections and the anti-inflammatory effect of this antibiotic. AZT kills senescent cells; furthermore, normal healthy cells thrived in the presence of AZT. The new interpretation is that the antibiotic is likely to kill "inflammatory" fibroblasts, which could be useful as a preventive treatment for older people, as well as treatment for people who already have the virus [28,29].

On the other hand, since AZT inhibit viral replication (this could be considered as having a side effect because of functionally inhibits the synthesis of cellular proteins) this antibiotic behave as anti -viral agent, which could be useful for the treatment and prevention of COVID-19. In addition, AZT is an immune modulator, and it has been reported to provide clinical benefit in inflammatory airway diseases [30,31]. There are numerous studies that report a possible antiviral activity of azithromycin against viruses as diverse as influenza virus, rhinovirus, respiratory syncytial virus and Zika [32,33].

In vitro AZT has been reported to block the internalization of influenza A (H1N1) virus in human lung epithelial cells during the early phase of infection. In addition, it has also been demonstrated, in an animal model, that administering intranasal AZT to mice infected with influenza A (H1N1) virus, observing a reduction in the viral load at the lung level. It has been communicated that AZT appears to act as a lysosomotropic agent, affecting lysosomal traffic and pH [34,35].

Among the possible effects of AZT would be included the interference with the ECA2 receptor and decreased virus binding [36], alkalinization of endosomes and lysosomes, with consequent inhibition of lysosomal enzymatic activities responsible for virus entry and replication cycle [37]. In respiratory syncytial virus-infected mice, prophylactic administration of AZT resulted in reduced weight loss, airway inflammation, cytokine levels, and mortality [38].

Different publications collect evidence of the anti-inflammatory properties of AZT in the airway, decreasing the levels of cytokines, closely related to lung damage [39], and of various interleukins. AZT has been published to alter the phenotype of macrophages, reducing the production of the pro-inflammatory cytokines IL-12 and IL-6 and increasing that of IL-10, which has an anti-inflammatory effect [40]. In addition, it would cause a global amplification of the host's antiviral responses mediated by interferon (34, 35) and seems to modify the airway microbiome [41]. The inhibition of SARS-CoV 2 replication in vitro by azithromycin has recently been demonstrated [42]. On the other hand, it has been suggested that other macrolides may have similar properties [43-45].

AZT has been used to treat chronic inflammatory diseases of the airways because it regulates cell-cell contact between airway epithelial cells. Hypersecretion of mucus in the airways is an important component of chronic respiratory diseases. Mucin 5AC (MUC5AC) is the major mucin produced by airway epithelial cells, and hypersecretion of MUC5AC is a sign of various inflammatory lung diseases. . Recently, matrix metallopeptidase 9 was found to be involved in mucus hypersecretion. Furthermore, AZT may inhibit the ability of TNF-α- to induce the production of interleukin (IL) -8 [11]. In patients with cystic fibrosis, many of whom have chronic treatment with AZT and other antibiotics, a low morbidity and mortality is being recorded for what would be expected as a high-risk pathology [46,47].

Disadvantages, risks and doubts of AZT use

At present, in adults and children, a confirmed case of COVID-19, requires a positive PCR test for SARS-CoV-2. However, the reality is that there may be a lack or limited capacity of tests in many places. Thus, the GP may feel the urge to prescribe antibiotics for acute respiratory infections (ARI), without sufficiently considering the consequences of inappropriate treatments. So, despite antibiotics are not recommended for treating uncomplicated ARI, antibiotic prescribing is widespread. Also, telemedicine visits that are frequent during COVID-19 outbreak, may Increase even more antibiotic overprescribing. COVID-19 antibiotics are reserved for patients suspected of having concomitant bacterial or fungal infections; but, in practice, AZT tends to be empirically prescribed (on basis of effects which seems to inhibit viral replication). However, there is still little scientific evidence to support its administration, and the consequences of inadequate prescription of antibiotics should be considered. In adults and paediatrics patients, a prudent and judicious use of antibiotics, including AZT, for ARI, is even more necessary during the COVID-19 pandemic [25].

