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ORIGINAL RESEARCH

Relationship Between Azithromycin and Cardiovascular Outcomes in Unvaccinated Patients With COVID-19 and Preexisting Cardiovascular Disease

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BACKGROUND: Empiric antimicrobial therapy with azithromycin is highly used in patients admitted to the hospital with COVID-19, despite prior research suggesting that azithromycin may be associated with increased risk of cardiovascular events.

METHODS AND RESULTS: This study was conducted using data from the ISACS-COVID-19 (International Survey of Acute Coronavirus Syndromes-COVID-19) registry. Patients with a confirmed diagnosis of SARS-CoV-2 infection were eligible for inclusion. The study included 793 patients exposed to azithromycin within 24 hours from hospital admission and 2141 patients who received only standard care. The primary exposure was cardiovascular disease (CVD). Main outcome measures were 30-day mortality and acute heart failure (AHF). Among 2934 patients, 1066 (36.4%) had preexisting CVD. A total of 617 (21.0%) died, and 253 (8.6%) had AHF. Azithromycin therapy was consistently associated with an increased risk of AHF in patients with preexisting CVD (risk ratio [RR], 1.48 [95% CI, 1.06–2.06]). Receiving azithromycin versus standard care was not significantly associated with death (RR, 0.94 [95% CI, 0.69–1.28]). By contrast, we found significantly reduced odds of death (RR, 0.57 [95% CI, 0.42–0.79]) and no significant increase in AHF (RR, 1.23 [95% CI, 0.75–2.04]) in patients without prior CVD. The relative risks of death from the 2 subgroups were significantly different from each other ($P_{interaction}$=0.01). Statistically significant association was observed between AHF and death (odds ratio, 2.28 [95% CI, 1.34–3.90]).

CONCLUSIONS: These findings suggest that azithromycin use in patients with COVID-19 and prior history of CVD is significantly associated with an increased risk of AHF and all-cause 30-day mortality.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT05188612.

Key Words: acute heart failure ■ azithromycin ■ cardiovascular diseases ■ COVID-19

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CLINICAL PERSPECTIVE

What Is New?

- COVID-19 is a hyperinflammatory syndrome leading to multiorgan injury and dysfunction, which may result in heart failure.
- Although azithromycin is one of the most commonly prescribed antibiotics during the COVID-19 pandemic, its use has been associated with an increased risk of cardiovascular complications and death in some prior studies on COVID-19–free populations.
- In this COVID-19 cohort study, after adjustment with inverse probability of treatment weighting, azithromycin therapy was consistently associated with an increased risk of acute heart failure and death in patients with preexisting cardiovascular disease, but not in those free of previous cardiovascular disease.

What Are the Clinical Implications?

- Patients with preexisting cardiovascular disease are perceived to be at higher risk of poor outcomes in COVID-19, but the reasons for worse outcomes are still unsettled.
- Azithromycin use may be a factor associated with poor outcomes in patients with prior cardiovascular disease.
- Prescribers should be aware of the potential association between azithromycin and cardiovascular outcomes.

Nonstandard Abbreviations and Acronyms

AHF	acute heart failure
ISACS	International Survey of Acute Coronavirus Syndromes

COVID-19 is commonly a form of severe respiratory infection from SARS-CoV-2 characterized by bilateral lung infiltrates with no clinical or objective evidence of other organ dysfunction. Nevertheless, in many cases, COVID-19 may also induce immune activation and fulminant cytokine release, resulting in excessive inflammation and extrapulmonary organ injury including adverse effects on the heart.[1–3] On this background, identifying which patient populations are at highest risk of developing cardiac complications is a high clinical priority. A better understanding is necessary of whether COVID-19 therapeutics being studied or currently in use are likely to provide harm rather than benefit.

Little is known about the relationship between cardiac manifestations of the disease and treatment options including antibiotic therapy. Azithromycin is the most consistently studied antibiotic for use in treating patients with COVID-19. It does not improve mortality within 28 days or affect the rate of intensive care unit admission as assessed by trials conducted in the overall population of hospitalized adults.[4–8] Yet, azithromycin is still recommended and widely used as empiric therapy in specific situations, such as the presence of a lobar infiltrate on a chest radiograph, leukocytosis, and elevated serum lactate levels.[9,10]

Based on the current concepts of viral pathogenesis and potential pneumonia coinfection, empiric azithromycin therapy for pulmonary SARS-CoV-2 pneumonia is rational. However, the same cannot be said for cardiac manifestations of SARS-CoV-2. A safety concern was raised by a study conducted on the Tennessee Medicaid beneficiaries that reported an association of azithromycin use with sudden cardiac death.[11] This risk was found to be higher among patients with a history of cardiovascular disease (CVD). In response to this and other published reports associating azithromycin use with an increased risk of cardiac death, the Food and Drug Administration in March 2013 released a statement to healthcare professionals warning that the risk of cardiac death may be increased among patients with a history of cardiac risk factors.[12] Questions raised by the Food and Drug Administration statement remained unanswered, because subsequent studies have produced conflicting results on the assumed association between azithromycin and cardiac events.[13,14] Divergences may, in part, be due to differences in the study populations, outcome measures, and methods of control for key confounders, such as the indication for azithromycin use.

We performed a comprehensive analysis of the relationship between azithromycin use and outcomes in CVD through a large multicenter cohort study of adults hospitalized for COVID-19 across 5 European countries. The aim was to establish whether azithromycin increases cardiac complications and whether its effect on cardiac complications and mortality varies according to the presence of previous CVD.

METHODS

Data Sharing Statement

To guarantee the confidentiality of personal and health information, only the authors had access to the data during the study. Access to the ISACS-COVID-19 (International Survey of Acute Coronavirus Syndromes-COVID-19) data is according to the information on the ISACS-Archives (NCT01218776) website. The source codes for this article are uploaded on GitHub.

We analyzed information from the ISACS-COVID-19 (NCT05188612) from December 2021 to February 2022. This study complied with the Declaration of Helsinki.

The local research ethics committee from each hospital approved the study. Because patient information was collected anonymously, institutional review boards waived the need for individual informed consent.

Participants

Details on the study design, sampling, and recruitment are described in Data S1. Briefly, this was a retrospective cohort study from 17 medical centers of 5 European countries participating in the ISACS-COVID-19 study: Croatia, Italy, Macedonia, Romania, and Serbia. The ISACS-COVID-19 registry includes sites in which investigators are committed to collecting good-quality data for a low-budget study over a planned 1-year follow-up period and did not aim for a strict proportionate sampling of the entire country. Patients vaccinated against COVID-19 and those with previous infection were excluded from the current analysis. The diagnosis of acute COVID-19 infection was defined by polymerase chain reaction testing evidence of SARS-CoV-2 RNA on nasopharyngeal swabs within 14 days before or up to 48 hours after admission. Field work was performed by staff from each of the country's health services under a common protocol developed by the University of Bologna, which also coordinated the recruitment of patients. All data were transferred to the Department of Electrical and Computer Engineering, University of California, Los Angeles, where final statistical analyses were performed.

Exposure

This retrospective cohort study was designed to examine the benefits and risks associated with azithromycin use within 24 hours from hospital admission compared with standard of care. Patients were scheduled to receive azithromycin 500 mg by mouth or intravenous injection once a day for 10 days. All patients included in the study received the scheduled treatment. Patients who were initiated on azithromycin >24 hours following admission were excluded due to the possible introduction of immortal time bias. Patients receiving medications had to survive to the time of administration of such medications.[15]

Antimicrobial agents and other medications that patients had taken were identified from the registry records. We identified frequent escalation from azithromycin use to other antimicrobial agents such as β-lactam antibiotics or sulfonamides. Patients with additional antimicrobial therapy during the study period were entered into the cohort (Table 1).

Standard of Care

An emergency such as a pandemic event is, by definition, associated with an undefined usual standard of care. In the current study, usual standard of care included several antiviral medications, corticosteroids, and additional antibiotics that had been dispensed at any time during hospitalization. Concomitant medications can be confounders. However, they should not bias the comparisons, because minor imbalances in medication use between azithromycin and standard of care groups after inverse probability of weighting are consistent with there being no major confounding effect of these variables.

Variables and Definitions

The following variables were extracted from the electronic health records: demographic characteristics (age and sex), cardiovascular risk factors (smoking, hypertension, hypercholesterolemia, diabetes, and obesity), preexisting CVD (myocardial infarction, chronic coronary syndrome, heart failure, percutaneous coronary intervention, coronary artery bypass graft, atrial fibrillation, pulmonary embolism, and hemorrhagic or ischemic stroke), preexisting pulmonary disease (asthmas and chronic obstructive pulmonary disease), chronic kidney disease, active cancer, and dementia (Table 1). We also noted the type of medications given during hospitalization. Definitions of the patient-level data on conventional risk factors and preexisting comorbidities are reported in Data S1. Diagnosis of COVID-19–related pneumonia was confirmed by chest radiography or chest computed tomography performed in emergency rooms.

Data on Laboratory Values

We assessed the following laboratory baseline parameters: leukocyte and platelet count (10^9 per liter), hemoglobin (grams per deciliter), serum biochemical tests including renal (creatinine, milligrams per deciliter) and liver (alanine aminotransferase, units per liter; aspartate aminotransferase, units per liter), C-reactive protein (milligrams per deciliter), D-dimer (nanograms per milliliter), and lactate dehydrogenase (units per liter). Reference values are reported in Data S1.

