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The evolution in pharmacology of infectious diseases 2022

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The evolution in pharmacology of infectious diseases: 2022

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Editorial: The evolution in pharmacology of infectious diseases: 2022

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Editorial on the Research Topic
The evolution in pharmacology of infectious diseases: 2022

1 The landscape of infectious diseases post-pandemic: a pharmacological perspective

The era post-2020 has marked a defining moment in the evolution of pharmacology, with a particular emphasis on infectious diseases (Baker et al., 2022). This period has been characterized by the relentless global campaign against COVID-19 and the emergence of a spectrum of other infectious pathogens (Tabish, 2020). It was during this time that the field of pharmacology experienced an unprecedented amalgamation of global research endeavors and the development of innovative therapeutic solutions. The research conducted in these years has not only paved the way for groundbreaking therapeutic strategies but has also illustrated the extraordinary capacity for resilience and innovation within the scientific community (Rijs and Fenter, 2020). These advancements, born out of necessity in the face of global health crises (Betz et al., 2023), have reshaped the landscape of infectious disease treatment and prevention.

This editorial offers an insightful examination and analysis of the significant advancements in the field of infectious diseases from 2022 to 2023, specifically focusing on how these developments have transformed our approaches to combating such diseases. At the heart of this exploration is an overview of 15 pivotal articles featured in our Research Topic. These articles, contributed by 120 distinguished experts worldwide, represent a critical blend of knowledge, combining a range of expert insights and discoveries. This collective wisdom is crucial for developing innovative strategies to treat infectious diseases and significantly enhances our methods for addressing global health challenges. Additionally, this editorial delves into the interplay between pathogens and recent advances in drug development, highlighting how this period has served as both a rigorous test of scientific resolve and a catalyst for notable progress in medical science. By exploring the dynamic relationship between emerging diseases and pharmacological innovations, it underscores how this era has been challenging yet instrumental in driving substantial advancements in the evolution of the medical field.

2 COVID-19 therapeutic developments: key research findings and innovations

In the ongoing struggle against COVID-19, the scientific community has been unwavering in its quest to uncover new therapeutic avenues. Umakanthan et al. made a significant contribution with their study on the unexpected benefits of Statins, commonly used for lipid management, in reducing mortality among hospitalized COVID-19 patients. This groundbreaking finding not only highlights the anti-inflammatory and immunomodulatory properties of statins but also opens new avenues for COVID-19 treatment, suggesting a broader application of these drugs beyond their traditional use. Complementing this, Barati et al.'s research on the combination of Noscapine and Licorice for cough relief in COVID-19 outpatients indicated a slight superiority over traditional treatments. The study is particularly pertinent as it addresses the symptomatic burden experienced by COVID-19 patients and emphasizes the importance of effective symptom management strategies in viral infections. It signifies the potential of integrating traditional remedies with modern pharmacological practices, a theme that resonates throughout our Research Topic. For instance, Maen et al.'s study on Thymoquinone formulation (NP-101) against SARS-CoV-2 revealed its potential as a novel treatment, pointing to new directions in antiviral therapy. This finding not only contributes to the ongoing efforts against COVID-19 but also underscores the importance of exploring natural compounds in drug development.

The exploration of combination therapy as a strategy for treating COVID-19 has gained traction, as highlighted by Akinbodale et al. (2022). This approach involves the concurrent use of multiple medications, targeting the virus from various perspectives to potentially enhance patient outcomes. A notable study by Dastan et al. examined the combined use of Tocilizumab, a biologic drug approved for treating moderate to severe rheumatoid arthritis in adults, and Baricitinib, an immunomodulatory medication for rheumatoid arthritis. Their research, focusing on severe COVID-19 cases, revealed that while this combination did not significantly reduce mortality rates, it was associated with a reduced necessity for Intensive Care Unit (ICU) admission. This outcome underscores the potential benefits of such combination therapies in certain patient groups and clinical settings. It also emphasizes the growing importance of personalized medicine in effectively managing COVID-19.

In a real-world study, Zhong et al. evaluated the efficacy and safety of Nirmatrelvir/Ritonavir co-administration in patients with rheumatic disease infected with SARS-CoV-2. This study found that using Nirmatrelvir/Ritonavir as part of standard or early treatment regimens led to a shorter time for symptom resolution compared to a control group. Even when this combination of drugs was administered after 5 days of symptom onset, it still offered benefits for rheumatic patients. The study highlighted the importance of early Nirmatrelvir/Ritonavir utilization and following the recommended regimen, showing favorable outcomes and an acceptable safety profile for immunosuppressed rheumatic patients. In addition, Wei et al.'s comparative analysis of Azvudine, originally used for HIV-1, and Nirmatrelvir/Ritonavir concluded that Azvudine offered similar safety but slightly better clinical benefits in hospitalized patients. This finding adds to the growing body of literature on effective COVID-19 treatments and highlights the importance of continual evaluation and comparison of therapeutic options in the rapidly evolving landscape of the pandemic. In a similar vein, Zhu's perspective on Azvudine, showcased its effectiveness in treating moderate COVID-19 cases. This work exemplifies the potential of drug repurposing in the pandemic era, providing a cost-effective solution to the global health crisis. It also underscores the importance of adaptability in pharmaceutical research, as existing drugs can be re-evaluated and repurposed to meet emerging health challenges.

Finally, the investigation into the cardiac impacts of Remdesivir by Hajimoradi et al. shed light on the complexities of COVID-19 treatment, particularly the incidence of sinus bradycardia in patients treated with this antiviral drug. This finding highlights the need for comprehensive monitoring during treatment, underscoring the multifaceted nature of COVID-19 and the importance of considering the broader implications of antiviral therapies.

3 Innovations beyond COVID-19

Moving beyond COVID-19, these years also witnessed significant advancements in the treatment of other infectious diseases. For instance, El-Mahdy et al.'s study on chronic hepatitis C patients treated with direct-acting antivirals showed marked improvements in liver function and antioxidant profiles. This work marks a milestone in the management of this chronic infection and highlights the progress made in understanding and treating hepatitis C.

In the realm of cystic fibrosis, a debilitating genetic disease, Rakhshan et al.'s evaluation of inhaled Amikacin as an adjunct therapy in treating *Pseudomonas aeruginosa* exacerbations represents a significant step forward. This clinical trial not only highlights innovation in treatment strategies but also emphasizes the importance of addressing the specific needs of patients with cystic fibrosis.

Drug repurposing continued to be a key theme in the research landscape, as exemplified by Porta et al.'s review on Chagas disease. This neglected tropical disease, caused by the protozoan parasite *Trypanosoma cruzi*, has been a longstanding public health challenge, particularly in Latin America. The review emphasized the urgent need for new treatment strategies and highlighted the potential of repurposing existing drugs as a cost-effective and expedient approach to address this issue. In the field of cutaneous leishmaniasis, another neglected tropical disease, Hakamifard et al.'s clinical trial on liposomal Clarithromycin, an antibiotic, combined with the antileishmanial agent Glucantime suggested a significant effect in reducing lesion size. This clinical trial is particularly relevant for regions where leishmaniasis remains a significant public health issue and where effective treatment options are sorely needed.

The challenge of drug resistance, a critical concern in modern medicine, was highlighted in Sichen et al.'s analysis of multidrug resistance in tuberculosis patients in Northeast China. This report

underscores the need for continued vigilance and innovation in the fight against drug-resistant infections, a challenge that continues to evolve and requires new strategies. Similarly, Wang et al.'s review on the complexities of non-tuberculosis mycobacteria skin infections called for a more nuanced understanding and tailored therapeutic strategies. This review reflects the evolving nature of infectious diseases and the corresponding need for adaptive and targeted treatment approaches.

In veterinary medicine, Lee et al.'s pharmacokinetic/pharmacodynamic study on Tylosin, a macrolide antibiotic, in pigs co-infected with *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* provided valuable insights into effective dosing strategies. This study not only advances our understanding of antimicrobial therapy in animal health but also reflects the interconnectedness of human and animal health, a concept integral to the One Health approach (Mackenzie and Jeggo, 2019).

4 Conclusion

The period from 2022 to 2023 in pharmacology has been characterized by a blend of innovation, repurposing, and deepened understanding of infectious diseases. The articles in our Research Topic have significantly contributed to this evolving landscape, where old and new therapies converge, providing hope and direction for future research and patient care. This era's developments serve as a beacon of progress, illuminating the path towards more effective, safe, and accessible treatments for infectious diseases globally. As we move forward, the lessons learned, and strategies developed during this time will undoubtedly continue to influence the field of pharmacology and the broader scientific community's approach to addressing health challenges.

Author contributions

EP: Conceptualization, Formal Analysis, Investigation, Supervision, Writing–original draft, Writing–review and editing. AS: Conceptualization, Formal Analysis, Investigation, Supervision, Writing–original draft, Writing–review and editing. KK: Conceptualization, Formal Analysis, Investigation, Supervision, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Akinbolade, S., Coughlan, D., Fairbairn, R., McConkey, G., Powell, H., Ogunbayo, D., et al. (2022). Combination therapies for COVID-19: an overview of the clinical trials landscape. *Br. J. Clin. Pharmacol.* 88 (4), 1590–1597. doi:10.1111/bcp.15089

Baker, R. E., Mahmud, A. S., Miller, I. F., Rajeev, M., Rasambainarivo, F., Rice, B. L., et al. (2022). Metcalf CJE. Infectious disease in an era of global change. *Nat. Rev. Microbiol.* 20 (4), 193–205. doi:10.1038/s41579-021-00639-z

Betz, U. A. K., Arora, L., Assal, R. A., Azevedo, H., Baldwin, J., Becker, M. S., et al. (2023). Game changers in science and technology - now and beyond. *Technol. Forecast. Soc. Change.* 193, 122588. doi:10.1016/j.techfore.2023.122588

Mackenzie, J. S., and Jeggo, M. (2019). The One health approach-why is it so important? *Trop. Med. Infect. Dis.* 4 (2), 88. doi:10.3390/tropicalmed4020088

Rijs, C., and Fenter, F. (2020). The academic response to COVID-19. *Front. Public Health* 8, 621563. doi:10.3389/fpubh.2020.621563

Tabish, S. A. (2020). COVID-19 pandemic: emerging perspectives and future trends. *J. Public Health Res.* 9 (1), 1786. doi:10.4081/jphr.2020.1786

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ORIGINAL RESEARCH
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The Effect of Statins on Clinical Outcome Among Hospitalized Patients With COVID-19: A Multi-Centric Cohort Study

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The coronavirus disease-2019 (COVID-19) is caused by SARS-CoV-2, leading to acute respiratory distress syndrome (ARDS), thrombotic complications, and myocardial injury. Statins, prescribed for lipid reduction, have anti-inflammatory, anti-thrombotic, and immunomodulatory properties and are associated with reduced mortality rates in COVID-19 patients. Our goal was to investigate the beneficial effects of statins in hospitalized COVID-19 patients admitted to three multi-specialty hospitals in India from 1 June 2020, to 30 April 2021. This retrospective study included 1,626 patients, of which 524 (32.2%) were antecedent statin users among 768 patients (384 statin users, 384 non-statin users) identified with 1:1 propensity-score matching. We established a multivariable logistic regression model to identify the patients' demographics and adjust the baseline clinical and laboratory characteristics and co-morbidities. Statin users showed a lower mean of white blood cell count (7.6×10^3/μL vs. 8.1×10^3/μL, $p < 0.01$), and C-reactive protein (100 mg/L vs. 120.7 mg/L, $p < 0.001$) compared to non-statin COVID-19 patients. The same positive results followed in lipid profiles for patients on statins. Cox proportional-hazards regression models evaluated the association between statin use and mortality rate. The primary endpoint involved mortality during the hospital stay. Statin use was associated with lower odds of mortality in the propensity-matched cohort (OR 0.52, 95% CI 0.33-0.64, $p < 0.001$). These results support the previous evidence of the beneficial effects of statins in reducing mortality in hospitalized COVID-19 patients.