Thus, for example, mass distribution of AZT to preschool children

has been shown to reduce infant mortality in sub-Saharan Africa, but at the cost of amplifying resistance to macrolides; the effects on the intestinal resistome, a reservoir of antimicrobial resistance genes in the body, of administration of azithromycin for a long period are unclear [48].

In patients with COVID-19, there is insufficient evidence to conclude any difference between macrolide use and standard of care. Larger trials are needed to determine the effects of macrolides on pulmonary and other outcomes in COVID-19 patients [15]. In this way, a live systematic review included only one randomized controlled trial that evaluated the use of AZT (and associated with hydroxychloroquine compared to hydroxychloroquine alone) for COVID-19; the results of this systematic review show that there is not enough evidence to conclude any difference between the intervention and control groups. All outcomes evaluated had a wide confidence interval, were assessed with a small sample size, and therefore had low or very low certainty of evidence [15]. Our study was retrospective and with a small sample size, so these same comments can be applied in principle, although our study shows the strength of matching for some confounding variables.

On the other hand, AZT can prolong the QT interval. Additionally, it should be considered that patients with severe COVID-19 are elderly and have pre-existing comorbidities, so adding a potentially risky drug may represent a challenge for the patient's health. In our study, ECGs were not systematically performed in the cases, but in those that were done (mainly in hospitalized patients), a greater frequency of increased QT interval was not found.

Finally, World Health Organization has recently published a summary of the evidence that updates information regarding the use of 79 therapeutic options for COVID-19, including AZT, concluding that AZT probably does not reduce mortality or mechanical ventilation and does not improve time to symptom resolution [5,49]. Our study agrees with no improvement in the time to resolution of symptoms, but not about mortality; Of course, the strength of the evidence shown by our small study only allows us to suggest that these results should be reviewed through studies with better designs. Perhaps it should be borne in mind that a lesson to learn in the COVID-19 pandemic is that the necessary short-term dependence on repurposing existing drugs will not often produce true successful outcomes [8].

Limitations and strengths of the study

Our study has some limitations and strengths:

1. It is a study of matched case controls

Developing specific selection criteria when selecting cases can lead to better control of potential confounders. However, matching has its disadvantages, since once specific variables are matched, they can no longer be measured or analyzed in the study. Matching based on variables that are modified by exposure or disease (such as symptoms) can lead to bias and reduce the validity of the study. Another possible disadvantage is that the matching is based on variables that are not really confusing, that is, they are not related both to the exposure and to the result, which causes a loss of efficiency and a decrease in the validity of the comparison (overmatching). Finally, it reduces the flexibility of the analysis and increases the complexity of the study [17].

2. It is a retrospective study

This type of study is subject to biases (errors that affect observations) in data collection; although these data were collected from the patients' medical records, in a single general practice consultation, and by their own GP.

3. It has a small sample size

Our study included a small sample; this limitation can lead to the estimation of parameters with little precision or it will be unable to detect differences between groups, even if they exist. But, it is necessary to take into account the balance between sample size and the requirements of the study in terms of economic, human and time resources.

Conclusion

AZT has been suggested as a potential treatment for COVID 19 patients due to its purported role in preventing bacterial superinfection and its immunomodulatory and anti-inflammatory effects. In the context of general medicine in Toledo (Spain), in patients with COVID-19 admitted to the hospital or treated as outpatients, AZT did not shorten the duration of clinical symptoms, but it was shown to be a protective factor against mortality. However, due to the limitations of the study design (retrospective) and the small sample size, the results should be considered with caution and probably, until this date, the use of AZT in COVID-19 should be restricted to patients in which there is an obvious antimicrobial indication. This study struggles between wanting a result as general as possible and being able to obtain that result, providing elements for reflection and future research that accepts or rejects the hypothesis that arises from these results.

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