Outcome Measures

The primary outcome measure was all-cause mortality within 30 days of hospital admission. The 30-day window was selected to enrich the data over those acquired during the index hospitalization while mitigating survivor bias. Secondary key outcomes were acute respiratory failure (ARF), acute heart failure (AHF), and acute kidney injury (AKI) during hospitalization. Hypoxemia (PaO_2/FiO_2 ratio [The ratio of the partial pressure of oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2)] ≤300 mm Hg), and the need for mechanical ventilation were grouped together for defining the

Table 1. Baseline Characteristics Stratified by Use of Azithromycin on Admission

Characteristic	Azithromycin, N=793	No azithromycin, N=2141	Standardized difference
Female sex	363 (45.8)	901 (42.1)	0.07
Age, y, mean (SD)	66.1 (15.8)	64.5 (15.8)	0.10*
Cardiovascular risk factors			
Diabetes	214 (27.0)	536 (25.0)	0.04
Hypertension	477 (60.2)	1363 (63.7)	−0.07
Hypercholesterolemia	233 (29.4)	601 (28.1)	0.03
Current smoking	73 (9.2)	210 (9.8)	−0.02
Former smoking	127 (16.0)	318 (14.9)	0.03
Obesity	170 (21.4)	496 (23.2)	−0.04
Comorbidities	463 (58.4)	1175 (54.9)	0.07
History of CVD	292 (36.8)	774 (36.2)	0.01
Chronic kidney disease	97 (12.2)	262 (12.2)	−0.0002
Chronic lung conditions	105 (13.2)	253 (11.8)	0.04
Active cancer	119 (15.0)	294 (13.7)	0.04
Dementia	109 (13.7)	218 (10.2)	0.11*
Clinical features			
Days before admission, mean (SD)	3.3 (3.8)	3.4 (4.9)	−0.03
Radiograph/CT signs of interstitial pneumonia	491 (61.9)	1462 (68.3)	−0.13*
ICU	146 (18.4)	623 (29.1)	−0.25*
Laboratory testing			
WBC count on admission, 10^9/L, mean (SD)	7.7 (3.9)	8.9 (7.3)	−0.21*
Hemoglobin on admission, g/dL, mean (SD)	12.9 (2.0)	13.0 (2.1)	−0.07
Platelet count on admission, 10^9/L, mean (SD)	232.0 (111.2)	231.8 (105.8)	0.002
Serum creatinine on admission, mg/dL, mean (SD)	1.1 (1.0)	1.2 (1.1)	−0.19*
CRP, mg/dL, mean (SD)	10.0 (9.0)	11.4 (10.3)	−0.14*
D-dimer, ng/mL, mean (SD)	3.3 (7.3)	4.1 (9.0)	−0.10*
AST, U/L, mean (SD)	79.6 (220.1)	118.3 (407.0)	−0.12*
ALT, U/L, mean (SD)	73.9 (109.6)	106.6 (303.1)	−0.14*
LDH, U/L, mean (SD)	433.2 (519.0)	568.0 (660.3)	−0.23*
In-hospital treatment			
Darunavir	12 (1.5)	12 (0.6)	0.09*
Lopinavir/ritonavir	18 (2.3)	52 (2.4)	−0.01
Remdesivir	59 (7.4)	244 (11.4)	−0.14*
Hydroxychloroquine	226 (28.5)	246 (11.5)	0.44*
Corticosteroids	475 (59.9)	1419 (66.3)	−0.13*
Oral anticoagulants	73 (9.2)	240 (11.2)	−0.07
Heparin	679 (85.6)	1761 (82.3)	0.09*
Antiplatelet treatment	141 (17.8)	576 (26.9)	−0.22*
β-lactam antibiotics	502 (63.3)	895 (41.8)	0.44*
Sulfonamides	12 (1.5)	37 (1.7)	−0.02
Diuretics	217 (27.4)	971 (45.4)	−0.38*

Data are reported as number (percent) or mean (SD) as appropriate, unless otherwise stated. ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CT, computed tomography; CVD, cardiovascular disease; ICU, intensive care unit; LDH, lactate dehydrogenase; and WBC, white blood cell.

*P <0.05.

occurrence of ARF, in line with previous observations reporting that many patients with hypoxemia had not received mechanical ventilation.[16,17] AKI was defined as an increase in serum creatinine by ≥0.3 mg/dL within 48 hours according to the Kidney Disease: Improving Global Outcomes definition.[18] The diagnosis of AHF was initially based on clinical evaluation and was confirmed by chest radiography or computed tomography. All end points were site-reported.

Statistical Analysis

Patient characteristics were stratified according to treatment group: azithromycin versus no azithromycin recipients. Baseline characteristics were reported as percentages for categorical variables and means with SD for continuous variables (Table 1). We had complete data on outcomes. Some patients had missing data on other variables (Data S1). We used multiple imputation with chained equation (MICE) as the imputation method to treat missing data (Data S1).[19] Estimates of odds ratios (ORs) or risk ratios (RRs) and associated 95% CIs were obtained using logistic regression or inverse probability of treatment weighting models, respectively. We used logistic models to evaluate the effect of preexisting CVD and AHF on 30-day mortality. Three models were run that incrementally added covariates. The first model included only demographics, cardiovascular risk factors, comorbidities, and clinical and biochemical features on hospital presentation (Table 1). Model 2 then additionally adjusted for use of corticosteroids. Model 3 included additional clinical therapeutic factors that have been suggested as potential reasons for variation in outcomes, specifically use of antiviral agents. By adjusting for patient characteristics and main therapeutic factors incrementally, we attempted to understand the contribution of CVD and AHF to outcomes. Inverse probability weights were calculated using the propensity score to create a sample in which the distribution of measured baseline covariates was independent from azithromycin use (Data S1).[20] Because of the instability that can be induced by extreme weights, stabilized weights were used that also preserve the original sample size. We created a threshold for weights to avoid the impacts of the outliers. We used 0.01 as the threshold of the propensity weighting. Standardized differences after weighting were calculated to ensure balanced treatment groups with respect to baseline characteristics. Groups were considered balanced when the standardized difference was <10% (Data S1).[21] Outcome comparisons between groups were made by *P* values from 2-sided tests ($P<0.05$). To account for differences in patient-level characteristics and illness severity among groups, we prespecified the following covariates for inclusion in the models: demographics, cardiovascular risk factors, comorbidities, clinical and biochemical features on hospital presentation, and in-hospital treatment (Table 1). Because factors associated with AHF include CVD, sensitivity analyses examined the risk of organ injury and mortality with azithromycin among individuals without a history of CVD. Because results might be partially confounded by use of antimicrobial agents in patients labeled as having standard of care, sensitivity analyses were also conducted including only patients with prescriptions of azithromycin (ie, excluding those with prescriptions of additional antimicrobial agents during hospitalization). To minimize concern about comparison of outcomes in subgroups, estimates were compared by test of interaction on the log scale.[22] A *P* value <0.05 was taken to indicate that the difference between the effects in azithromycin recipients versus nonrecipients was unlikely to have occurred simply by chance (Data S1). All statistical analyses were performed using R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We identified a cohort of 4462 patients with COVID-19. There were 1528 patients who were excluded because they were initiated on azithromycin >24 hours following admission, leaving a final population of 2934 patients. Among the remaining 2934 patients, 1066 (36.4%) had preexisting CVD. A total of 617 (21.0%) died, and 253 (8.6%) had AHF. Overall, 793 (27.0%) received azithromycin (Figure S1).

Characteristics of the Study Cohort

The demographic and baseline clinical characteristics of the study population are presented in Table 1. Azithromycin users, compared with standard of care, were significantly (standardized difference ≥0.10) older and had higher rates of dementia (13.7% versus 10.2%), but were less likely to present with radiograph or computed tomography signs of interstitial pneumonia (61.9% versus 68.3%). In addition, patients exposed to azithromycin were more likely to receive hydroxychloroquine (28.5% versus 11.5%), but less likely to receive antiviral medications including remdesivir (7.4% versus 11.4%), antiplatelet agents (17.8% versus 26.9%), and diuretics (27.4% versus 45.4%). Patients receiving azithromycin were also less likely to have had an intensive care unit encounter (18.4% versus 29.1%) during hospitalization. Unadjusted outcomes are reported in Tables S1 through S3.

Balancing Covariates and Outcomes

The characteristics of patients receiving azithromycin prescriptions and those of the propensity-score weighted controls were well balanced (Table 2). Patients who received azithromycin had a similar rate

Table 2. Inverse Probability of Treatment Weighting: Clinical Factors Associated With Outcomes. Results Stratified by Use of Azithromycin

Clinical factor	Azithromycin, N=792	No azithromycin, N=2141	Standardized difference
Female sex	42.5	43.0	−0.01
Age, y, mean (SD)	65.0 (15.6)	64.8 (15.9)	0.01
Cardiovascular risk factors			
Diabetes	25.5	25.5	−0.001
Hypertension	64.7	62.7	0.04
Hypercholesterolemia	31.5	29.0	0.05
Current smoking	10.4	9.7	0.02
Former smoking	14.0	15.0	−0.03
Obesity	23.4	23.2	0.01
Comorbidities			
Chronic kidney disease	13.8	12.4	0.04
Chronic lung conditions	11.7	12.1	−0.01
Active cancer	14.3	14.3	0.002
Dementia	10.2	11.0	−0.03
Clinical features on admission			
Radiograph/CT signs of interstitial pneumonia	65.2	66.4	−0.02
Laboratory testing			
WBC count on admission, 10^9/L, mean (SD)	8.4 (4.4)	8.5 (6.6)	−0.02
Hemoglobin on admission, g/dL, mean (SD)	13.0 (2.1)	13.0 (2.1)	−0.02
Platelet count on admission, 10^9/L, mean (SD)	231.5 (113.9)	231.7 (106.7)	−0.002
Serum creatinine on admission, mg/dL, mean (SD)	1.3 (1.5)	1.2 (1.0)	0.07
CRP, mg/dL, mean (SD)	11.1 (9.9)	11.0 (10.0)	0.01
D-dimer, ng/mL, mean (SD)	4.0 (9.8)	3.9 (8.5)	0.02
AST, U/L, mean (SD)	97.6 (271.9)	107.9 (370.9)	−0.03
ALT, U/L, mean (SD)	80.9 (118.7)	87.9 (267.3)	−0.05
LDH, U/L, mean (SD)	500.9 (533.2)	532.1 (596.1)	−0.06
In-hospital treatment			
Darunavir	1.2	1.0	0.02
Lopinavir/ritonavir	2.6	2.4	0.01
Remdesivir	9.9	10.3	−0.01
Hydroxychloroquine	16.8	16.9	−0.002
Corticosteroids	62.6	64.5	−0.04
Oral anticoagulants	11.6	10.8	0.03
Heparin	81.5	83.1	−0.04
Antiplatelet treatment	24.9	21.7	0.08
β-lactam antibiotics	45.5	47.8	−0.05
Sulfonamides	2.5	1.8	0.05
Diuretics	40.9	40.4	0.01
Outcome			***P* value**
Primary outcome: 30-d mortality	18.5	20.9	0.140
Risk ratio (95% CI)	0.86 (0.70–1.06)		0.150
Secondary outcome: AHF	11.0	8.3	0.030
Risk ratio (95% CI)	1.67 (1.05–1.79)		0.020

Data are reported as percent or mean (SD) as appropriate, unless otherwise stated. AHF indicates acute heart failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CT, computed tomography; LDH, lactate dehydrogenase; and WBC, white blood cell.

of death than patients who did not receive it (18.5% versus 20.9%; RR, 0.86 [95% CI, 0.70–1.06]), but they were at increased risk of AHF (11.0% versus 8.3%; RR, 1.67 [95% CI, 1.05–1.79]). When we compared azithromycin use versus no antibiotic treatment, results were similar to those of the primary analyses (Table S4). Azithromycin was still significantly associated with an increased risk of AHF (RR, 1.54 [95% CI, 1.07–2.22]), but the risk of death was not significantly different between the 2 groups (RR, 1.02 [95% CI, 0.78–1.32]).