Keywords: COVID-19, statins, cohort, mortality, propensity

INTRODUCTION

The coronavirus disease (COVID-19) derives from the novel coronavirus SARS-CoV-2, which has caused a global pandemic since March 2020 (Umakanthan et al., 2020). The hospitalized patients suffering from COVID-19 have presented with a range of clinical manifestations that include mild respiratory illness to severe respiratory failure (Parasher, 2021).

Based on the categorical classification of severity of illness and complications, the management of COVID-19 has ranged from intubation to mechanical ventilation combating acute respiratory distress syndrome (ARDS) (Umakanthan et al., 2022). The initial phase of COVID-19 management depended on the patient's investigational outcome of lung function test, pulmonary vascular resistance, and vascular endothelial function (Huertas et al., 2020; Potus et al., 2020). COVID-19 patients also exhibited a higher risk of systemic complications that include cardiovascular system (myocardial infarction, myocarditis), cerebrovascular events (hemorrhagic infarctions, encephalopathy), and thromboembolic events. The COVID-19 patients' often presented with significant co-morbidities that included diabetes, hypertension, obesity, and a previous episode of ischemic heart disease (Umakanthan and Lawrence, 2022). A retrospective study conducted in China, revealed that out of 173 severe COVID-19 patients, the prevalence of hypertension, diabetes mellitus, coronary vascular disease, and cerebrovascular disease was significantly higher than non-severe diseases (Guan et al., 2020). The cholesterol-lowering drugs, statins, have shown to decrease the risk of atherosclerotic induced complications such as ischemic heart disease and its related complications (Feingold et al., 2000). Statins act in various modes that include antithrombotic, anti-inflammatory, and enhanced endothelial function (Pinal-Fernandez et al., 2018). These effects can prove beneficial in reducing mortality in COVID-19 patients, as shown in a retrospective study conducted in China (Zhang et al., 2020). Statins exhibit their cholesterol-lowering effect by inhibiting the mevalonate pathway and reduces lipid levels and enhances vascular endothelial function, causing a significant reduction in mortality due to complications arising from coronary artery disease (Zhou and Liao, 2009). The molecular pathogenesis for statins include inhibiting miR-133a expression, decreasing C-reactive protein levels (CRP), interfering with Kruppel-like factor-2 signaling, and modulating high mobility group box 1/ toll-like receptor 4(HMGB1/TLR4) pathway (Gu et al., 2020).

Given the antagonist mentioned above effects of COVID-19 on the immune-inflammatory status of the host and the agonist action of statins in these patients, we performed a retrospective cohort study to investigate the effects of statins in COVID-19 hospitalized patients' In India. The present study is the first type to be conducted on an Indian population, and it highlights the salient beneficial effects of statins in a cohort group.

MATERIALS AND METHODS

Data Sources

In India, all citizens are assigned a unique identification number using a biometric ID system at an individual level (Unique Identification Authority of India, 2009). For this study, we collected data from three multispecialty hospitals in India. The patients' data was verified using the unique identity number. Then, the patients' demographic details and other relevant clinical and laboratory details were obtained from the medical records department. A superior level of confidentiality and electronically secured data storage systems were utilized throughout the study.

Study Design and Population

This retrospective cohort study involved COVID-19 patients who were hospitalized and treated in three multispecialty hospitals in India from the time interval of 1 June 2020, to 30 April 2021.

Inclusion and Exclusion Criteria

The inclusion criteria for the study population were COVID-19 patients hospitalized, discharged, or died during the period mentioned above. The confirmation of COVID-19 was based on positive results obtained from the SARS-CoV-2 polymerase chain reaction (PCR) test of respiratory (nasopharynx, oropharynx) specimens and oxygenation saturation (SpO2) of ≤93% or PaO2/FiO2 < 300 mmHg. Imaging studies (Chest computed tomography) supporting PCR test results were included. These testing were all conducted in the hospitals mentioned above. Patients below the age of 18 and above 80 years, those without proper medical records documentation, patients admitted for only 1 day, and those suffering from *tuberculosis*, hepatitis B, HIV, and carcinomas requiring immunomodulatory therapies were excluded. The initial case-finding tally of 1834 patients was identified and reviewed based on the inclusion criteria. Of these patients, following this scrutiny, a total of 1,626 patients were identified for our study.

Data Extraction

The patient data was identified using an electronic data search at the medical records department in three hospitals. No manual abstraction was performed due to COVID-19 restrictions. Patient demographics and clinical data were filled in using the most commonly occurring clinical signs and symptoms. The same selection format was followed to collect laboratory results. Treatment history and clinical outcome were followed until the end of the study period, patients' death in-hospital, or until the patients' discharge. The data was stored using alphanumerical code, and the patient's name was de-identified.

Intervention

Baseline information, including age, gender, body mass index (BMI), and co-morbidities, were recorded. Clinical co-morbidities included hypertension, diabetes mellitus, coronary artery disease, chronic lung disease, chronic kidney disease, stroke, and heart failure. We included features of clinical presentations during the patient's hospitalization (i.e., presence of fever, dyspnea, cough, chest pain, fatigue, and O2 saturation). Several laboratory parameters at the time of hospital admission were also collected from the electronic medical records, including white blood count (WBC), D-dimer, C-reactive protein (CRP),

TABLE 1 | Missing laboratory markers in the propensity-matched cohort.

Laboratory marker	Statin use (384)	Non-statin use (384)
WBC	1 (0.2%)	4 (1%)
D-dimer	92 (23.9%)	111 (28.9%)
CRP	38 (9.8%)	43 (11.1%)
ESR	80 (20.8%)	74 (19.2%)
Total cholesterol	34 (8.8%)	47 (12.2%)
LDL	38 (9.8%)	59 (15.3%)
HDL	38 (9.8%)	59 (15.3%)
Triglycerides	5 (1.3%)	11 (2.8%)

WBC, white blood cells; CRP, C-reactive protein; ESR, erythrocyte sedimentation rates; LDL, low density lipoprotein; HDL, high density lipoprotein.

erythrocyte sedimentation rate (ESR), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides. As statins act by lowering lipid levels, we collected lipid values from COVID-19 patients from inpatient and outpatient medical records and then averaged them for each patient throughout the study. Some patients failed to present the laboratory values collected during their clinical checkups; the data was validated by involving patients in whom these were available. The details for missing laboratory values are provided in **Table 1**. In this study population, 524 patients (32.2%) were on statin therapy, and the remaining 1,102 (67.7%) were not on statins. Among 768 patients, a 1:1 propensity-score matching (384 statin users and 384 non-statin users) was done. The comparison between statin and non-statin patients was further categorized. A detailed medical record search for individual patient demographics, clinical features, COVID-19 disease, duration of statin use, and underlying co-morbidities was elicited either from medical records or from the patients/family members and then documented. The data was reviewed by the treating physicians, pulmonologists, and radiologists to exclude any data inaccuracies.

Patient and Public Involvement

None of the patients and the public were involved during the design, data analysis, and manuscript preparation.

Ethics Approval

The Cross-Hospital Ethics Review Board approved this study, and the waiver was granted for informed consent as patient data were de-identified.

Statin Exposure and Study Outcomes

Antecedent statin use in this cohort study was defined as "medical data record with statin being prescribed by the patient's physician as a routine home medication due to their previous history of hypertension, coronary artery disease, and stroke." The statin in these patients was appeased from the patient or the family members, dependents, and pharmacies at the time of hospitalization. The primary endpoint was defined as "mortality during a hospital stay," and the secondary endpoint was defined as "invasive mechanical ventilation during a hospital stay." Our study outcome also included the duration of hospital stay, number of days on a ventilator, and use of hemodialysis.

Statistical Analyses

This study's characteristic variables included the basic demographics, associated co-morbidities, antecedent statin use, common clinical and laboratory findings, and clinical outcomes. The statistical estimates were previewed as number (percentage) [n (%)] for nominal variables, medians, and inter-quantile ranges (IQR) for measuring variables. The differences between this study's unmatched and matched cohorts were examined using the two-sided independent *t*-test and the Chi-square test.

We constructed Cox proportional-hazard regression models to estimate the association between statin, use, and primary endpoint. Before building the Cox model, the Schoenfeld residual analysis test detected the proportional hazard (PH) for all confounders. The PH means "the ratio of the hazards for any two variables is constant over time."

The univariable logistic regression was performed for each confounding factor. The variables that had a significant association with the mortality hazard were probed into the multivariate Cox regression. To adjust the bias resulting from the confounders, we implemented four strategies; multivariate Cox regression, weighted PH Cox, PH Cox after the propensity score matching, and the PH Cox adjusted with propensity score (**Figure 1**). In our study, individual matching was impossible due to the variations in the drug types used in COVID-19 therapy and other confounding variables, including the clinical and laboratory characteristics.

A confounder is a significant factor in causing variance and bias in an observational study. Due to the absence of randomization of patients in the treatment category, the cohort patients are not identical in their clinical, laboratory characteristics, and treatment strategy. To minimize the effects of the confounding factor, we implemented propensity score methods. The propensity score is a statistical analysis to estimate treatment's pure effect and reduce or eliminate the bias caused by other confounding variables. All confounding variables' effects were summarized into a scoring pattern, referred to as the "propensity score." The individual propensities were estimated using the multivariate logistic regression model that included all the confounding covariates, mixed Cox model, Cox model with time-varying exposure, and marginal-structural model.

We formulated a multivariable logistic regression model to determine the confounding factors. The patients with statin index treatment were considered a dependent variable, and the baseline covariates were considered an independent variable. The propensity score matching was implemented using a nearest-neighbor strategy with the specification of caliper width equal to 0.1 of the standard deviation of the logit propensity score. All the baseline variables in the propensity-matched cohort were evaluated by implementing descriptive analysis. Laboratory results and patient clinical outcomes were stratified based on statin use in the cohort group of hospitalized COVID-19 patients.