Incidence of Primary and Secondary Outcomes Stratified by Preexisting CVD

Compared with standard of care, patients with preexisting CVD who received azithromycin were at significantly higher risk of AHF (22.8% versus 16.6%; RR, 1.48 [95% CI, 1.06–2.06]) but not of death within 30 days (26% versus 27.2%; RR, 0.94 [95% CI, 0.69–1.28]) (Table 3). By contrast, among patients with no prior CVD, azithromycin significantly reduced 30-day mortality compared with standard of care (10.6% versus 17.2%; RR, 0.57 [95% CI, 0.42–0.79]) (Table 4). In line with this finding, patients who were prescribed azithromycin did not show an increased risk of AHF (4.6% versus 3.7%; RR, 1.23 [95% CI, 0.75–2.04]). The relative risk for the outcome of death was considerably lower in patients free of history of CVD compared with those with preexisting CVD ($P_{\text{interaction}}$=0.01) (Figure 1 and Tables S5 and S6).

Associations Between CVD and Cardiovascular Outcomes

Preexisting CVD was significantly associated with higher odds of 30-day mortality (OR, 1.92 [95% CI, 1.26–2.93]) in a model adjusted for age, sex, cardiovascular risk factors, comorbidities, and clinical and biochemical findings on admission (Figure 2). Significant association was also observed between AHF and death (OR, 2.28 [95% CI, 1.34–3.90]) (Figure 3). These associations did not differ according to treatment with corticosteroids or antiviral agents (Figures 2 and 3).

Use of Azithromycin at Admission and Other Measures of Illness Severity

After inverse probability of treatment weighting, patients who received azithromycin were less likely than their counterparts with standard of care to develop AKI (13.1% versus 17.3%; RR, 0.72 [95% CI, 0.57–0.92]) and ARF (48.1% versus 52.4%; RR, 0.84 [95% CI, 0.71–0.99]) (Table S7). Among patients with preexisting CVD, the results were similar to those observed in the overall population of patients. Azithromycin was still significantly associated with a reduced hazard of AKI (13.6% versus 23.2%; RR, 0.52 [95% CI, 0.36–0.76]) and ARF (RR, 0.67 [95% CI, 0.51–0.87]) during hospitalization (Figure 4 and Table S8).

DISCUSSION

Our propensity score–based weighted subgroup analysis indicates that the effect of azithromycin in the treatment of patients hospitalized with COVID-19 differs between the population as a whole and a population comprising only patients without a prior history of CVD. For the totality of patients included in this study, there was no decreased risk of death among patients who took azithromycin, but there was an increased risk of AHF. The absolute excess risk of AHF and death compared with standard of care varied considerably according to the CVD status. Among patients without CVD, azithromycin was significantly associated with a reduced risk of death, with an absolute difference of 6.6% compared with standard of care. In this group of patients, azithromycin users did not show significant increase in the rate of AHF compared with nonusers. By contrast, azithromycin therapy in patients with preexisting CVD was consistently associated with an excess risk of AHF, and the reduced risk of death seen in the group of patients free of CVD did not persist. Finally, we found a statistically significant association between azithromycin use and reduced odds of AKI and ARF, which was not dependent on the presence of CVD.

In summary, any potential positive effect of azithromycin on AKI and mortality among patients with COVID-19 would have been diluted by its negative effect among patients presenting with preexisting CVD. In the absence of other data on the efficacy of azithromycin in patients with no prior CVD, our findings raise strong concerns about the appropriate use of azithromycin therapy in patients with COVID-19.

Subgroup analyses are generally considered to be exploratory rather than definitive for several reasons, including the lack of randomization, the hypotheses by which such analyses are planned, and the statistical methods used to identify interactions. Our study addressed each of the above-reported concerns. Concern about nonrandomized treatment allocation was addressed by creating a quasirandomized sample using a parametric balancing strategy by inverse probability of treatment weighting models. There was a specific prior suspicion of the existence of an outcome interaction between azithromycin therapy and cardiovascular deaths in non–COVID-19 populations.[11,12] We identified the interaction between cardiovascular health status and azithromycin therapy by comparing the RRs of azithromycin users versus nonusers in 2 distinct analyses including and excluding patients with prior cardiovascular disorders. The relative risks of

Table 3. Inverse Probability of Treatment Weighting: Clinical Factors Associated With Outcomes in Patients With Prior Cardiovascular Disease

Clinical factor	Azithromycin, N=290	No azithromycin, N=774	Standardized difference
Female sex	42.9	43.3	−0.008
Age, y, mean (SD)	71.3 (11.9)	72.4 (11.8)	−0.05
Cardiovascular risk factors			
Diabetes	36.0	35.8	0.005
Hypertension	84.7	83.4	0.03
Hypercholesterolemia	48.8	45.5	0.06
Current smoking	9.0	9.3	−0.008
Former smoking	20.7	21.2	−0.01
Obesity	28.7	27.2	0.03
Comorbidities			
Chronic kidney disease	21.3	22.6	−0.03
Chronic lung conditions	19.3	17.2	0.05
Active cancer	15.9	14.9	0.02
Dementia	15.4	18.5	−0.08
Clinical features on admission			
Radiograph/CT signs of interstitial pneumonia	61.8	61.8	−0.0002
Laboratory testing			
WBC count on admission, 10^9/L, mean (SD)	8.8 (4.6)	8.9 (5.2)	−0.02
Hemoglobin on admission, g/dL, mean (SD)	12.5 (2.1)	12.6 (2.2)	−0.05
Platelet count on admission, 10^9/L, mean (SD)	230.0 (101.7)	226.2 (102.3)	0.03
Serum creatinine on admission, mg/dL, mean (SD)	1.2 (1.1)	1.3 (1.3)	−0.08
CRP, mg/dL, mean (SD)	11.1 (10.1)	11.3 (9.9)	−0.01
D-dimer, ng/mL, mean (SD)	4.0 (7.1)	4.1 (8.3)	−0.02
AST, U/L, mean (SD)	131.9 (463.9)	135.4 (506.3)	−0.007
ALT, U/L, mean (SD)	80.4 (134.2)	95.7 (187.5)	−0.06
LDH, U/L, mean (SD)	493.3 (652.4)	537.4 (611.5)	−0.06
In-hospital treatment			
Darunavir	0.8	0.7	0.02
Lopinavir/ritonavir	2.0	1.6	0.02
Remdesivir	13.0	11.4	0.04
Hydroxychloroquine	13.8	13.5	0.01
Corticosteroids	58.9	62.3	−0.07
Oral anticoagulants	20.8	19.9	0.02
Heparin	76.9	80.0	−0.07
Antiplatelet treatment	37.2	35.5	0.03
β-lactam antibiotics	48.2	49.3	−0.02
Sulfonamides	3.2	1.9	0.08
Diuretics	49.6	48.8	0.01
Outcome			***P* value**
Primary outcome: 30-d mortality	26.0	27.2	0.694
Risk ratio (95% CI)	0.94 (0.69–1.28)		0.695
Secondary outcome: AHF	22.8	16.6	0.029
Risk ratio (95% CI)	1.48 (1.06–2.06)		0.021

Data are reported as percent or mean (SD) as appropriate, unless otherwise stated. Results stratified by use of azithromycin. AHF indicates acute heart failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CT, computed tomography; LDH, lactate dehydrogenase; and WBC, white blood cell.

Table 4. Inverse Probability of Treatment Weighting: Clinical Factors Associated With Outcomes in Patients Without Prior Cardiovascular Disease

Clinical factor	Azithromycin, N=500	No azithromycin, N=1367	Standardized difference
Female sex	43.8	42.3	0.03
Age, y, mean (SD)	60.6 (16.3)	60.6 (15.9)	0.004
Cardiovascular risk factors			
Diabetes	19.6	19.8	−0.005
Hypertension	51.3	51.2	0.003
Hypercholesterolemia	21.0	20.0	0.02
Current smoking	10.9	10.1	0.02
Former smoking	10.4	11.6	−0.03
Obesity	21.2	21.3	−0.003
Comorbidities			
Chronic kidney disease	5.7	6.8	−0.04
Chronic lung conditions	9.0	9.4	−0.01
Active cancer	13.1	13.6	−0.01
Dementia	6.9	6.8	0.005
Clinical features on admission			
Radiograph/CT signs of interstitial pneumonia	67.2	69.3	−0.04
Laboratory testing			
WBC count on admission, 10^9/L, mean (SD)	8.0 (4.3)	8.3 (7.3)	−0.05
Hemoglobin on admission, g/dL, mean (SD)	13.2 (2.1)	13.2 (2.1)	0.004
Platelet count on admission, 10^9/L, mean (SD)	236.9 (117.5)	234.6 (108.7)	0.02
Serum creatinine on admission, mg/dL, mean (SD)	1.0 (0.9)	1.0 (0.8)	−0.01
CRP, mg/dL, mean (SD)	10.4 (9.1)	10.8 (10.0)	−0.04
D-dimer, ng/mL, mean (SD)	4.0 (10.4)	3.7 (8.7)	0.03
AST, U/L, mean (SD)	75.1 (159.9)	82.9 (208.5)	−0.03
ALT, U/L, mean (SD)	82.7 (150.0)	91.0 (215.2)	−0.04
LDH, U/L, mean (SD)	507.3 (558.6)	528.5 (586.7)	−0.03
In-hospital treatment			
Darunavir	1.4	1.4	−0.002
Lopinavir/ritonavir	2.5	2.9	−0.02
Remdesivir	8.4	9.7	−0.04
Hydroxychloroquine	18.9	18.9	−0.0006
Corticosteroids	62.5	65.8	−0.06
Oral anticoagulants	7.2	5.7	0.06
Heparin	84.2	84.9	−0.02
Antiplatelet treatment	19.5	18.2	0.03
β-lactam antibiotics	47.2	47.0	0.005
Sulfonamides	2.1	1.8	0.02
Diuretics	33.4	35.6	−0.04
Outcome			***P* value**
Primary outcome: 30-d mortality	10.6	17.2	<0.001
Risk ratio (95% CI)	0.57 (0.42–0.79)		<0.001
Secondary outcome: AHF	4.6	3.7	0.433
Risk ratio (95% CI)	1.23 (0.75–2.04)		0.413

Data are reported as (percent) or mean (SD) as appropriate. Results are stratified by use of azithromycin. AHF indicates acute heart failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CT, computed tomography; LDH, lactate dehydrogenase; and WBC, White blood cell.