We constructed a logistic regression on the propensity-matched cohort with the control group as a reference to address the primary and secondary endpoints. We analyzed and examined if the effect estimate remained consistent by utilizing logistic regression with multivariable adjustment on the total cohort. We modified the

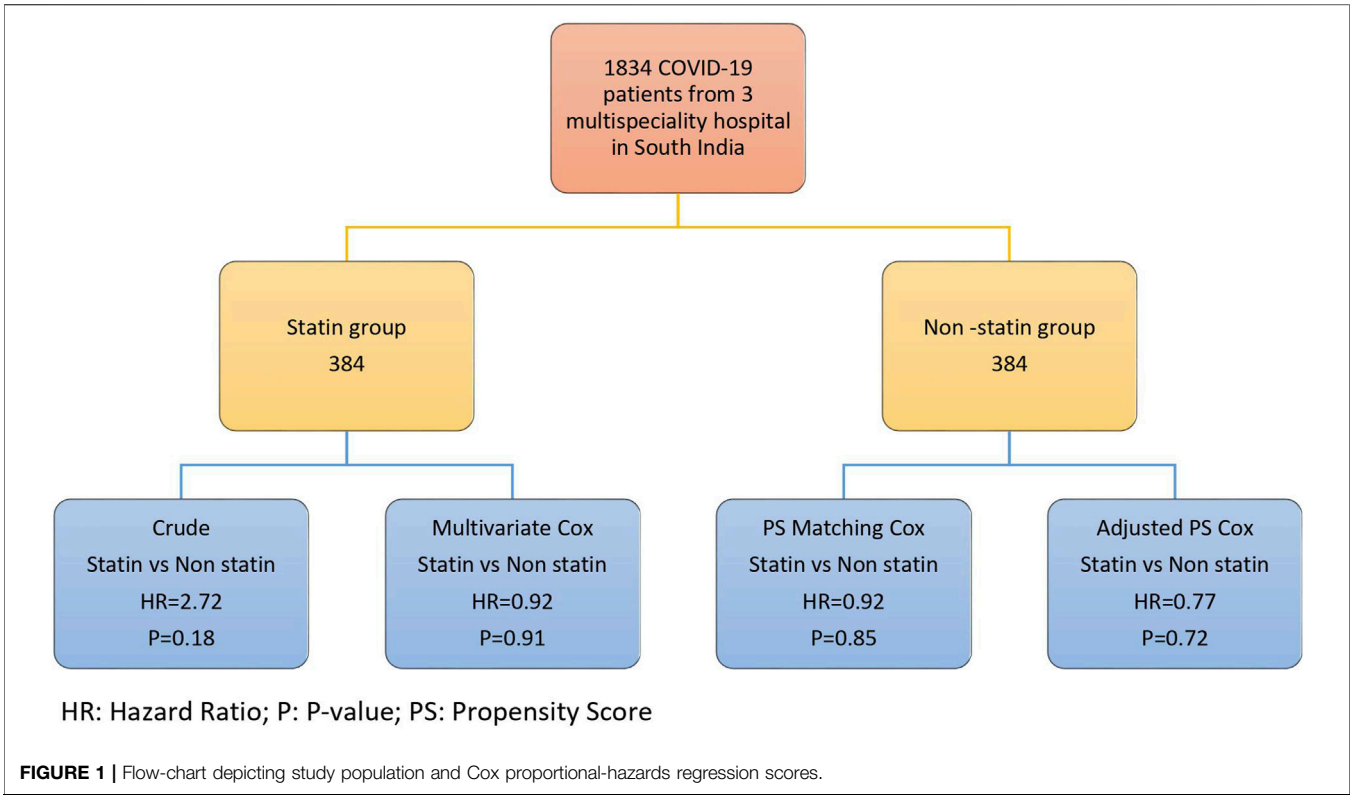

FIGURE 1 | Flow-chart depicting study population and Cox proportional-hazards regression scores.

multivariable models for variables that have been previously studied concerning COVID-19 mortality, including the baseline covariates. Statistical package for social science (SPSS) software 2.0 was used for statistical analyses (Arbogast et al., 2013; Ranganathan et al., 2017).

Sensitivity Analyses

Sensitivity analyses were performed by defining the recent statin users as "either antecedent statin or in-hospital statin use." We analyzed the data by defining statin users as "in-hospital statin users." By implementing these amended definitions, we estimated the relation of any recent statin users with the primary endpoint using multivariable logistic regression. We also utilized the subgroup analyses to evaluate the association of antecedent statin use with the primary endpoint in a subset of patients where statins are generally prescribed (e.g., history of hypertension, coronary artery disease, and stroke).

Missing Data

Data search revealed missing BMI and prior statin use in 19 and 15% of the registered patients. We utilized multiple imputations with predictive mean matching to adjust the missing data entry variables. We imputed 100 datasets, estimated the odds ratios on each imputed data variable, and averaged the one hundred estimated values to acquire the pooled estimates. Rubin's rules were implemented to calculate the model estimates and the standard errors (Lu et al., 2008). The patients' co-morbidities and lipid results were missing in 18 and 55% of the cohort, and these were presented only at baseline. The remaining variables were missing in less than 5% of the cohort study.

RESULTS

General Patient Characteristics

Out of 1,626 patients included in our study, 524 (32.2%) had a history of antecedent statin use before hospital admission. Based on the average age of the patients, statin users (median age of 63 years with IQR 55-79) were older in comparison to non-statin users (median age of 59 years with IQR 45-77) ($p < 0.001$). COVID-19 was marginally more in males (55.84%) and in statin users (52.4%) ($p = 0.05$) (**Table 2**).

Prevalence of Co-morbid Variables

Patients using statins had a significant higher prevalence of co-morbidities such as hypertension (75.0 vs. 58.0%), diabetes mellitus (62.5 vs. 40.3%), coronary artery disease (47.5 vs. 37.1%), and chronic lung disease (39.1 vs. 24.3%) in comparison to patients without statin intake ($p < 0.001$ for all). Furthermore, patients using statins were more likely to experience chronic kidney disease (37.2 vs. 15.9%), cerebrovascular accidents (11.0 vs. 2.9%) ($p < 0.01$ for both) (**Table 2**).

Variation in the Incidences of Clinical Manifestations

At the time of initial clinical presentation, patients on statins had a high incidence of dyspnea, cough, and fatigue (95.8, 93.3, and 92.7%, respectively) in comparison to non-statin patients (78.0, 81.7, and 76.4% respectively. There were no notable differences in the patients' presentation with chest pain and low O2 saturation among statin and non-statin users (**Table 2**).

TABLE 2 | Characteristic baseline demographics and clinical manifestations in unmatched and matched (propensity) cohorts.

	Unmatched			Matched		
Total *N*= 1626	**Statin use 524 (32.2%)**	**Non-statin use 1102 (67.7%)**	***p* value**	**Statin use (*n*=384)**	**Non-statin use (*n*=384)**	***p* value**
Demographics Age (years)	63 (55-79)	59 (45-77)	<0.001	62 (54-76)	64 (55-77)	0.16
BMI (kg/m^2)	28.2 (24.1-31.8)	27.8(24.4-32.0)	0.24	28.3 (24.6-32.4)	27.2 (23.8-31.8)	0.62
Gender						
Male	275 (52.4%)	633 (57.4%)	0.05	211 (54.9%)	223 (58.0%)	1.0
Female	249 (47.5%)	469 (42.5%)		173 (45.0%)	161 (41.9%)	
Co-morbidities						
HTN	393 (75.0%)	640 (58.0%)	<0.001	242 (63.0%)	273 (71.0%)	0.45
DM	328 (62.5%)	445 (40.3%)	<0.001	212 (55.2%)	224 (58.3%)	0.54
CAD	249 (47.5%)	409 (37.1%)	<0.001	62 (16.1%)	58 (15.1%)	0.7
CLD	205 (39.1%)	268 (24.3%)	<0.001	93 (24.2%)	96 (25.0%)	0.45
CKD	195 (37.2%)	176 (15.9%)	<0.01	84 (21.8%)	80 (20.8%)	0.75
CVA	58 (11%)	33 (2.9%)	<0.01	35 (9.1%)	33 (8.5%)	0.86
Heart failure	220 (41.9%)	364 (33.0%)	0.96	64 (16.6%)	62 (16.1%)	0.88
Liver disease	36 (6.8%)	44 (3.9%)	0.98	36 (9.3%)	28 (7.2%)	0.70
Clinical presentations						
Fever (^{o}c)	37.45 ± 1.16	37.41 ± 1.04	0.787	37.31 ± 1.09	37.22 ± 1.04	0.60
Dyspnoea n (%)	502 (95.8%)	860 (78.0%)	<0.01	320 (83.3%)	312 (81.2%)	0.88
Cough n (%)	489 (93.3%)	901 (81.7%)	<0.01	303 (78.9%)	297 (77.3%)	0.87
Chest pain n(%)	26 (4.9%)	54 (4.9%)	<0.001	35 (9.1%)	39 (10.1%)	0.88
Fatigue n (%)	486 (92.7%)	843 (76.4%)	<0.01	296 (77.0%)	301 (78.3%)	0.86
O_2 saturation ($SpO2_2$ ≤93%)	157 (29.9%)	332 (30.1%)	<0.001	114 (29.6%)	108 (28.1%)	0.88

BMI, body mass index; HTN, hypertension; DM, diabetes mellitus; CAD, coronary artery disease; CLD, chronic lung disease; CKD, chronic kidney disease; CVA, cerebrovascular accident.

TABLE 3 | Laboratory values in propensity-matched cohorts.

	At admission		At discharge		
Laboratory values	**Statin use**	**Non-statin use**	**Statin use**	**Non- statin use**	***p* value**
WBC count (10^3/μL)	7.9 (5.5–11.4)	8.6 (5.4–12.0)	7.6 (5.2–11.1)	8.1 (5.6–11.6)	<0.01
D-dimer (μg/ml)	2.3 (1.3–4.1)	2.8 (1.4–5.2)	2.1 (1.1–3.8)	2.5 (1.2–4.8)	0.37
CRP (mg/ml)	106 (50–178)	127.6 (82.2–198.8)	100.0 (48.1–172.2)	120.7 (72.2–196.6)	<0.001
ESR (mm/hr)	72 (41–103)	79.8 (38.6–98.2)	67.5 (36.3–98.1)	66.5 (34.4–93.6)	0.84
Total cholesterol (mg/dl)	161.1 (122.2–191.1)	168.8 (137.4–206.6)	154.2 (118.3–186.0)	164.6 (133.0–203.7)	<0.01
LDL (mg/dl)	81.2 (61.2–112.4)	93.4 (67.4–114.4)	79.1 (58.0–110.2)	92.0 (68.0–115.0)	<0.01
HDL (mg/dl)	42.0 (36.2–56.4)	38.2 (32.6–46.4)	44.0 (34.4–56.4)	40.0 (32.2–53.3)	0.25
Triglycerides (mg/dl)	148.2 (102.4–198.4)	156.4 (102.4–212.6)	144.0 (99.2–192.3)	158.0 (94.7–216.7)	0.25

WBC, white blood cells; CRP, C-reactive protein; ESR, erythrocyte sedimentation rates; LDL, low density lipoprotein; HDL, high density lipoprotein.

Variations in Laboratory Findings Based on Propensity-Matched Cohort Incidences Were Compared During Admission and Discharge in Statin and Non-Statin Users

At the time of discharge the laboratory results revealed that the patients with statins showed a lower mean of WBC count (7.6 × 10^3/μL vs. 8.1 × 10^3/μL, $p < 0.01$), and C-reactive protein (100 mg/L vs. 120.7 mg/L, $p < 0.001$) compared to non-statin COVID-19 patients. The same positive results followed in lipid profiles for patients on statins (**Table 3**). Patients with antecedent statin use showed no significant differences for mechanical ventilation (20 vs. 24.2%, p 0.07), and hemodialysis (5.4 vs. 7%, p 0.41), and in length of hospital stay and days on a ventilator (**Table 4**).

Clinical Outcomes of Propensity-Matched Cohort

Comparison and variation in clinical outcomes in the propensity-matched cohort between statin and non-statin use are presented in **Table 4**. The mortality in statin users was 17.1% compared to 31% in non-statin users.

Multivariable Adjusted Overall Cohorts

The clinical outcome in the multivariable-adjusted overall cohort [odds ratio (OR) 0.55, 95% confidence interval (CI) 0.37-0.63] revealed that statin users had a significant effect on the primary endpoint (mortality during hospitalization). The odds ratio was defined as "a measure of association between the statin use/exposure and the clinical outcome." The secondary endpoint was attained in 77 (20%) patients receiving

TABLE 4 | Clinical outcome in propensity matched cohorts.

Clinical Variable	Statin use (*n* = 384)	No statin use (*n* = 384)	*p* value
Length of hospital stay (days)	6.0 (3.0–11.0)	6.0 (4.0–12.0)	0.27
Mechanical ventilation n (%)	77 (20.0%)	93 (24.2%)	0.07
Days on ventilator	13.5 (3.2–31.0)	12.8 (2.0–34.1)	0.77
Mortality after hospitalization n (%)	66 (17.1%)	119 (31%)	<0.001
Haemodialysis n (%)	21 (5.4%)	27 (7.0%)	0.41

TABLE 5 | Clinical outcome in multivariable adjusted cohorts and propensity matched cohorts.