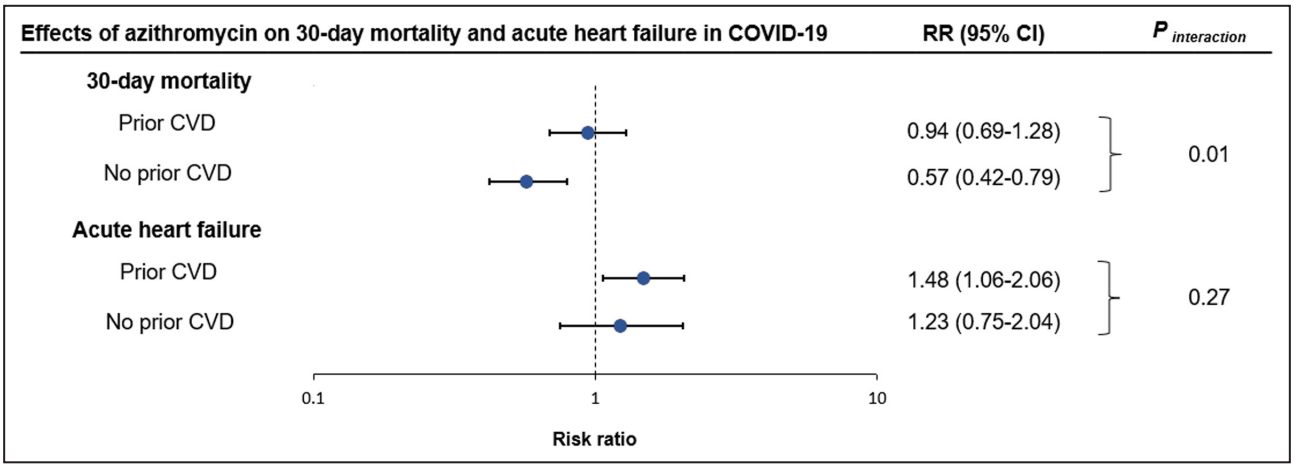

Figure 1. Effects of azithromycin on 30-day mortality and acute heart failure in COVID-19.
CVD indicates cardiovascular disease; and RR, risk ratio.

death from these subgroups were significantly different from each other as assessed by comparing their estimates with interaction tests on the log scale.[22] The magnitude of the interaction between cardiovascular health status and azithromycin therapy and the precision in its estimation suggest that the association we have identified is not a statistical artifact, but rather a clinically relevant finding.

To our knowledge, there are no published data on interactions between CVD status and azithromycin therapy in COVID-19 with which to compare our results, because previous randomized controlled trials of azithromycin therapy did not report results stratified by CVD.[23] Moreover, the study protocol of such trials did not exclude patients with concomitant use of additional antimicrobial agents. Antibiotics can lead to renal injuries, and as such, they can be confounders of the primary outcome of mortality.[24] Finally, the antiviral and anti-inflammatory properties of azithromycin are suited to patients with early- but not advanced-stage disease. Upon high suspicion for infection, administration within the first 24 to 48 hours of azithromycin therapy guarantees the greatest chances for therapeutic success.[25] By contrast, previous trials have enrolled patients nearing ≥8 days since symptom onset, which might not represent the optimal target population.

The above limitations were addressed in the current study. Definition of the standard of care included information on additional antimicrobial treatment. Thus, we may exclude that the widespread use of additional antibiotic therapy might have abrogated some antibacterial benefit of azithromycin interfering with the study power to find a difference. The median duration of symptoms at hospital admission was 3.8 days in

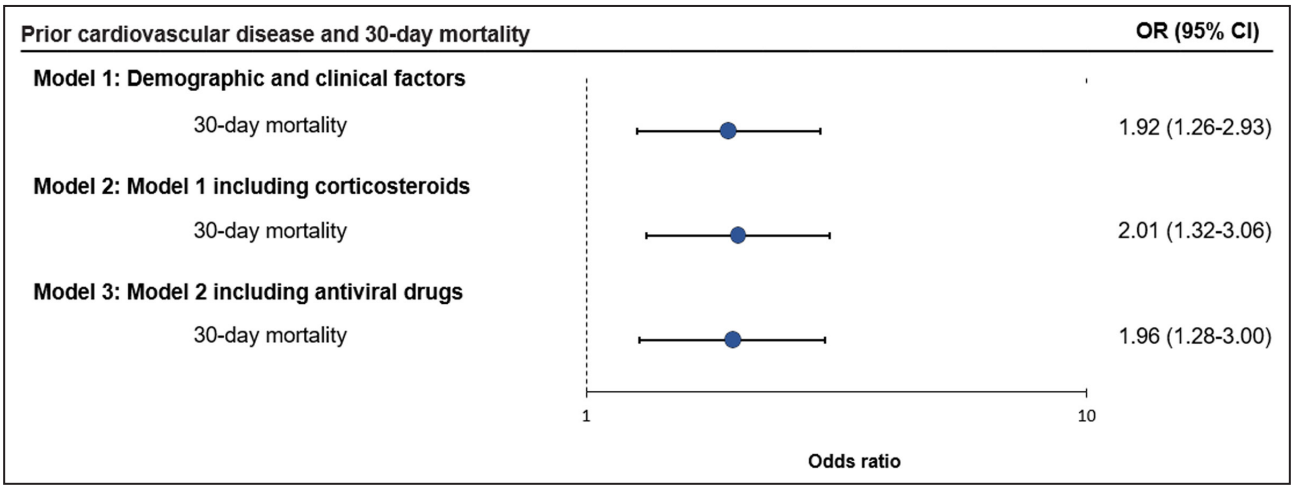

Figure 2. Prior cardiovascular disease and 30-day mortality.
Sequential logistic regression for the effect of prior cardiovascular disease on 30-day mortality. The following covariates are sequentially included in the adjusted models. Adjusted Model 1: demographics, cardiovascular risk factors, comorbidities, and clinical and biochemical features on hospital presentation only. Adjusted Model 2: Model 1 and use of corticosteroids. Adjusted Model 3: Model 2 and use of antiviral agents. OR indicates odds ratio.

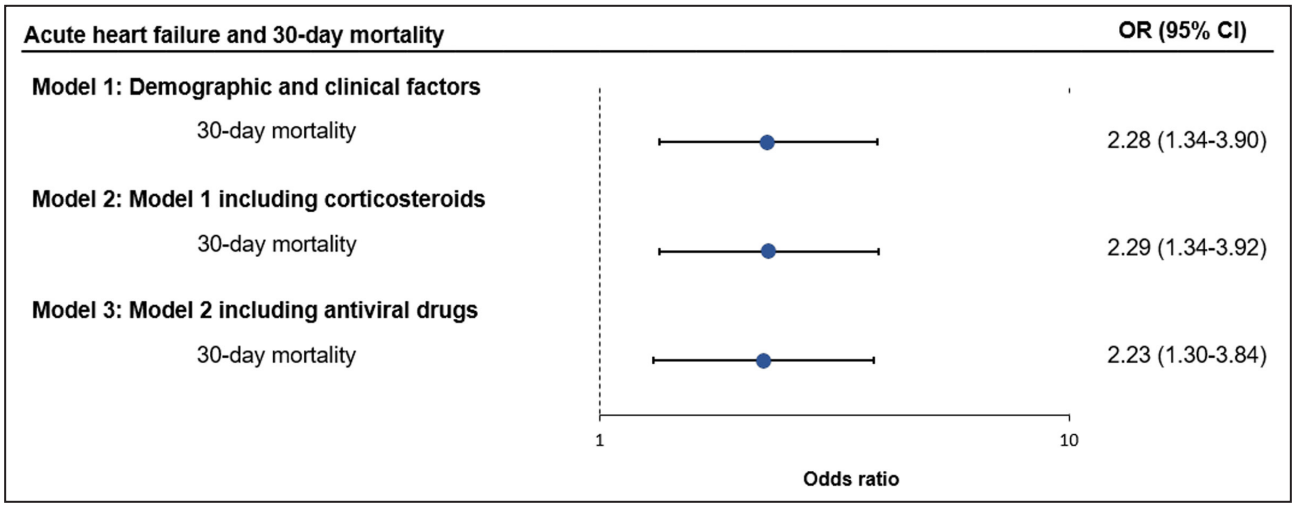

Figure 3. Acute heart failure and 30-day mortality.
Sequential logistic regression for the effect of acute heart failure on 30-day mortality. The following covariates are sequentially included in the adjusted models. Adjusted Model 1: demographics, cardiovascular risk factors, comorbidities, and clinical and biochemical features on hospital presentation only. Adjusted Model 2: Model 1 and use of corticosteroids. Adjusted Model 3: Model 2 and use of antiviral agents. OR indicates odds ratio.

the azithromycin group and 4.9 days in the standard care group. In COVID-19, the viral load is highest in the first 5 days of infection but decreases rapidly thereafter, being undetectable after 8 days.[26] We are therefore confident that our results have validity to exclude that delayed azithromycin therapy may have influenced negative results.

Our study was prompted by the evidence that azithromycin may cause cardiac toxicity in some prior studies on COVID-19–free populations.[11,27] Suggested mechanisms for the increased risk of death among patients receiving azithromycin may involve cardiac arrythmias in the context of QT interval prolongation[28] or an increased incidence of heart failure as a consequence of a metabolic cardiac dysfunction.[29,30] These findings need careful consideration because, despite these clinical observations, the cardiac effects in humans as well as the mechanistic basis for the reported AHF remain poorly defined. Recent experimental work has tried to match azithromycin tested in vitro to the clinical situation. Such studies reported a reduction of the cardiac contractile force in the presence of an azithromycin dosage of 500 mg/d. It is likely that azithromycin acts on multiple ion channels of cardiomyocytes whose inotropic effects can be further reduced by the concurrence of myocardial injury caused by COVID-19.[31] Moreover, the response of the immune system to infection might trigger the development of

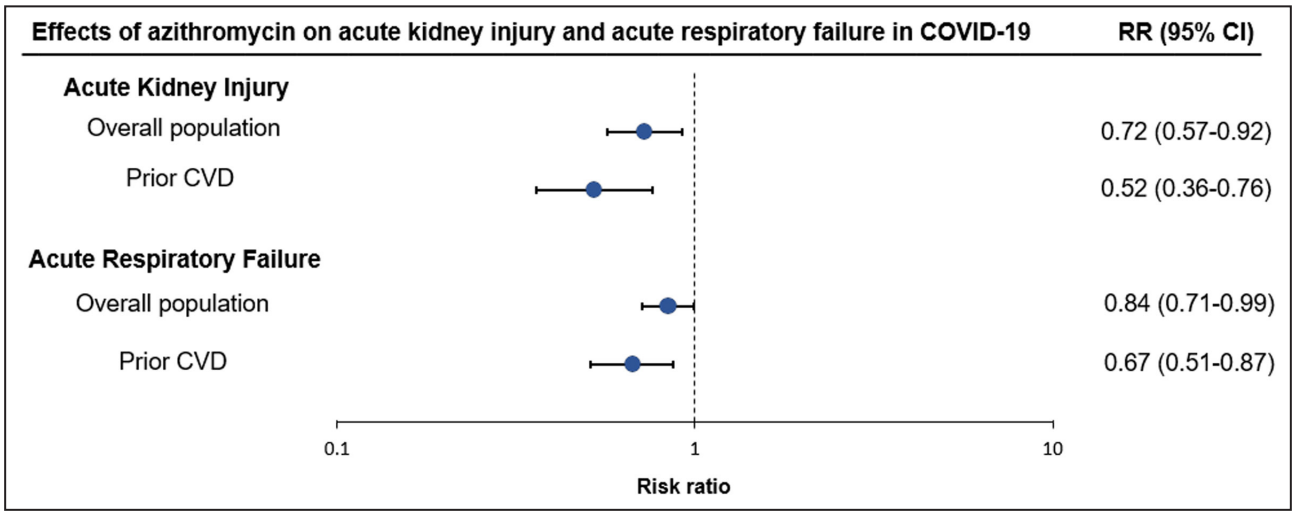

Figure 4. Effects of azithromycin on acute kidney injury and acute respiratory failure in COVID-19.
CVD indicates cardiovascular disease; and RR, risk ratio.

stress-induced cardiomyopathy-associated cardiac dysfunction.[32] Taken together, these findings suggest a kind of interaction between azithromycin and COVID-19 that promotes heart failure. Given that ≈25% of patients hospitalized with COVID-19 develop AHF, the potential contribution of azithromycin to outcomes should be taken into consideration.

The current study tried to address a further issue: Why would azithromycin be associated with an increased risk of cardiac events but similar risk of mortality compared with standard of care in COVID-19? The key point to understand is that some beneficial effects of azithromycin are possible. Numerous clinical trials have proven its efficacy in chronic obstructive pulmonary disease, bronchiectasis, and pneumonia.[13] Recent work has proven its efficacy in major adverse kidney events in patients who are critically ill with sepsis-associated AKI.[33] The present study provided confirmatory evidence of such findings in COVID-19: the rates of AKI and ARF were 28% and 16% lower, respectively, among patients who received azithromycin compared with those who did not, and ARF and AKI are commonly associated with COVID-19.[34] Although there is proof of azithromycin's potential therapeutic efficacy, it is uncertain if this benefit can balance the mortality risk due to the adverse cardiovascular effects. In the current study, the increased risk of AHF in the population as a whole did not persist after restricting the analysis to patients without prior cardiovascular events. The incidence of mortality from COVID-19 in these patients was lower than that observed with standard of care. These findings are therefore consistent with a net clinical benefit associated with azithromycin therapy only when its use is restricted to patients without a history of prior CVD. Yet, it should be reminded that studies on AKI are susceptible to inclusion bias, because there remain unresolved issues with the definition of AKI in its specific correlation with baseline renal function and renal recovery.[35] Moreover, we used the most current classification system for ARF, which is the Berlin consensus definition and its modifications.[17,36] At present, however, a patient's COVID-19–associated respiratory failure is typically classified with a scale that was developed by the World Health Organization, which is largely based on the amount of respiratory support provided.[10] Thus, results from large randomized controlled trials on the effect of azithromycin in patients with no prior CVD, but with AKI or ARF remain the gold standard at this stage of the pandemic.

Some study limitations need to be acknowledged. First, potential confounding and bias by intent to treat cannot be ruled out given the study's observational nature. Inverse probability of treatment weighting and landmark analysis for selection of treatment given on hospital admission have minimized such confounding. Although, at this stage of the pandemic, randomized clinical trials to assess the effectiveness of established drugs in the treatment of COVID-19 and its fatal complications are feasible but difficult; we detected harm to individual patients. As such, azithromycin should not be prioritized as key target of antimicrobial stewardship programs.

Second, all patients in our cohort are White, precluding any assessment of racial variations in response to SARS-CoV-2 infection. Third, not infrequently there is clinical confusion about whether patients have AHF, pneumonia, or both. Given that COVID-19 primarily causes viral pneumonia, the pulmonary edema that is observed in these patients is often regarded as noncardiogenic. We are unable to determine the extent to which these conditions may have been improperly differentiated. Nonetheless, it is unlikely that these potential misclassifications differentially affect results of patients with and without prior CVD and thus are unlikely to modify the outcome differences that we found. Fourth, the virus continues to evolve, and as new variants emerge, the epidemiology of cardiovascular manifestations in COVID-19 might change over time. Although, we were unable to identify the SARS-CoV-2 variants of interest through sequencing, we may attribute the clinical manifestation of COVID-19 reported in our study as mainly related to the Omicron variant surge or its subvariants. Earliest samples of Omicron B.1.1.529 were documented in multiple countries on November 2021. Omicron was still the dominant variant circulating globally, accounting for >98% of viral sequences in February 2022. The current investigation collected data from December 2021 to February 2022.

In conclusion, among patients hospitalized with COVID-19, those with prior CVD had higher AHF and mortality rates than those without CVD. Azithromycin use may be a factor associated with poor outcomes in patients with prior CVD. Prescribers should be aware of the potential association between azithromycin and cardiovascular outcomes.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Data S1
Tables S1–S8
Figure S1
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SUPPLEMENTAL MATERIAL

Data S1.

SUPPLEMENTAL METHODS

The International Survey of Acute Coronary Syndromes (ISACS) COVID-19.

In response to the COVID-19 crisis, ISACS-TC (International Survey of Acute Coronary Syndromes in Transitional Countries; NCT01218776)[37] has promoted a new registry including the existing and additional centres from the same geographic areas to support clinical research to prevent, and treat the COVID-19 illness. (International Survey of Acute Coronavirus Syndromes-COVID-19 [ISACS COVID-19], NCT05188612)

The characteristics of each active enrolling centre are described below.

Characteristics of centers included in ISACS COVID-19, stratified by country

Center name	City	Total capacity	ICU capacity	Center type
Italy				
IRCCS Azienda Ospedaliero-Universitaria di Bologna, St Orsola University Hospital	Bologna	≥450	0-20	Academic Hospital
AOU Policlinico "Gaetano Martino"	Messina	≥450	20-60	Academic Hospital
Macedonia				
University Clinic for infectious diseases	Skopje	0-150	0-20	Academic Hospital
University Clinic for cardiology	Skopje	0-150	0-20	Academic Hospital
PHI Specialised Hospital for Geriatric and Paliative medicine	Skopje	150-300	0-20	Non-Academic Hospital
Institute of Respiratory Diseases in Children - Kozle	Skopje	0-150	0-20	Non-Academic Hospital al
Specialized hospital for prevention, treatment and rehabilitation of cardiovascular diseases	Ohrid	0-150	0-20	Non-Academic Hospital
Serbia				

Hospital Medical Center Bezanijska kosa	Belgrade	150-300	20-60	Academic Hospital
Clinic for Anesthesia, Covid Hospital Batajnica,	Belgrade	≥450	≥60	Non-Academic Hospital
University Clinical Center Nis	Nis	≥450	≥60	Academic Hospital
Institute for Cardiovascular Diseases Dedinje	Belgrade	0-150	20-60	Academic Hospital
Clinical Center of Serbia	Belgrade	≥450	≥60	Academic Hospital
Institute for cardiovascular Diseases Sremska Kamenica	Novi Sad	150-300	20-60	Academic Hospital
Clinical Hospital Center Dragiša Mišović	Belgrade	300-450	20-60	Academic Hospital
Romania				
Emergency Clinical Hospital of Bucharest	Bucharest	≥450	20-60	Academic Hospital
Croatia				
University Hospital Centre Zagreb	Zagreb	≥450	≥60	Academic Hospital
University Hospital Dubrava	Zagreb	≥450	20-60	Academic Hospital

Definition of conventional risk factors and pre-existing comorbidities

Smoking habits were self-reported. We defined current smokers as individuals who smoked 100 cigarettes in his or her lifetime and who smoked cigarettes, cigars, and cigarillos at the time of the index event. Participants who have smoked at least 100 cigarettes in their lifetime but who were not active smokers at the time of the index event were labelled as former smokers regardless of time since they quit.[38] Former smokers were defined as those patients who had a history of tobacco smoking, but were not active smokers at the time of the index event. Hypertension, hypercholesterolemia, and diabetes mellitus were assessed by documentation of medical history prior to admission in the database. Obesity was defined as a BMI ≥ 30.0 kg/m2 according to World Health Organization.[39]. Chronic Kidney disease was defined as a glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 based on the Chronic Kidney Disease Epidemiology Collaboration creatinine equation or need for dialysis.[40]. Active cancer was defined as cancer diagnosed within the previous six months, recurrent,

regionally advanced or metastatic cancer, anti-cancer treatment administered within six months, or haematological cancer with incomplete remission.[41] Diagnosis of dementia was based on clinical evaluation. It required a history of cognitive decline and impairment in daily activities, with corroboration from a close friend or family member, and a mental status examination by a clinician to delineate impairments in memory, language, attention, visuospatial cognition, executive function, and mood.[42] The types of chronic lung conditions that were diagnosed in our population included exclusively asthma and chronic obstructive pulmonary disease.