	OR*	95% CI	*p*-value
Primary endpoint (mortality during hospital stay)			
PS-matched	0.52	0.33-0.6 4	<0.001
Multivariable (PS-matched)	0.53	0.35-0.67	<0.001
Multivariable (overall)	0.55	0.37-0.63	
Secondary endpoint (invasive mechanical ventilation during hospital stay)			
PS-matched	0.80	0.64-1.02	<0.001
Multivariable (PS-matched)	0.89	0.68-1.20	<0.001
Multivariable (overall)	0.76	0.58-1.00	

*PS, propensity score; OR, odds ratio; CI, confidence interval. * Odds ratio (OR) was defined as "a measure of association between the statin use/exposure and the clinical outcome".*

statins compared to 93 (24.2%) non-statin users. Statin users also showed reduced odds in the multivariable-overall (OR 0.76, 95%CI 0.58-1.00), but this was statistically not significant (**Table 5**).

Statistical Comparison Between at Risk, Death, and Discharge Status in the Propensity-Matched Cohort

The hospitalized COVID-19 patients were followed over a 5-day interval for over 30 days (**Figure 2**). The incidence rate of mortality during the 30-day follow-up was 0.09 cases per 100-person-day in statin users (the mortality rate was 17.1%) in comparison to 0.11 cases per 100-person-day in non-statin users (the mortality rate was 31%) (**Table 6**). The matched statin users had more severe baseline clinical characteristics and higher proportions of pre-existing co-morbidities. We used the mixed-Cox model without involving the time-varying exposures in our propensity score matched (PSM) cohort; the statins significantly reduced the mortality incidence (aHR:0.25, 95% CI: 0.12-0.38, p = 0.001) (**Table 6**). The graph (**Figure 2**) shows the survival pattern among COVID-19 patients between statin and non-statin users. The "at-risk" COVID-19 cases were defined as the total number of patients minus deaths and the "survival" COVID-19 cases were defined as the total number of at-risk COVID-19 patients minus deaths.

Statin Frequency, Regimens, and Dosage

In our study, physicians prescribed Rosuvastatin, the most preferred statin, to COVID-19 patients (**Figure 3**). It was observed that Rosuvastatin was most effective than the other statin group in reducing LDL, triglycerides, and total cholesterol and increasing HDL levels. As presented in **Table 7**, in COVID-19 patients with co-morbidities, Rosuvastatin was the most preferred statin to be prescribed by the physicians in COVID-19 patients with diabetes mellitus (51.8%). In comparison,

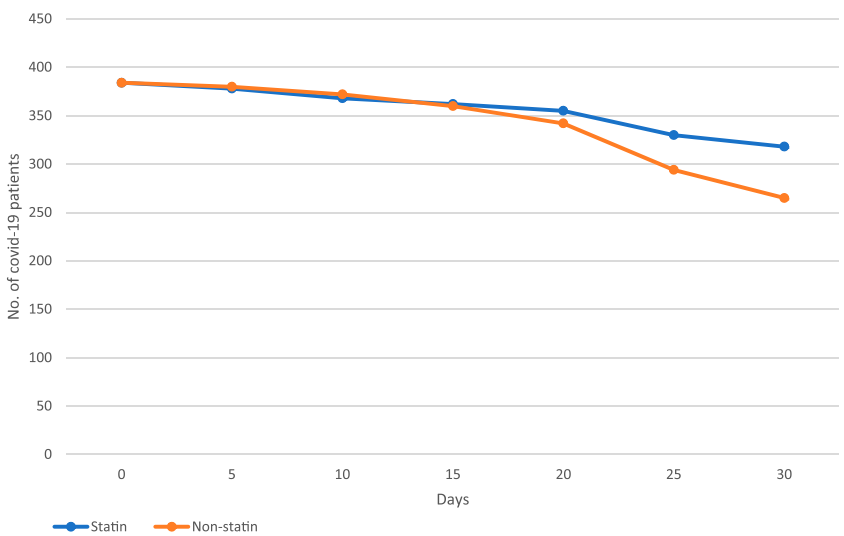

FIGURE 2 | Survival curves depicting statin and non-statin use after Propensity score matched.

TABLE 6 | Incidence rate and hazard ratios to evaluate the association between in-hospital statin therapy and mortality.

Statin versus Non-statin[a]	Unmatched							Matched				
	Crude incidence			Cox model time-varying exposure		Marginal-structural model		Crude incidence after PSM			Mixed Cox model	
	IR	IRR (95%CI)	*p* value	aHR (95%CI)	p value[b]	aHR (95%CI)	*p* value	IR	IRR (95%CI)	*p* value	aHR (95%CI)	*p* value
	0.09 vs. 0.11	0.39 (0.21–0.57)	0.023	0.31 (0.14-0.48)[c]	0.0004	0.39 (0.25-0.53)[c]	0.017	0.08 vs. 0.14	0.23 (0.09–0.36)	<0.001	0.25 (0.12-0.38)[d]	0.001

[a]*There were 524 and 1102 COVID-19 hospitalized patients in unmatched statin and non-statin groups respectively. After PSM with 1:1 ratio, there were 384 and 384 COVID-19 hospitalized patients in matched statin and non-statin groups, respectively.*
[b]*SPSS statistical analyses 2.0 was used to calculate the* p *values.*
[c]*The hazard ratio was adjusted for age, gender, BMI, co-morbidities (HTN, CAD, CLD, CKD, CVA, and heart failure), laboratory values (WBC count, D-dimer, CRP, ESR and SpO2), mechanical ventilation, hemodialysis, and duration of hospitalization.*
[d]*aHR was calculated based on mixed-effect Cox model with adjustment of age, gender, CAD, increase D-dimer, increase CRP, increase ESR at admission.*
IR, Incidence rate; IRR, Incidence rate ratio; aHR, adjusted hazard ratio.

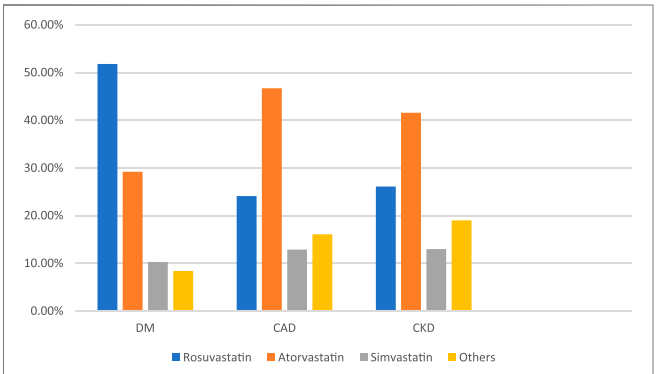

FIGURE 3 | Preferred statin in COVID-19 patients.

atorvastatin was the most preferred statin in CAD and CKD patients (46.7 and 41.6%, respectively). The highest tolerable dose for Rosuvastatin was 40 mg, and for atorvastatin and simvastatin, it was 80mg, respectively. However, Rosuvastatin was prescribed at a 10 mg daily dose, whereas atorvastatin and simvastatin were prescribed at a 20 mg dose for 6 weeks.

DISCUSSION

This cohort study evaluated the effect and outcome of statin use (antecedent and in-hospital) on patients' clinical manifestation, laboratory results, and clinical outcome. The principal findings in this analysis show that: 1. COVID-19 patients have commonly used statins (32.2%) prior to hospital admission due to their impending co-morbidities (hypertension, diabetes mellitus, coronary artery disease), 2. COVID-19 patients were older males, with a higher BMI, 3. the total lipid profile, WBC count, and C-reactive protein, were more favorable in statin users, and 4. the clinical outcome was significantly better in statin users.

Several literature studies have postulated the role of statins in COVID-19 patients (Hope et al., 2019; Fan et al., 2020; Pal et al., 2021; Wu et al., 2021). In our study, the predominance of COVID-19 in the elderly population can be elucidated for two reasons. One, the elderly population is more likely to be associated with co-morbidities such as hypertension, diabetes mellitus, and coronary artery disease, in contrast to the younger and middle age group. Two, the elderly population is more immune-compromised. These factors form precursor mediums in developing inflammatory-triggered cytokine storm syndrome, which further complicates the host defense mechanism and increases the probability of hospitalization, as observed in our study. The prevalence of hypertension in our hospitalized COVID-19 patient cohort was 75%. The proposed pathogenesis for this common coexistence is elevated levels of IL-6, tumor necrosis factor α (TNF α), and granulocyte-macrophage colony-stimulating factor resulting in COVID-19 induced cytokine storm. The cytokine storm further induces an imbalance in the renin-angiotensin system (RAS) and the NADH/NADPH oxidase causing aggravated pulmonary damage (Mahmudpour et al., 2020). The resulting underlying pulmonary pathology triggers the occurrence of respiratory symptoms, as observed in our study.

TABLE 7 | Preferred statin in a COVID-19 patient with co-morbidity.

COVID-19 patients with co-morbidities	Rosuvastatin	Atorvastatin	Simvastatin	Others	Total
Diabetes mellitus	110 (51.8%)	62 (29.2%)	22 (10.3%)	18 (8.4%)	212
CAD	15 (24.1%)	29 (46.7%)	8 (12.9%)	10 (16.1%)	62
CKD	22 (26.1%)	35 (41.6%)	11 (13%)	16 (19%)	84

CAD, coronary artery disease; CKD, chronic kidney disease.

The higher percentage of diabetes mellitus in our hospitalized COVID-19 patients is elucidated by the relationship between elevated glucose levels and SARS-CoV-2 replication. The enhanced viral replication seen in COVID-19 diabetic patients is due to sustained glycolysis, increased mitochondrial reactive oxygen species production, and activation of hypoxia-inducible factor 1 α. Our study shows that COVID-19 patients with diabetes mellitus are at a higher risk for hospitalization and have a higher mortality rate due to dysregulated immune response (Hammad et al., 2019). Other causes described in the literature for higher prevalence of diabetes in COVID-19 patients are reduced macrophage function, decreased mobilization of polymorphonuclear leukocytes, and inhibition of tumor necrosis alpha activity on T-cells (Galicia-Garcia et al., 2020; Mahmudpour et al., 2020). Statins inhibit the major protease (Mpro) and RNA-dependent RNA-polymerase (RdRp), thereby lowering serum IL-6 and modulating macrophage activity (Coperchini et al., 2020; Costela-Ruiz et al., 2020; Pawlos et al., 2021). These actions provide an ameliorated immune environment and reduce the severity of COVID-19 illness, further reflected by laboratory inflammatory markers (CRP, WBC count) as observed in our study (Ponti et al., 2020).

COVID-19 also triggers the risk of coronary vascular disease in patients with hypercholesterolemia and atherosclerosis (Grzegorowska and Lorkowski, 2020; Nishiga et al., 2020; Basu-Ray et al., 2021). Statins generally lower LDL-cholesterol by inhibiting the HMG-CoA reductase enzymes in the liver, further inhibiting HMG-CoA conversion into mevalonate and reducing the total cholesterol (Feingold et al., 2000). recent studies at the molecular level have shown that statins suppress TLR4/MyD88/NF kB signaling (Senol et al., 2021). These molecular level changes make the patient move into a protective anti-inflammatory state. The role of an intracellular inflammasome NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) is also well established. This intracellular sensor is enabled by oxidized LDL and TNF α, causing activation and release of cytokines in patients with metabolic dysfunction, resulting in cardiovascular complications in COVID-19 patients. Statins inhibit NLRP3 inflammasome activation by suppressing the oxidized LDL, and TNF α, and improving the cardiovascular functional outcomes in COVID-19 patients (Umakanthan et al., 2021b; Koushki et al., 2021). These beneficial effects of statins on cholesterol levels have been observed in our lipid profile of matched cohort patients.

The role of COVID-19 in causing endothelial dysfunction is well established (Gavriilaki et al., 2020; Bonaventura et al., 2021). This effect is mainly attributed to its microvasculature immune-inflammatory process, reflected by higher laboratory D-dimer levels (Gencer et al., 2020). Higher D-dimer levels impart the extent of thrombus formation and predict the risk of thrombo-embolic complications (Al-Ani et al., 2020). Statins improve endothelial functions by enervating TGF ß, VEGF and reducing serum PAI-1 levels (Tsujinaka et al., 2017). They exert their anti-thrombotic effects by reducing the tissue factor expression and downregulating the blood coagulation cascade. This endothelial function enhancing property of statins has been reflected in many studies by the lower D-dimer levels in COVID-19 statin users, however in our study, the D-dimer levels for statin users vs. non-statin users were not significantly different ($p = 0.37$). (Vuorio and Kovanen, 2020; Ferrari et al., 2021).

The relation between hypertriglyceridemia and the risk of developing atherosclerosis has been contrasting. The initial studies revealed that patients with severe hypertriglyceridemia had a higher risk of developing atherosclerosis. However, the recent data suggest that patients with severe hypertriglyceridemia have a large-sized lipoprotein, hence restricting its entry into the arterial intima (Nelson, 2013; Prasad et al., 2019). Thus, based on this observation in our study, patients with moderate elevations of triglyceride are at higher risk of developing atherosclerosis. In our matched cohort, statin showed a more successful cholesterol-lowering effect (higher HDL and lower triglyceride) in patients with hypertriglyceridemia than in patients without elevated triglyceride levels.

This observational study aims to estimate the effects of statins on the treatment outcome of hospitalized COVID-19 patients. Our study's selection of patients on statins was influenced by subjective characteristics (e.g., co-morbidities). Due to the subjective selection, the statin-treated patients' baseline characteristics differed from the non-statin patients. The logistic regression model determined these systemic differences in the baseline covariates and the confounding factor-induced biased results between statin users and non-statin users. In our study results, rosuvastatin was the most preferred statin to be prescribed by the clinician. In a meta-analysis performed by Weng et al., they discussed that rosuvastatin (at 10 mg) and atorvastatin (at 20 mg) were the two most effective statins that could reduce LDL and raise HDL levels (Weng et al., 2010). The STELLAR trial and the PULSAR study demonstrated a remarkable reduction in LDL, total cholesterol, and triglycerides compared to other statins (Clearfield et al., 2006; Welty et al., 2016). Many patients with a high risk of coronary vascular disease generally fail to attain the recommended LDL goal. However, the PULSAR trial demonstrated that with rosuvastatin at 10 mg, many patients could attain the LDL goal of less than 100 mg/dl. These patients had either atherosclerosis, type 2 diabetes mellitus, or were at elevated risk of coronary vascular disease (Clearfield et al., 2006).

Numerous studies have favored the use of statins in COVID-19 patients (Fan et al., 2020; Zhang et al., 2020; Wu et al., 2021). The National Health Institute, United States has recommended that COVID-19 patients continue statin therapy to prevent and treat cardiovascular disease (Vuorio and Kovanen, 2020). This safe and cost-effective drug has been proven beneficial during hospitalization and linked with better clinical outcome (Zhang et al., 2020). The diagnosis of COVID-19 in adult patients with cardiovascular disease has triggered clinicians to initiate statin therapy (Madjid et al., 2020).

The endothelial dysfunction caused by COVID-19 is well combatted by statin-induced improved endothelial function, thereby reducing the risk of thrombotic complications (Gavriilaki et al., 2020). Retrospective meta-analyses and cohort studies have shown better clinical outcomes in COVID-19 patients with statin use (Fan et al., 2020; Zhang et al., 2020; Wu et al., 2021). This includes investigating such

patients for the severity of the infection and in-hospital mortality. However, such results need a cautious interpretation since most statin users are old males with hypertension, diabetes mellitus, and cardiovascular disease; these are proven factors to exacerbate COVID-19 prognosis (Scheen, 2021). The effects of these confounding factors need to be eliminated by rigorous statistical analyses as done in our study (Arbogast et al., 2013). Statins modulate and modify ACE2 levels and produce epigenetic modifications, thereby preventing the progression to Acute Respiratory Distress Syndrome (ARDS) and also limit the severity of COVID-19, as shown in COVID-19 literature studies (Castiglione et al., 2020; Pawlos et al., 2021).

Our multi-centric study analyses during the COVID-19 pandemic have demonstrated that prior statin use significantly reduces hospital mortality rates. The literature studies on the effect of statins on hospitalized COVID-19 patients validate the results presented in our manuscript. A study done by Zhang et al. showed some variations in their results compared to our study due to the differences in the study size (<10% of hospitalized patients were statin users) and the genetic form of the Chinese population (Zhang et al., 2020). Most studies originated from China, and few recent meta-analyses showed the reflection of statin effects in the European and North American patient population. In a study conducted in the US, statin use showed a remarkable reduction in mortality rates in hospitalized COVID-19 patients, as displayed in our cohort study (Kow and Hasan, 2020; Wu et al., 2021). This US study compared the demographic, clinical, and laboratory variables between statin users and non-users by implementing the student t-test. The hospital discharge events have competed with in-hospital mortality using competing events analysis. Confounding bias was reduced by applying a multivariable regression model and propensity score analysis to conclude the inverse relationship between statin use and mortality rates. However, the findings and results varied in study size, and type of statin regimens (Saeed et al., 2020).

Limitations of the Study

Our study was conducted from the time interval of 1 June 2020, to 30 April 2021. Since Genesequencing was not available in these three multispecialty hospitals where the study was conducted, we could not incorporate the details of our study's gene sequencing and variants results. Furthermore, the samples from COVID-19-positive patients had to be sent to regional gene sequencing laboratories, which would require ethical clearance from the National Regional Research Centre for the results obtained to be used in our study. Since intra-mural or extra-mural grants did not fund our study, we could not afford to bear the enormous financial burden for this aspect.

The detailed age-wise separation was not reflected in detail in our manuscript as the other age groups were not statistically significant to provide a positive impact through a graphical representation using plot analysis by the statistical analyzer software as used in our study (both joint-plot and Forest plot analysis were attempted for this purpose). Hence, we decided only to present the age range that positively impacted our study, as shown above.

CONCLUSION

This is the first multi-centric cohort study conducted in India during the worst wave of the COVID-19 pandemic. We initially involved 1626 COVID-19 patients, followed by a 1:1 propensity-score matching, and eventually, 768 patients were included. After adopting appropriate statistical methods to minimize confounding, we observed that statins users had favorable laboratory findings and clinical outcomes compared to non-statin users. These findings may further strengthen the focus on the beneficial effects of administering statins in COVID-19 patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The Cross-Hospital Ethics Review Board approved this study and waiver was granted for informed consent as patient's data were de-identified.

AUTHOR CONTRIBUTIONS

SU—Concept and study design, methodology, formal analysis, writing original draft, review and editing final draft. SS and SJ—Supervision, Concept and study design, methodology, formal analysis, devising investigation tools, data analysis, and final version edit and review. MM, JD, and SP—Methodology, formal analysis, devising investigation tools, data collection leaders, validation of data source and contents. RR—Statistical analysis and writing statistical component of the manuscript. AC, VS, MY, AB, SA, CS, VM, and RS—Data collection and tabulation.

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REFERENCES

Al-Ani, F., Chehade, S., and Lazo-Langner, A. (2020). Thrombosis Risk Associated with COVID-19 Infection. A Scoping Review. *Thromb. Res.* 192, 152–160. doi:10.1016/j.thromres.2020.05.039

Arbogast, P. G., and VanderWeele, T. J. (2013). "Considerations for Statistical Analysis," in *Developing a Protocol for Observational Comparative Effectiveness Research: A User's Guide*. P. Velentgas, N. A. Dreyer, P. Nourjah, Editors et al. (Rockville, MD: Agency for Healthcare Research and Quality US). Available from: https://www.ncbi.nlm.nih.gov/books/NBK126192/.

Basu-Ray, I., Almaddah, N., Adeboye, A., and Soos, M. P. (2021). "Cardiac Manifestations of Coronavirus," in *StatPearls [Internet]* (Treasure Island FL: StatPearls Publishing). Available from: https://www.ncbi.nlm.nih.gov/books/NBK556152/.

Bonaventura, A., Vecchié, A., Dagna, L., Martinod, K., Dixon, D. L., Van Tassell, B. W., et al. (2021). Endothelial Dysfunction and Immunothrombosis as Key Pathogenic Mechanisms in COVID-19. *Nat. Rev. Immunol.* 21, 319–329. doi:10.1038/s41577-021-00536-9

Castiglione, V., Chiriacò, M., Emdin, M., Taddei, S., and Vergaro, G. (2020). Statin Therapy in COVID-19 Infection. *Eur. Heart J. Cardiovasc Pharmacother.* 6 (4), 258–259. doi:10.1093/ehjcvp/pvaa042

Clearfield, M. B., Amerena, J., Bassand, J. P., Hernández García, H. R., Miller, S. S., Sosef, F. F., et al. (2006). Comparison of the Efficacy and Safety of Rosuvastatin 10 Mg and Atorvastatin 20 Mg in High-Risk Patients with Hypercholesterolemia--Prospective Study to Evaluate the Use of Low Doses of the Statins Atorvastatin and Rosuvastatin (PULSAR). *Trials* 7, 35. doi:10.1186/1745-6215-7-35

Coperchini, F., Chiovato, L., Croce, L., Magri, F., and Rotondi, M. (2020). The Cytokine Storm in COVID-19: An Overview of the Involvement of the Chemokine/chemokine-Receptor System. *Cytokine Growth Factor Rev.* 53, 25–32. doi:10.1016/j.cytogfr.2020.05.003

Costela-Ruiz, V. J., Illescas-Montes, R., Puerta-Puerta, J. M., Ruiz, C., and Melguizo-Rodríguez, L. (2020). SARS-CoV-2 Infection: The Role of Cytokines in COVID-19 Disease. *Cytokine Growth Factor Rev.* 54, 62–75. doi:10.1016/j.cytogfr.2020.06.001

Fan, Y., Guo, T., Yan, F., Gong, M., Zhang, X. A., Li, C., et al. (2020). Association of Statin Use with the In-Hospital Outcomes of 2019-Coronavirus Disease Patients: A Retrospective Study. *Front. Med.* 7, 584870. doi:10.3389/fmed.2020.584870

Feingold, K. R., Anawalt, B., and Boyce, A. (2000). "Cholesterol Lowering Drugs,". K. R. Feingold, B. Anawalt, and A. Boyce. Editors *Endotext [Internet]* (South Dartmouth (MA): MDText.com, Inc.). Available from: https://www.ncbi.nlm.nih.gov/books/NBK395573.