Data on laboratory values

All participants underwent venous blood sampling on hospital admission. Reference values are reported below.

Reference values for laboratory testing

	Reference values
Laboratory findings on hospital admission	
Leukocyte count, (10^9/L)	4.0-11.0
Hemoglobin, (g/dL)	Male=13.5 - 17.2
	Female=11.8 – 15.8
Platelet count, (10^9/L)	160 - 370
Serum creatinine levels, (mg/dL)	0.50 – 1.20
Peak laboratory findings during hospitalization	
C-reactive protein, (mg/dL)	<0.5
Aspartate aminotransferase, (U/L)	Male <50, Female <35
Alanine aminotransferase, (U/L)	Male <50, Female <35
Lactate dehydrogenase, (U/L)	<248
D-dimer, (ng/mL)	<0.55

Multiple Imputation using Chained Equation (MICE) algorithm

Multiple Imputation using Chained Equation (MICE) algorithm is an efficient and popular method to fill in missing data where each missing value on some records is replaced by a value obtained from

related cases in the whole set of records. Thus, imputation for clinical features was conducted using the chained equations across other features.[19] More specifically, MICE algorithm sequentially imputes the missing values of clinical features based on both observed values and previously imputed values. This sequential imputation is conducted via chained equations.

We tried multiple imputations using the MICE algorithm for the initial analyses to address the uncertainty in the imputation process. More specifically, we generated multiple imputed datasets and check whether the conclusions are consistent across the different imputed datasets. If the conclusions are consistent across multiple imputed datasets, we use a single imputed dataset (by MICE algorithm) as the final dataset to report the results of statistical analyses in the paper.

Inverse Propensity Score Weighting Analysis

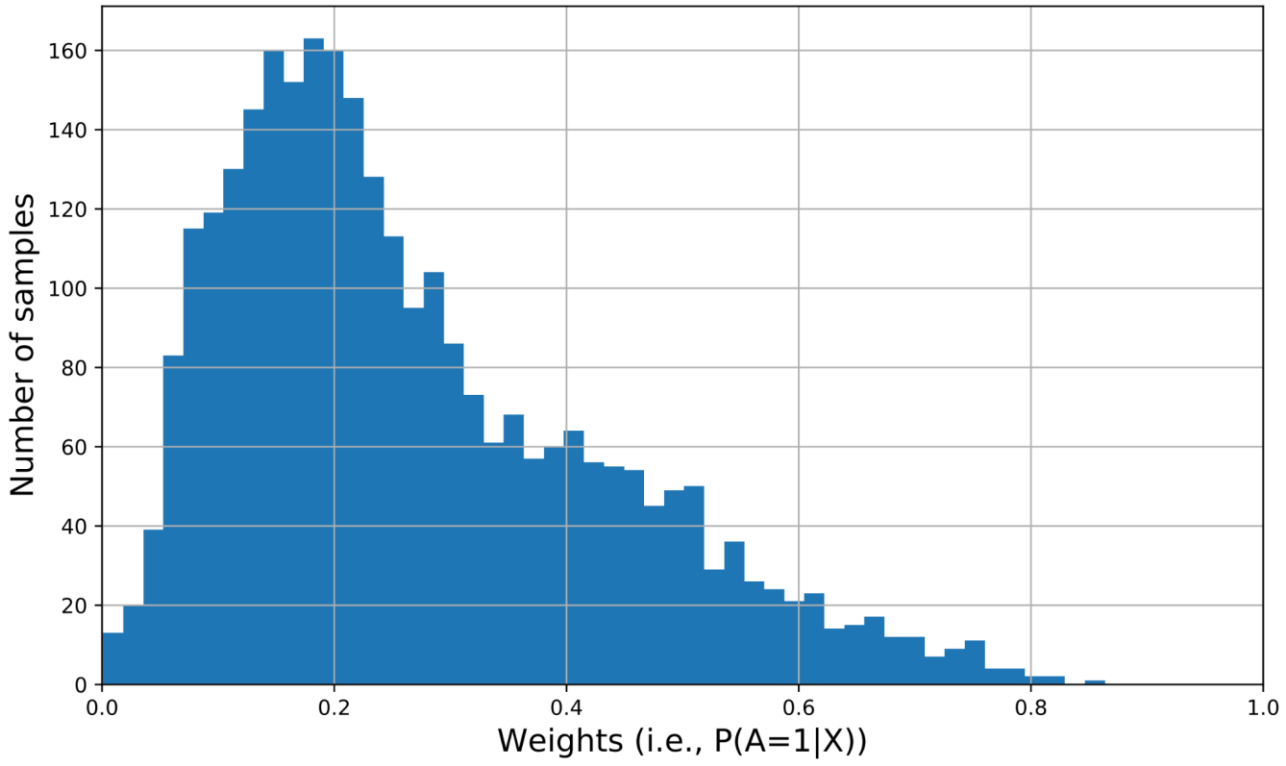
We used Inverse Propensity Score Weighting (IPW) to balance the distribution of covariates between two patient groups. Note that we use Logistic Regression to estimate the propensity scores ({P}(Z=1 | x)) If *e* denotes the estimated propensity score (i.e. e=\hat{P}(Z=1 | x), where the patient x is included in patient group 1; then, 1-e = \hat{P}(Z=0 | x)), then the original sample is weighted by the following weights: Z/e+(1−Z)/ 1−e where Z represents the patient group. For instance, women (Z=1) are assigned a weight equal to the reciprocal of the propensity score (1/e), while men (Z=0) are assigned a weight equal to the reciprocal of one minus the propensity score (1/1-e). The weighting procedure for each sample balances the covariate distributions between two patient groups.[20]

In details, we computed the propensity scores using logistic regression: (i) coefficients of the terms were used, (ii) we did not use the interaction terms between variables, (iii), we checked that the distributions of each feature were well distributed between two groups after inverse propensity score weighting using standardized differences.

Coefficients of terms used in the propensity score estimation	
Intercept	-1.2251
Female sex	-0.0182
Age, mean (SD)	0.0075
Cardiovascular risk factors	
Diabetes	0.1927
Hypertension	-0.1072
Hypercholesterolemia	0.1835
Current smoking	0.1686
Former smoking	0.2187
Obesity	0.0956
Comorbidities	
Chronic kidney disease	0.3288
Chronic lung conditions	0.1004
Active cancer	0.0480
Dementia	0.0837
Clinical features on admission	
X-ray/ CT signs of interstitial pneumonia	-0.2536
WBC count on admission, 10^9/L [mean (SD)]	-0.0269
Hb on admission, g/dL [mean (SD)]	-0.0227
Platelet count on admission, 10^9/L [mean (SD)]	0.0008
Serum creatinine on admission, mg/dL [mean (SD)]	-0.1404
CRP, mg/dL [mean (SD)]	-0.0087
D-dimer, ng/mL [mean (SD)]	0.0067
AST, U/L [mean (SD)]	0.0003
ALT, U/L [mean (SD)]	-0.0007

LDH, U/L [mean (SD)]	-0.0003
In-hospital treatment	
Darunavir	0.0804
Lopinavir/Ritonavir	-0.7288
Remdesivir	-0.1317
Hydroxychloroquine	1.0842
Corticosteroids	0.0255
Oral anticoagulants	-0.2066
Heparin	0.1816
Antiplatelet treatment	-0.5033
β-lactam antibiotics	0.9770
Sulfonamides	-0.2771
Diuretics	-0.7265

The weight distributions (P(A=1|X)) is described below in terms of the histogram



Inverse probability of treatment weighting method can potentially result in unstable and biased estimates if some of the weights are very high. To avoid excessive weights, we compared results with other methods for handling confounding. We included probability of treatment variables in a multivariable model. We also used XGBoost, a decision-tree-based ensemble machine learning algorithm, as an alternative multivariable model for estimating the probability of treatment. Conclusions from theses analyses were the same as our current results. Further, we created a threshold for weights to avoid the impacts of the outliers (we use 0.01 as threshold). Therefore, the inverse probability of treatment weighting analyses presented in the current analysis were quite stable.

Computation of Relative Risk and its Confidence Interval

In a two-group cohort study, the risk ratio (RR, also called relative risk), is usually applied to compare risks of a health event between two independent binomial populations that differ by a demographic characteristic (i.e. sex, age) or by the level of exposure to a specific drug or risk factor. In such types of studies, data can be summarized in a confusion matrix as follows:

	Risk of Designated Outcome		
	Yes	**No**	**Total**
Exposed	a	b	a+b (H_1)
Unexposed	c	d	c+d (H_0)
Total	a+c	b+d	

Where H_1 and H_0 correspond to the total number of exposed and unexposed patients, respectively, whereas a and c represent the number of exposed and unexposed patients at risk for the designated outcome, respectively.

RR is defined as the ratio between the risk of outcome in exposed patients (H_1) and the risk of outcome in unexposed patients ($H_{0,}$) which can be summarized as:

$$RR = \frac{\left({}^{a}/_{H_1}\right)}{\left({}^{c}/_{H_0}\right)}$$

When applying this equation to an IPTW balanced population, ${}^{a}/_{H_1}$ will be assigned a weight equal to the reciprocal of the propensity score $\left(\frac{1}{e}\right)$ and ${}^{c}/_{H_0}$ will be weighted by the reciprocal of one minus the propensity score ($\frac{1}{(1-e)}$).