Ferrari, F., Martins, V. M., Teixeira, M., Santos, R. D., and Stein, R. (2021). COVID-19 and Thromboinflammation: Is There a Role for Statins? *Clinics* 76, e2518. doi:10.6061/clinics/2021/e2518

Galicia-Garcia, U., Jebari, S., Larrea-Sebal, A., Uribe, K. B., Siddiqi, H., Ostolaza, H., et al. (2020). Statin Treatment-Induced Development of Type 2 Diabetes: From Clinical Evidence to Mechanistic Insights. *Int. J. Mol. Sci.* 21 (13), 4725. doi:10.3390/ijms21134725

Gavriilaki, E., Anyfanti, P., Gavriilaki, M., Lazaridis, A., Douma, S., and Gkaliagkousi, E. (2020). Endothelial Dysfunction in COVID-19: Lessons Learned from Coronaviruses. *Curr. Hypertens. Rep.* 22 (9), 63. doi:10.1007/s11906-020-01078-6

Gencer, S., Lacy, M., Atzler, D., van der Vorst, E. P. C., Döring, Y., and Weber, C. (2020). Immunoinflammatory, Thrombohaemostatic, and Cardiovascular Mechanisms in COVID-19. *Thromb. Haemost.* 120 (12), 1629–1641. doi:10.1055/s-0040-1718735

Grzegorowska, O., and Lorkowski, J. (2020). Possible Correlations between Atherosclerosis, Acute Coronary Syndromes and COVID-19. *J. Clin. Med.* 9 (11), 3746. doi:10.3390/jcm9113746

Gu, H., Wang, C., Li, J., Yang, Y., Sun, W., Jiang, C., et al. (2020). High Mobility Group Box-1-toll-like Receptor 4-phosphatidylinositol 3-kinase/protein Kinase B-Mediated Generation of Matrix Metalloproteinase-9 in the Dorsal Root Ganglion Promotes Chemotherapy-Induced Peripheral Neuropathy. *Int. J. Cancer* 146 (10), 2810–2821. doi:10.1002/ijc.32652

Guan, W.-j., Ni, Z.-y., Hu, Y., Liang, W.-h., Ou, C.-q., He, J.-x., et al. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720. doi:10.1056/NEJMoa2002032

Hammad, M. A., Syed Sulaiman, S. A., Aziz, N. A., and Mohamed Noor, D. A. (2019). Prescribing Statins Among Patients with Type 2 Diabetes: The Clinical Gap between the Guidelines and Practice. *J. Res. Med. Sci.* 24, 15. doi:10.4103/jrms.JRMS_100_18

Hope, H. F., Binkley, G. M., Fenton, S., Kitas, G. D., Verstappen, S. M. M., and Symmons, D. P. M. (2019). Systematic Review of the Predictors of Statin Adherence for the Primary Prevention of Cardiovascular Disease. *PLoS One* 14 (1), e0201196. doi:10.1371/journal.pone.0201196

Huertas, A., Montani, D., Savale, L., Pichon, J., Tu, L., Parent, F., et al. (2020). Endothelial Cell Dysfunction: a Major Player in SARS-CoV-2 Infection (COVID-19)? *Eur. Respir. J.* 56 (1), 2001634. doi:10.1183/13993003.01634-2020

Koushki, K., Shahbaz, S. K., Mashayekhi, K., Sadeghi, M., Zayeri, Z. D., Taba, M. Y., et al. (2021). Anti-inflammatory Action of Statins in Cardiovascular Disease: the Role of Inflammasome and Toll-like Receptor Pathways. *Clin. Rev. Allerg. Immunol.* 60, 175–199. doi:10.1007/s12016-020-08791-9

Kow, C. S., and Hasan, S. S. (2020). Meta-analysis of Effect of Statins in Patients with COVID-19. *Am. J. Cardiol.* 134, 153–155. doi:10.1016/j.amjcard.2020.08.004

Lu, Y., Liu, D. X., and Tam, J. P. (2008). Lipid Rafts Are Involved in SARS-CoV Entry into Vero E6 Cells. *Biochem. Biophys. Res. Commun.* 369 (2), 344–349. doi:10.1016/j.bbrc.2008.02.023

Madjid, M., Safavi-Naeini, P., Solomon, S. D., and Vardeny, O. (2020). Potential Effects of Coronaviruses on the Cardiovascular System: A Review. *JAMA Cardiol.* 5 (7), 831–840. doi:10.1001/jamacardio.2020.1286

Mahmudpour, M., Roozbeh, J., Keshavarz, M., Farrokhi, S., and Nabipour, I. (2020). COVID-19 Cytokine Storm: The Anger of Inflammation. *Cytokine* 133, 155151. doi:10.1016/j.cyto.2020.155151

Nelson, R. H. (2013). Hyperlipidemia as a Risk Factor for Cardiovascular Disease. *Prim. Care* 40 (1), 195–211. doi:10.1016/j.pop.2012.11.003

Nishiga, M., Wang, D. W., Han, Y., Lewis, D. B., and Wu, J. C. (2020). COVID-19 and Cardiovascular Disease: from Basic Mechanisms to Clinical Perspectives. *Nat. Rev. Cardiol.* 17, 543–558. doi:10.1038/s41569-020-0413-9

Pal, R., Banerjee, M., Yadav, U., and Bhattacharjee, S. (2021). Statin Use and Clinical Outcomes in Patients with COVID-19: An Updated Systematic Review and Meta-Analysis. *Postgrad. Med. J.* 98, 354–359. doi:10.1136/postgradmedj-2020-139172

Parasher, A. (2021). COVID-19: Current Understanding of its Pathophysiology, Clinical Presentation and Treatment. *Postgrad. Med. J.* 97 (1147), 312–320. doi:10.1136/postgradmedj-2020-138577

Pawlos, A., Niedzielski, M., Gorzelak-Pabiś, P., Broncel, M., and Woźniak, E. (2021). COVID-19: Direct and Indirect Mechanisms of Statins. *Int. J. Mol. Sci.* 22 (8), 4177. doi:10.3390/ijms22084177

Pinal-Fernandez, I., Casal-Dominguez, M., and Mammen, A. L. (2018). Statins: Pros and Cons. *Med. Clin. Barc.* 150 (10), 398–402. doi:10.1016/j.medcli.2017.11.030

Ponti, G., Maccaferri, M., Ruini, C., Tomasi, A., and Ozben, T. (2020). Biomarkers Associated with COVID-19 Disease Progression. *Crit. Rev. Clin. Lab. Sci.* 57 (6), 389–399. doi:10.1080/10408363.2020.1770685

Potus, F., Mai, V., Lebret, M., Malenfant, S., Breton-Gagnon, E., Lajoie, A. C., et al. (2020). Novel Insights on the Pulmonary Vascular Consequences of COVID-19. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 319 (2), L277–L288. doi:10.1152/ajplung.00195.2020

Prasad, M., Sara, J., Widmer, R. J., Lennon, R., Lerman, L. O., and Lerman, A. (2019). Triglyceride and Triglyceride/HDL (High Density Lipoprotein) Ratio Predict Major Adverse Cardiovascular Outcomes in Women with Non-obstructive Coronary Artery Disease. *J. Am. Heart Assoc.* 8 (9), e009442. doi:10.1161/JAHA.118.009442

Ranganathan, P., Pramesh, C. S., and Aggarwal, R. (2017). Common Pitfalls in Statistical Analysis: Logistic Regression. *Perspect. Clin. Res.* 8 (3), 148–151. doi:10.4103/picr.PICR_87_17

Saeed, O., Castagna, F., Agalliu, I., Xue, X., Patel, S. R., Rochlani, Y., et al. (2020). Statin Use and In-Hospital Mortality in Patients with Diabetes Mellitus and COVID-19. *J. Am. Heart Assoc.* 9 (24), e018475. doi:10.1161/JAHA.120.018475

Scheen, A. J. (2021). Statins and Clinical Outcomes with COVID-19: Meta-Analyses of Observational Studies. *Diabetes Metab.* 47 (6), 101220. doi:10.1016/j.diabet.2020.101220

Senol, S. P., Temiz-Resitoglu, M., Guden, D. S., Sari, A. N., Sahan-Firat, S., and Tunctan, B. (2021). Suppression of TLR4/MyD88/TAK1/NF-Kb/cox-2 Signaling Pathway in the Central Nervous System by Bexarotene, a Selective RXR Agonist, Prevents Hyperalgesia in the Lipopolysaccharide-Induced Pain Mouse Model. *Neurochem. Res.* 46 (3), 624–637. doi:10.1007/s11064-020-03197-7

Tsujinaka, H., Itaya-Hironaka, A., Yamauchi, A., Sakuramoto-Tsuchida, S., Shobatake, R., Makino, M., et al. (2017). Statins Decrease Vascular Epithelial Growth Factor Expression via Down-Regulation of Receptor for Advanced Glycation End-Products. *Heliyon* 3 (9), e00401. doi:10.1016/j.heliyon.2017.e00401

Umakanthan, S., Bukelo, M. M., and Gajula, S. S. (2022). The Commonwealth Caribbean COVID-19: Regions Resilient Pathway During Pandemic. *Front. pub. health* 10, 844333. doi:10.3389/fpubh.2022.844333

Umakanthan, S., and Lawrence, S. (2022). Predictors of COVID-19 Vaccine Hesitancy in Germany: a Cross-Sectional, Population-Based Study. *Postgrad. Med. J.* 3, 141365. doi:10.1136/postgradmedj-2021-141365

Umakanthan, S., Sahu, P., Ranade, A. V., Bukelo, M. M., Rao, J. S., Abrahao-Machado, L. F., et al. (2020). Origin, Transmission, Diagnosis and Management of Coronavirus Disease 2019 (COVID-19). *Postgrad. Med. J.* 96 (1142), 753–758. doi:10.1136/postgradmedj-2020-138234

Umakanthan, S., Senthil, S., John, S., Madhavan, M. K., Das, J., Patil, S., et al. (2021). The Protective Role of Statins in COVID-19 Patients: a Retrospective Observational Study. *Transl. Med. Commun.* 6 (1), 22. doi:10.1186/s41231-021-00102-4

Unique Identification Authority of India (2009). Government of India, Home Page. Available at: https://uidai.gov.in.(Accessed January 28 2009).

Vuorio, A., and Kovanen, P. T. (2020). Statins as Adjuvant Therapy for COVID-19 to Calm the Stormy Immunothrombosis and beyond. *Front. Pharmacol.* 11, 579548. doi:10.3389/fphar.2020.579548

Welty, F. K., Lewis, S. J., Friday, K. E., Cain, V. A., and Anzalone, D. A. (2016). A Comparison of Statin Therapies in Hypercholesterolemia in Women: A Subgroup Analysis of the STELLAR Study. *J. Womens Health (Larchmt)* 25 (1), 50–56. doi:10.1089/jwh.2015.5271

Weng, T. C., Yang, Y. H., Lin, S. J., and Tai, S. H. (2010). A Systematic Review and Meta-Analysis on the Therapeutic Equivalence of Statins. *J. Clin. Pharm. Ther.* 35 (2), 139–151. doi:10.1111/j.1365-2710.2009.01085.x

Wu, C. C., Lee, A. J., Su, C. H., Huang, C. Y., Islam, M. M., and Weng, Y. C. (2021). Statin Use Is Associated with a Decreased Risk of Mortality Among Patients with COVID-19. *J. Clin. Med.* 10 (7), 1450. doi:10.3390/jcm1007145

Zhang, X. J., Qin, J. J., Cheng, X., Shen, L., Zhao, Y. C., Yuan, Y., et al. (2020). In-hospital Use of Statins Is Associated with a Reduced Risk of Mortality Among Individuals with COVID-19. *Cell. Metab.* 32 (2), 176–e4. doi:10.1016/j.cmet.2020.06.015

Zhou, Q., and Liao, J. K. (2009). Statins and Cardiovascular Diseases: from Cholesterol Lowering to Pleiotropy. *Curr. Pharm. Des.* 15 (5), 467–478. doi:10.2174/138161209787315684

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Remdesivir associated sinus bradycardia in patients with COVID-19: A prospective longitudinal study

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Background: Remdesivir is effective against SARS-Cov-2 with little evidence of its adverse effect on the cardiac system. The aim of the present study is investigating the incidence of bradycardia in COVID-19 patients treated with Remdesivir.

Methods: This prospective longitudinal study was conducted in a tertiary center on COVID-19 patients for Remdesivir therapy. The objectives were to investigate the incidence of sinus bradycardia, and also the association between their demographics, underlying diseases, and the disease severity with developing bradycardia in COVID-19 patients treated with Remdesivir.

Results: Of 177 patients, 44% were male. The mean (±standard deviation) age of patients was 49.79 ± 15.16 years old. Also, 33% were hospitalized due to more severe symptoms. Oxygen support was required for all hospitalized subjects. A total of 40% of the patients had comorbidities, with the most common comorbidity being hypertension. The overall incidence of bradycardia (heart rate<60 bpm) in patients receiving Remdesivir was 27%, of whom 70% had extreme bradycardia (heart rate <50 bpm). There was also a statistically significant reduction in heart rate after five doses of Remdesivir compared to the baseline heart rates. In the multivariable model, none of the covariates including age above 60 years, female sex, CRP>50 mg/L, O2 saturation<90%, underlying cardiovascular disease, hypertension and diabetes mellitus, and beta-blockers were associated with Remdesivir-induced bradycardia. No association was found between the COVID-19 severity indicators and bradycardia.

Conclusion: As sinus bradycardia is a prevalent adverse cardiac effect of Remdesivir, it is recommended that all COVID-19 patients receiving Remdesivir, be evaluated for heart rate based on examination; and in the case of bradyarrhythmia, cardiac

monitoring should be performed during administration to prevent adverse drug reactions.

KEYWORDS

remdesivir, bradycardia, COVID-19, arrhythmia, ECG, cardiotoxicity, SARS-CoV-2

1 Introduction

The Coronavirus disease 2019 (COVID-19) first appeared in Wuhan, China, in December 2019 and was stated a pandemic by the world health organization (WHO) in March 2020. It rapidly spread around the world and has accounted for millions of global deaths since then. (Chen et al., 2020; Ganesh et al., 2021). The disease is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), an ribonucleic acid (RNA) virus from *Corona* Viridae family. The infection causes respiratory illness and varies widely in severity from asymptomatic or mild infection to severe pneumonia and subsequent fatal complications, including acute respiratory distress syndrome (ARDS), multiple organ failure, and death. (Grein et al., 2020; Zeng et al., 2021).

Among the antiviral drugs introduced and tested for the treatment of COVID-19, Remdesivir has been particularly used to treat the infection and long-COVID syndrome (Jacinto et al., 2021) during the pandemic after demonstrating its *in-vivo* and *in-vitro* inhibitory effects against SARS-CoV-2. (Beigel et al., 2020; Gordon et al., 2020; Gubitosa et al., 2020).

Remdesivir is a nucleotide analog that implicates in viral RNA and inhibits RNA polymerase and viral replication in a wide spectrum of viruses, including SARS-CoV-2, and is potently active in primary human epithelial cells in lung airways. (Gubitosa et al., 2020; Gupta et al., 2020; Gottlieb et al., 2022).

Various studies have indicated its inhibitory effect against the SARS-Cov-2, and it has been approved as an efficient antiviral treatment for hospitalized SARS-Cov-2 patients with moderate to severe infection in all variants of concern. (Beigel et al., 2020; Goldman et al., 2020; Grein et al., 2020; Pasquini et al., 2020; Wang et al., 2020; Pallotto et al., 2021a; Barkas et al., 2021; Brunetti et al., 2021; Gottlieb et al., 2022). A recent randomized controlled trial on non-hospitalized patients infected with COVID-19 who were at higher risk of disease progression showed that Remdesivir treatment reduced the risk of hospitalization and death by 87% compared to placebo. (Gottlieb et al., 2022).

Although its beneficial role in the treatment of COVID-19 has been valued and well described in the literature, evidence on its adverse effect (ADR), especially on the cardiovascular system, is scarce, and the available studies are mainly limited to hepatic, renal, and dermal adverse drug reactions of the drug. (Sarkar et al., 2020; Pallotto et al., 2021a; Pallotto et al., 2021b; Kow et al., 2021). Bradycardia, hypotension, QT interval prolongation, atrial fibrillation, and even cardiac arrest are among the most frequently reported cardiovascular complications attributed to Remdesivir in the literature. (Beigel et al., 2020; Grein et al., 2020; Gupta et al., 2020; Wang et al., 2020). Two potential mechanisms have been proposed for these adverse cardiac effect. First, the Remdesivir active metabolite resembles adenosine triphosphate (ATP). Adenosine may inhibit sinus node automaticity and atrioventricular (AV) node conduction by its chronotropic and dromotropic effects and transiently increases the central vagal tonicity in the heart and also the myocardial repolarization time. These effects may lead to arrhythmias, sinus bradycardia, corrected QT interval (QTc) prolongation and AV node blockage, as have been recently described in the literature. The second mechanism is the Remdesivir affinity to human mitochondrial RNA polymerase, which may possibly result in mitochondrial cardiomyocyte dysfunction and toxicity. (Kumar et al., 2021a; Ching and Lee, 2021; Day et al., 2021; Jacinto et al., 2021; Sanchez-Codez et al., 2021; Touafchia et al., 2021).

Currently, there is limited data on the cardiac adverse effect of Remdesivir except for a few case reports and case series. (Gubitosa et al., 2020; Gupta et al., 2020; Barkas et al., 2021; Ching and Lee, 2021; Day et al., 2021; Jacinto et al., 2021; Selvaraj et al., 2021).

Further comprehensive studies are required to clarify the exact association between Remdesivir and adverse cardiac effects that may lead to bradycardia and other cardiac complications in COVID-19 patients receiving this medication. The present study investigates the incidence of bradycardia in SARS-Cov-2 patients who received Remdesivir and examines the effect of demographic characteristics, underlying risk factors, and the infection severity on developing sinus bradycardia as the most prevalent cardiac complication of Remdesivir. (Lucijanic and Bistrovic, 2022). The results could provide a foundation for future precautions in treating COVID-19 patients receiving Remdesivir.

2 Materials and methods

2.1 Study population

This prospective longitudinal study was conducted using data from patients admitted to Dr. Masih Daneshvari hospital -a tertiary care center for lung diseases-in Tehran, Iran, from 19 August 2021, to 7 November 2021. The patients were randomly selected from daily systemic lists of registered patients with a COVID-19 diagnosis. The inclusion criteria were 1) aged 18 years or older, 2) a confirmed diagnosis of COVID-19 infection according to positive polymerase chain reaction (PCR) test results or chest computed tomography (CT) scan findings compatible with COVID-19 diagnosis, and 3) indication for receiving Remdesivir (Rezaei et al., 2021; Mirenayat et al., 2022). (Coronavirus Disease 2019, 2021). The exclusion criteria were 1) having rhythms other than sinus at baseline electrocardiogram (ECG), 2) a heart rate (HR) < 60 beats per minute (bpm) at baseline, and 3) using a cardiac pacemaker or cardiac resynchronization therapy (CRT) device and implantable-cardioverter defibrillator (ICD).

The priory sample size was calculated 166 using the formula $n = p(1-p)(\frac{z_{1-\alpha/2}+z_{1-\beta}}{p-p_0})^2$. The predicted incidence of bradycardia with Remdesivir P) was considered 20% and the P_0 calculated 28.7% based on the kumar et al. study (Kumar et al., 2021a). The power considered 80% and the type 1 error considered 5%. Initially, data was collected from 188 patients as it was predicted some patients probably miss follow-up sessions and finally data of 177 patients who met the criteria and completed follow-up sessions was analyzed.

2.2 Data collection and follow-up

Data on patients' demographic information, medical history, drug history, clinical condition, therapeutic management, laboratory values, and oxygen-support requirements were collected *via* an assessment form by clinicians (Baghaei et al., 2020). Patients' age, sex, comorbidities (e.g., diabetes mellitus, hypertension, and cardiovascular diseases), basal laboratory findings representative of infection severity including D-Dimer, CRP, absolute lymphocyte count (ALC), oxygen therapy requirement and O2 saturation, temperature, potassium, sodium, BUN, Cr level, outpatient or inpatient status, and using Tocilizumab, beta-blockers, and anti-arrhythmic drugs were variables included in the multivariable analysis. All vital sign measurements were performed immediately before and after Remdesivir administration and baseline ECG was performed for all patients enrolled the study before any therapy initiation. All patients underwent five sessions of Remdesivir administration, including 100 mg Remdesivir daily following a 200 mg intravenous loading dose. Dexamethasone and venous thromboembolism prophylaxis were also administered to all patients with different dosages according to the disease severity. The heart rates of patients were examined by a pulse oximeter as soon as Remdesivir administration was finished in each session. It was reconfirmed by a second measurement and the mean of two measurements were obtained. The Heart rate below 60 bpm was considered as bradycardia and the heart rate below 50 bpm was considered as extreme bradycardia. Second ECG was conducted if bradycardia detected on examination to determine the cardiac rhythm of patients in each session. A final ECG was performed for all patients in the study after the fifth dose of Remdesivir. Characteristics of baseline and final electrocardiograms were measured and reported by two cardiologists. The characteristics of baseline and final ECGs including the ventricular rate, PR duration, QRS width, QT interval duration, and QTc were extracted by two cardiologists and the baseline and final ECG characteristics were compared using the Wilcoxon rank test (p-value = .05). Severe bradycardia was defined as heart rate <50 bpm. (Drumheller et al., 2022). QTc was calculated through Bazett's formula ($QTc = \frac{QT}{\sqrt{RR}}$) (Bazett, 1997). QTc>460 ms in women and QTc>440 ms in men were considered as the prolongation of the QTc interval. Absolute QTc≥500 ms (millisecond) was considered as extreme QTc prolongation. (Russo et al., 2020). Patients who developed bradycardia were followed 2 weeks after drug cessation for their heart rate to investigate if this was a temporary effect.

2.3 Ethical approval and consent to participate

An informed consent form was reviewed and signed by all patients before participation. The study obtained the approval of the Iran National Committee for Ethics in Biomedical Research and followed the national standards for performing Medical Research in Iran (Ethic code: IR. SBMU.NRITLD.REC.1400.050, approval date: 2021-09-26), and the ethical guidelines outlined in the 1975 Helsinki Declaration.

2.4 Aims and objectives

The primary objective of the present study was to investigate the incidence of sinus bradycardia in COVID-19 patients receiving Remdesivir treatment. The secondary objective was to investigate the association of patients' underlying risk factors and diseases and also the severity of the COVID-19 infection with developing bradycardia in these patients.

2.5 Statistical analysis

The Kolmogorov-Smirnov and Shapiro-Wilk normality tests were used to examine the distribution of variables. Quantitative data were described by the median and interquartile range (IQR). For qualitative data, the frequency and percentage were calculated. For comparing means (or medians) between two groups, the T-test or Mann-Whitney U test were used for quantitative variables. To determine if the difference between observed and expected data is due to chance or due to a relationship between the qualitative variables, we used chi-square (or exact fisher tests) and Odds ratio for measuring (quantify) the strength (size) of association between them. Friedman and Wilcoxon signed-rank tests were used to investigate the changes within the repeated measured variables. To explain the relationship between bradycardia and underlying factors logistic regression analysis was assessed through multivariable analyses. The data was analyzed using statistical package for the social sciences (SPSS) software version 22, and a p-value below .05 was considered statistically significant in all analyses.