In order to compute the lower and upper (1-α) confidence limit RR_L for RR, we operate in the assumption of log normal distribution.[43] In particular, the variate $\log\frac{\left({}^{a}/_{H_1}\right)}{\left({}^{c}/_{H_0}\right)} = \log {}^{a}/_{H_1} - \log {}^{c}/_{H_0}$ is approximately normally distributed with approximate mean log(RR) and estimated variance $\frac{1-\left({}^{a}/_{H_1}\right)}{a} + \frac{1-({}^{c}/_{H_0})}{c}$.

It follows that RR_L can be computed by solving the following equation:

$$\frac{\left[\log(\frac{{}^{a}/_{H_1}}{{}^{c}/_{H_0}}) - \log(RR_L)\right]}{\left[\frac{1-\left({}^{a}/_{H_1}\right)}{a} + \frac{1-({}^{c}/_{H_0})}{c}\right]^{1/2}} = z_{1-\alpha}$$

Where $z_{1-\alpha}$, is the 100(1-α) percentage point of the N(O, 1) distribution

Comparison of means and prevalences in the weighted sample

To evaluate the balance of the baseline covariate distributions between treatment and control groups, standardized difference (SD) is widely used in inverse probability of treatment weighting (IPTW) framework. For the baseline analysis, we use standard SD which is defined as follows: $\frac{m_t - m_c}{\sqrt{\frac{s_t^2 + s_c^2}{2}}}$ for continuous variables and $\frac{m_t - m_c}{\sqrt{\frac{m_t(1-m_t)+m_c(1-m_c)}{2}}}$ for binary variable where m_t, m_c are sample mean of the

variables for treatment and control group, and s_t^2, s_c^2 are sample variance of the variables for treatment and control group, respectively. For IPTW analysis, we use weighted SD where m_t, m_c are replaced to weighted sample mean of the variables for treatment and control group, and s_t^2, s_c^2 are replaced to weighted sample variance of the variables for treatment and control group, respectively. Weights are determined by the inverse probability of treatment received. In general, 0.1 is the reasonable threshold to determine whether two distributions are balanced (i.e., if SD > 0.1, the baseline covariate is imbalanced).[21]

Interaction test

The comparison of two estimated quantities, each with its standard error, is a general method that can be applied widely. We compared the risk ratios of primary and secondary outcomes from subgroups stratified by use of azithromycin. These measures were always analyzed on the log scale because the distributions of the log ratios tend to be closer to normal than of the ratios themselves. If the estimates are *E*1 and *E*2 with standard errors SE(*E*1) and SE(*E*2), then the difference *d*=*E*1 - *E*2 has standard error SE(*d*)=Ö[SE(*E*1)2 + SE(*E*2)2] i.e., the square root of the sum of the squares of the separate standard errors. The ratio *z*=*d*/SE(*d*) gives a test of the null hypothesis that in the population the difference *d* is zero, by comparing the value of *z* to the standard normal distribution. The 95% confidence interval (CI) for the difference is *d*-1.96SE(*d*) to *d*+1.96SE(*d*).[11] Bland and Altman are explicit in explaining that the method they describe only applies to comparisons of two independent estimates.[22] As documented in our interaction test results, the two groups are "disjoint" and each estimate (both mean and confidence interval of RR) is independently computed. For example, as can be observed in **Table S5**, Group 1 is represented by patients with preexisting cardiovascular disease and Group 2 by patients without preexisting cardiovascular disease. The two groups are completely disjoint and there are no common individuals. Furthermore, the mean and confidence interval of RR for each group was computed independently as shown in Table 3 and Table 4. Therefore, those are

not relied on the same covariate adjustment. As such, the two estimates were independent as required by the interaction test proposed by Bland and Altman.

SUPPLEMENTAL RESULTS

Interaction tests

We tested **(Table S5)** whether there is a significant interaction between risk ratios (azithromycin users versus non-users) for 30-day mortality derived from separate analyses: patients with and without preexisting cardiovascular disease. We obtained the logs of the risk ratios and their confidence intervals (rows 2 and 4). As 95% confidence intervals were obtained as 1.96 standard errors (SE) either side of the estimate, the SE of each log relative risk was obtained by dividing the width of its confidence interval by 2×1.96 (row 6). The estimated difference in log relative risks was *d*=E1- E2= 0.50 (row 7) and its standard error 0.23 (row 8). From these two values, we tested the interaction and estimated the ratio of the relative risks (with confidence interval). The test of interaction was the ratio of *d* to its standard error: z= 2.22, which gave a P value 0.01 when we referred it to a table of the normal distribution (row 10). The estimated interaction effect was exp =1.65 (row 11). The confidence interval for this effect was 0.06 to 0.94 on the log scale (row 9). Transforming back to the relative risk scale, we got 1.06 to 2.57 (row 12). We repeated the interaction test for the outcomes of acute heart failure **(Table S6).**

Table S1. Outcomes stratified by use of azithromycin

Outcome	Azithromycin N=793	No Azithromycin N=2,141	Standardized difference
Primary outcome: 30-day mortality, n (%)	134 (16.9)	483 (22.6)	-0.1426
Risk Ratio (95% CI)	0.70 (0.56 - 0.86)		-0.1426
Secondary outcome: AHF, n (%)	68 (8.6)	185 (8.6)	-0.0023
Risk Ratio (95% CI)	0.99 (0.74 - 1.33)		-0.0023

Table S2. Outcomes stratified by use of azithromycin; patients with prior cardiovascular disease

Outcome	Azithromycin N=292	No Azithromycin N=774	Standardized difference
Primary outcome: 30-day mortality, n (%)	77 (26.4)	228 (29.5)	-0.0689
Risk Ratio (95% CI)	0.86 (0.63 - 1.16)		-0.0689
Secondary outcome: AHF, n (%)	48 (16.4)	134 (17.3)	-0.0233
Risk Ratio (95% CI)	0.94 (0.65 - 1.35)		-0.0233

Table S3. Outcomes stratified by use of azithromycin; patients without prior cardiovascular disease

Outcome	Azithromycin N=501	No Azithromycin N=1,367	Standardized difference
Primary outcome: 30-day mortality, n (%)	57 (11.4)	255 (18.7)	-0.2048
Risk Ratio (95% CI)	0.56 (0.41 - 0.76)		-0.2048
Secondary outcome: AHF, n (%)	20 (4.0)	51 (3.7)	0.0136
Risk Ratio (95% CI)	1.07 (0.63 - 1.81)		0.0136

Table S4. Inverse probability of treatment weighting: clinical factors associated with outcomes. Results stratified by use of azithromycin or absence of antibiotic treatment

	Azithromycin N=793	**No Antibiotics N=775**	**Standardized difference**
Female sex	44.1	45.2	-0.02
Age, mean (SD)	64.8 (16.0)	64.8 (16.3)	0.005
Cardiovascular risk factors			
Diabetes	24.9	24.8	0.002
Hypertension	62.0	62.2	-0.005
Hypercholesterolemia	32.5	32.0	0.01
Current smoking	9.6	9.6	-0.0008
Former smoking	15.4	15.3	0.002
Obesity	21.6	21.4	0.003
Comorbidities			
Chronic kidney disease	12.9	12.0	0.02
Chronic lung conditions	10.8	10.7	0.003
Active cancer	14.4	14.7	-0.008
Dementia	11.9	12.0	-0.003
Clinical features on admission			
X-ray/CT signs of interstitial pneumonia	54.5	54.0	0.01
Lab testing			
WBC count on admission, 10^9/L [mean (SD)]	8.3 (4.59)	8.3 (6.4)	-0.01
Hb on admission, g/dL [mean (SD)]	12.8 (2.2)	12.9 (2.2)	-0.04
Platelet count on admission, 10^9/L [mean (SD)]	229.6 (114.3)	234.5 (103.3)	-0.04
Serum creatinine on admission, mg/dL [mean (SD)]	1.3 (1.5)	1.1 (1.1)	0.05
CRP, mg/dL [mean (SD)]	9.4 (8.7)	9.0 (8.6)	0.04
D-dimer, ng/mL [mean (SD)]	3.4 (7.6)	3.4 (8.2)	-0.007
AST, U/L [mean (SD)]	90.9 (285.6)	94.7 (305.3)	-0.01
ALT, U/L [mean (SD)]	76.1 (150.8)	78.9 (191.6)	-0.01
LDH, U/L [mean (SD)]	447.2 (543.5)	445.6 (474.5)	0.003
In-hospital treatment			
Darunavir	0.8	0.3	0.06

Lopinavir/Ritonavir	1.7	1.9	-0.01
Remdesivir	12.7	10.9	0.05
Hydroxychloroquine	18.2	16.2	0.05
Corticosteroids	56.6	55.5	0.02
Oral anticoagulants	11.1	11.3	-0.006
Heparin	77.7	78.1	-0.007
Antiplatelet treatment	21.4	21.9	-0.01
Diuretics	27.0	26.7	0.005
Outcomes			**P value**
Primary outcome: 30-day mortality	17.1	16.9	0.903
Risk Ratio (95% CI)	1.02 (0.78 – 1.32)		0.903
Secondary outcome: AHF	10.0	6.7	0.019
Risk Ratio (95% CI)	1.54 (1.07 – 2.22)		0.020

Data are reported as % or mean (SD), unless otherwise stated.

Abbreviations: AHF=Acute heart failure; ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; CRP= C-reactive protein; CT=computed tomography; Hb= Hemoglobin; LDH= Lactate dehydrogenase; WBC=White blood cells

Table S5. Interaction test: calculations for comparing two estimated risk ratios (Azithromycin users versus non-users) for 30-day mortality using inverse probability of treatment weighting: preexisting cardiovascular disease vs no prior cardiovascular disease

		Group 1 **[Pre-existing cardiovascular disease]** **(Patients n = 1,064)**	**Group 2** **[No prior cardiovascular disease]** **(Patients n = 1,867)**
1	**RR**	0.94	0.57
2	**log RR**	-0.06	-0.56
3	**95% CI for RR**	0.69 – 1.28	0.42 – 0.79
4	**95% CI for log RR**	-0.37-0.25	-0.87-(-0.24)
5	**Width of CI**	0.62	0.63
6	**SE (=width / (2*1.96))**	0.16	0.16
	Difference between log risk ratios		
7	**d (=$E_1 - E_2$)**	0.50	
8	**SE (d)**	0.23	
9	**CI (d)**	0.06-0.94	
10	**Test of Interaction**	2.22 **(P value: 0.01)**	
	Ratio of risk ratios		
11	**RRR (=exp(d))**	1.65	
12	**CI (RRR)**	1.06-2.57	

Table S6. Interaction test: calculations for comparing two estimated risk ratios (Azithromycin users versus non-users) for acute heart failure using inverse probability of treatment weighting: preexisting cardiovascular disease vs no prior cardiovascular disease

		Group 1 **[Pre-existing cardiovascular disease]** **(Patients n = 1,064)**	**Group 2** **[No prior cardiovascular disease]** **(Patients n = 1,867)**
1	**RR**	1.48	1.23
2	**log RR**	0.39	0.21
3	**95% CI for RR**	1.06 – 2.06	0.75 – 2.04
4	**95% CI for log RR**	0.06-0.72	-0.29-0.71
5	**Width of CI**	0.66	1
6	**SE (=width / (2*1.96))**	0.17	0.26
	Difference between log risk ratios		
7	**d (=$E_1 - E_2$)**	0.19	
8	**SE (d)**	0.31	
9	**CI (d)**	-0.42-0.79	
10	**Test of Interaction**	0.60 **(P value: 0.27)**	
	Ratio of risk ratios		
11	**RRR (=exp(d))**	1.20	
12	**CI (RRR)**	0.66-2.19	

Table S7. Inverse probability of treatment weighting: acute respiratory failure and acute kidney injury in the overall population stratified by use of azithromycin.

	Azithromycin N=792	**No Azithromycin N=2,141**	**Standardized difference**
Female sex	42.5	43.0	-0.01
Age, mean (SD)	65.0 (15.6)	64.8 (15.9)	0.01
Cardiovascular risk factors			
Diabetes	25.5	25.5	-0.001
Hypertension	64.7	62.7	0.04
Hypercholesterolemia	31.5	29.0	0.05
Current smoking	10.4	9.7	0.02
Former smoking	14.0	15.0	-0.03
Obesity	23.4	23.2	0.01
Comorbidities			
Chronic kidney disease	13.8	12.4	0.04
Chronic lung conditions	11.7	12.1	-0.01
Active cancer	14.3	14.3	0.002
Dementia	10.2	11.0	-0.03
Clinical features on admission			
X-ray/ CT signs of interstitial pneumonia	65.2	66.4	-0.02
Lab testing			
WBC count on admission, 10^9/L [mean (SD)]	8.4 (4.4)	8.5 (6.6)	-0.02
Hb on admission, g/dL [mean (SD)]	13.0 (2.1)	13.0 (2.1)	-0.02
Platelet count on admission, 10^9/L [mean (SD)]	231.5 (113.9)	231.7 (106.7)	-0.002
Serum creatinine on admission, mg/dL [mean (SD)]	1.3 (1.5)	1.2 (1.0)	0.07
CRP, mg/dL [mean (SD)]	11.1 (9.9)	11.0 (10.0)	0.01
D-dimer, ng/mL [mean (SD)]	4.0 (9.8)	3.9 (8.5)	0.02
AST, U/L [mean (SD)]	97.6 (271.9)	107.9 (370.9)	-0.03
ALT, U/L [mean (SD)]	80.9 (118.7)	87.9 (267.3)	-0.05

LDH, U/L [mean (SD)]	500.9 (533.2)	532.1 (596.1)	-0.06
In-hospital treatment			
Darunavir	1.2	1.0	0.02
Lopinavir/Ritonavir	2.6	2.4	0.01
Remdesivir	9.9	10.3	-0.01
Hydroxychloroquine	16.8	16.9	-0.002
Corticosteroids	62.6	64.5	-0.04
Oral anticoagulants	11.6	10.8	0.03
Heparin	81.5	83.1	-0.04
Antiplatelet treatment	24.9	21.7	0.08
β lactam antibiotics	45.5	47.8	-0.05
Sulfonamides	2.5	1.8	0.05
Diuretics	40.9	40.4	0.01
Outcome			**P value**
Secondary outcome: ARF	48.1	52.4	0.040
Risk Ratio (95% CI)	0.84 (0.71 – 0.99)		0.040
Secondary outcome: AKI	13.1	17.3	0.004
Risk Ratio (95% CI)	0.72 (0.57 – 0.92)		0.010

Data are reported as % or mean (SD), unless otherwise stated.

Abbreviations: AKI=Acute kidney injury; ALT=Alanine aminotransferase; ARF=Acute respiratory failure; AST=Aspartate aminotransferase; CRP=C-reactive protein; CT=computed tomography; Hb= Hemoglobin; LDH= Lactate dehydrogenase; WBC=White blood cells

Table S8. Inverse probability of treatment weighting: acute respiratory failure and acute kidney injury in patients with preexisting cardiovascular disease stratified by use of azithromycin.

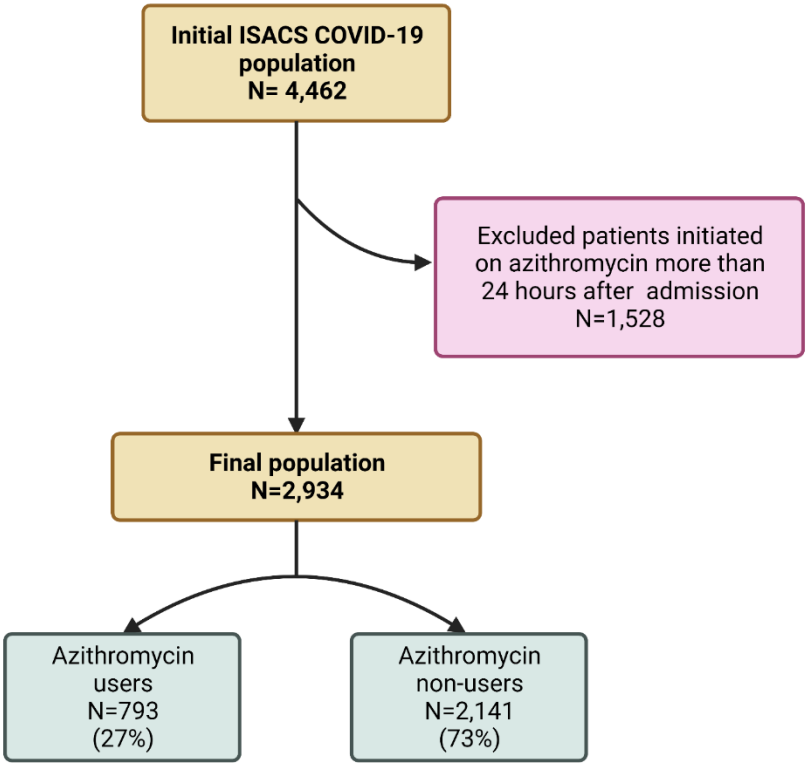
	Azithromycin N=290	**No Azithromycin N=774**	**Standardized difference**
Female sex	42.9	43.3	-0.008
Age, mean (SD)	71.3 (11.9)	72.4 (11.8)	-0.05
Cardiovascular risk factors			
Diabetes	36.0	35.8	0.005
Hypertension	84.7	83.4	0.03
Hypercholesterolemia	48.8	45.5	0.06
Current smoking	9.0	9.3	-0.008
Former smoking	20.7	21.2	-0.01
Obesity	28.7	27.2	0.03
Comorbidities			
Chronic kidney disease	21.3	22.6	-0.03
Chronic lung conditions	19.3	17.2	0.05
Active cancer	15.9	14.9	0.02
Dementia	15.4	18.5	-0.08
Clinical features on admission			
X-ray/CT signs of interstitial pneumonia	61.8	61.8	-0.0002
Lab testing			
WBC count on admission, 10^9/L [mean (SD)]	8.8 (4.6)	8.9 (5.2)	-0.02
Hb on admission, g/dL [mean (SD)]	12.5 (2.1)	12.6 (2.2)	-0.05
Platelet count on admission, 10^9/L [mean (SD)]	230.0 (101.7)	226.2 (102.3)	0.03
Serum creatinine on admission, mg/dL [mean (SD)]	1.2 (1.1)	1.3 (1.3)	-0.08
CRP, mg/dL [mean (SD)]	11.1 (10.1)	11.3 (9.9)	-0.01
D-dimer, ng/mL [mean (SD)]	4.0 (7.1)	4.1 (8.3)	-0.02
AST, U/L [mean (SD)]	131.9 (463.9)	135.4 (506.3)	-0.007
ALT, U/L [mean (SD)]	80.4 (134.2)	95.7 (187.5)	-0.06
LDH, U/L [mean (SD)]	493.3 (652.4)	537.4 (611.5)	-0.06

In-hospital treatment			
Darunavir	0.8	0.7	0.02
Lopinavir/Ritonavir	2.0	1.6	0.02
Remdesivir	13.0	11.4	0.04
Hydroxychloroquine	13.8	13.5	0.01
Corticosteroids	58.9	62.3	-0.07
Oral anticoagulants	20.8	19.9	0.02
Heparin	76.9	80.0	-0.07
Antiplatelet treatment	37.2	35.5	0.03
β lactam antibiotics	48.2	49.3	-0.02
Sulfonamides	3.2	1.9	0.08
Diuretics	49.6	48.8	0.01
Outcome			**P value**
Secondary outcome: ARF	47.6	57.7	0.003
Risk Ratio (95% CI)	0.67 (0.51 – 0.87)		0.003
Secondary outcome: AKI	13.6	23.2	<0.001
Risk Ratio (95% CI)	0.52 (0.36 – 0.76)		<0.001

Data are reported as % or mean (SD), unless otherwise stated.

Abbreviations: AKI=Acute kidney injury; ALT=Alanine aminotransferase; ARF=Acute respiratory failure; AST=Aspartate aminotransferase; CRP=C-reactive protein; CT=computed tomography; Hb= Hemoglobin; LDH= Lactate dehydrogenase; WBC=White blood cells.

Figure S1. Study flow chart.



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