3 Results

3.1 Patients' characteristics

Of the total 188 patients, two were excluded due to arrhythmia at baseline ECG, and nine were excluded because of missing follow-up information or final ECG or incomplete courses of Remdesivir therapy. Overall, the data of 177 patients, of which 44% were male, were analyzed. The baseline clinical characteristics of the patients are shown in Tables 1, 2. The Mean ± standard deviation (SD) age of patients was 49.79 ± 15.16 years (minimum 19, maximum 88). Of the total patients, 33% were hospitalized due to more severe symptoms, of which 98% were admitted to the COVID-19 ward and 2% to the intensive care unit (ICU). The rest of the patients (67%) were outpatients with less severe symptoms who were admitted to the hospital to receive Remdesivir and were discharged after each session of drug administration (Rezaei et al., 2021; Mirenayat et al., 2022). The most common symptoms at admission were cough (80%) and dyspnea (56%). Oxygen support was required for 33% of patients, of whom 18% were supplied with oxygen through a high flow nasal cannula (HFNC), 13% through a non-rebreather face mask, and 2% through bi-level positive airway pressure (BiPAP). None of the studied patients were intubated. A total of 40% of patients had comorbidities, with the most common comorbidity being hypertension, with a prevalence of 14%. Also, 13% of patients were diabetic, and 9% had an underlying cardiovascular disease. Patients' medication history at admission showed that 12.4% used beta-blockers. The median of onset of symptoms to admission for patients was 8.13 ± 3.76 days.

The overall incidence of bradycardia (heart rate<60) in patients receiving Remdesivir was 27%, and 19% of patients developed extreme bradycardia (heart rate <50 bpm). None of the patients developed

TABLE 1 Comparing medians of quantitative variables between two groups of patients (heart rate<60 and heart rate≥60) using Mann-Whitney U test.

Characteristic	Heart Rate<60 bpm								*p*-value
	No				Yes				
	Mean	Standard Deviation	Median	IQR	Mean	Standard Deviation	Median	IQR	
Age, year	50	15	47	21	49	14	48	21	.754
BMI	29.0	7.6	26.6	7.0	26.1	3.9	26.0	6.2	.227
Temperature, ˚C	36.669	.638	36.500	.900	36.627	.528	36.700	.550	.698
BP(systolic)	118	9	120	10	117	12	118	15	.516
BP(diastolic)	74.78	8.89	75.00	10.00	74.40	8.36	72.50	10.00	.657
HR, bpm	97	17	96	21	97	14	98	16	.826
O2_sat, %	92	5	93	5	91	5	92	8	.253
WBC	6462.30	3320.10	5665.00	3160.00	7308.50	3436.51	6150.00	3900.00	.100
Hb, g/dL)	13.501	1.907	13.650	2.400	13.704	1.382	13.500	2.050	.972
Plt	204	86	185	107	207	81	186	81	.763
Lymph	1,203	549	1,054	576	1,185	463	1,079	536	.833
Neut	4,779	2,931	3815	2,872	5840	3463	4,760	4,246	.071
K,mmol/L	4.024	.417	4.000	.600	4.112	.395	4.100	.500	.274
D_Dimer, ng/mL	1778.833	1,258.294	1,363.500	1,437.000	1,348.400	882.836	1,309.000	1,078.000	.855
Troponin, ng/mL	.058	.052	.040	.075	.020	.000	.020	.000	.273
LDH, IU/L	630.560	198.785	653.000	332.000	660.900	261.091	605.000	397.000	.841
Bs, mg/dL	180.118	90.376	135.000	121.000	173.250	104.460	121.500	100.000	.705
BUN, mg/dL	37.452	24.871	31.500	15.000	37.250	17.712	31.000	16.000	.689
Cr, mg/dL	1.059	.610	1.000	.210	1.016	.234	1.000	.400	.935
AST, U/L	39	24	33	24	48	38	32	34	.348
ALT, U/L	44	40	30	33	51	43	35	27	.244
ALK.ph, U/L	174	68	160	61	170	73	163	48	.737
Ca, mg/dL	9.113	1.527	9.250	1.000	9.617	.483	9.700	.900	.529
Mg, mg/dL	2.287	.338	2.300	.600	2.225	.560	2.150	.650	.497
PT, seconds	12.837	2.800	12.250	.700	16.655	23.410	12.200	1.200	.447
INR	1.080	.273	1.020	.070	1.056	.073	1.010	.105	.776
ESR, mm/hr	47.667	29.200	51.500	53.000	47.000	3.606	48.000	7.000	1.000
CRP,mg/L	21	19	15	37	24	18	18	33	.412
CPK, mcg/L	150	225	67	58	141	127	89	141	.427

IQR: Interquartile Range, BMI: Body mass index, BP: Blood Pressure, HR: Heart Rate, O2_sat: Oxygen saturation,WBC: White Blood Cell, Hb: Hemoglobin, Plt: Platelet, Lymph: Lymphocyte, Neut: Neutrophil, K: Potassium, LDH: Lactate dehydrogenase, BS: Blood Sugar, BUN: Blood Urea Nitrogen, Cr: Creatinine, AST: Aspartate transaminase, ALT: Alanine transaminase, ALK. ph: Alkaline phosphatase, Ca: Calcium, Mg: Magnesium, PT: Prothrombin Time, INR: International Normalized Ratio, ESR: Erythrocyte sedimentation rate, CRP: C-Reactive Protein, CPK: Creatine Phosphokinase, *p*-value<.05 was considered statistically significant.

p-value<.05 was considered statistically significant.

bradycardia in the first session of Remdesivir treatment. 2% developed bradycardia in the second session, 7% in the third session, 6% in the fourth session and 21% in the last session. The mean heart rates of patients in each session of Remdesivir treatment are shown in Figure 1. All except one patient with HR 25 bpm had asymptomatic bradycardia. Sinus bradycardia lasted up to 2 weeks after Remdesivir discontinuation. There were no significant differences in primary clinical characteristics in the bradycardia patients and others, and developing bradycardia did not affect the clinical outcome of patients in the study. Nevertheless, the CRP levels were not suggestive of developing bradycardia in patients in the present study (*p*-value = .41). All studied patients had a favorable

TABLE 2 Evaluating the association between qualitative variables and bradycardia using chi-square or fisher's exact test* and Odds ratio (OR) for measuring the strength (size) of association between them.

Characteristics			Heart rate<60		Total	*p*-Value	OR
			No	Yes			
Sex	Male	Count	58	19	77	.495	1.2
		% of Total	33.0%	10.8%	43.8%		
	Female	Count	70	29	99		
		% of Total	39.8%	16.5%	56.3%		
Age (Years)	Under 60	Count	92	37	129	.534	0.7
		% of Total	52.6%	21.1%	73.7%		
	60 and above	Count	35	11	46		
		% of Total	20.0%	6.3%	26.3%		
		% of Total	71.8%	26.4%	98.3%		
	Critical	Count	3	0	3		
		% of Total	1.7%	.0%	1.7%		
Oxygen therapy	Nasal cannula	Count	20	11	31	.491*	
		% of Total	36.4%	20.0%	56.4%		
	Reservoir mask	Count	13	7	20		
		% of Total	23.6%	12.7%	36.4%		
	BiPAP	Count	4	0	4		
		% of Total	7.3%	.0%	7.3%		
Admission	Outpatient	Count	92	27	119	.058	1.9
		% of Total	52.0%	15.3%	67.2%		
	Inpatient	Count	37	21	58		
		% of Total	20.9%	11.9%	32.8%		
Need of oxygen support	No	Count	89	28	117	.190	1.6
		% of Total	51.1%	16.1%	67.2%		
	Yes	Count	38	19	57		
		% of Total	21.8%	10.9%	32.8%		
		% of Total	67.5%	28.4%	95.9%		
Comorbidities	Yes	Count	50	19	69	.848	0.9
		% of Total	29.1%	11.0%	40.1%		
	No	Count	76	27	103		
		% of Total	44.2%	15.7%	59.9%		
Hypertension	No	Count	107	41	148	.481	0.7
		% of Total	62.2%	23.8%	86.0%		
	Yes	Count	19	5	24		
		% of Total	11.0%	2.9%	14.0%		
Diabetes	No	Count	110	39	149	.667	1.2
		% of Total	64.0%	22.7%	86.6%		
	Yes	Count	16	7	23		
		% of Total	9.3%	4.1%	13.4%		

(Continued on following page)

TABLE 2 (*Continued*) Evaluating the association between qualitative variables and bradycardia using chi-square or fisher's exact test* and Odds ratio (OR) for measuring the strength (size) of association between them.

Characteristics			Heart rate<60		Total	*p*-Value	OR
			No	Yes			
Cardiovascular disease	No	Count	119	38	157	.028*	3.6
		% of Total	69.2%	22.1%	91.3%		
	Yes	Count	7	8	15		
		% of Total	4.1%	4.7%	8.7%		
Hypercholesterolemia	Yes	Count	1	2	3	.175*	0.2
		% of Total	.6%	1.2%	1.7%		
	No	Count	125	44	169		
		% of Total	72.7%	25.6%	98.3%		
Thyroid disease	Yes	Count	3	0	3	.565*	
		% of Total	1.7%	.0%	1.7%		
	No	Count	123	46	169		
		% of Total	71.5%	26.7%	98.3%		
Chronic kidney disease	Yes	Count	2	0	2	.999*	
		% of Total	1.2%	.0%	1.2%		
	No	Count	124	46	170		
		% of Total	72.1%	26.7%	98.8%		
		% of Total	3.1%	1.3%	4.4%		
Anti-arrhythmia	No	Count	122	41	163	.056*	8.9
		% of Total	73.1%	24.6%	97.6%		
	Yes	Count	1	3	4		
		% of Total	.6%	1.8%	2.4%		
		% of Total	68.9%	25.1%	94.0%		
Azithromycin	No	Count	127	47	174	.999*	1.3
		% of Total	71.8%	26.6%	98.3%		
	Yes	Count	2	1	3		
		% of Total	1.1%	.6%	1.7%		
Furosemide	No	Count	113	40	153	.763	1.1
		% of Total	67.7%	24.0%	91.6%		
	Yes	Count	10	4	14		
		% of Total	6.0%	2.4%	8.4%		
Beta-blocker	No	Count	115	40	155	.297	1.6
		% of Total	65.0%	22.6%	87.6%		
	Yes	Count	14	8	22		
		% of Total	7.9%	4.5%	12.4%		

p-value<.05 was considered statistically significant. Note: chi-square test used unless otherwise noted. *fisher's exact test used.

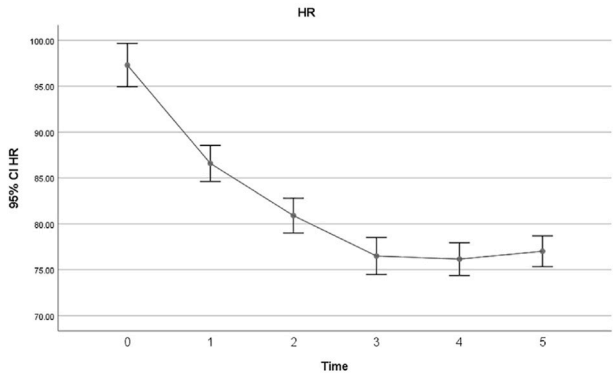

FIGURE 1
time (second, s), heart rate (beats per minute, bpm).

prognosis regardless of developing bradycardia, and no case of mortality or intubation during hospitalization was observed.

A change of 5 mm Hg was observed in the mean diastolic blood pressure after Remdesivir administration, which was statistically significant (p-value = .001). However, changes in the mean systolic blood pressure were not significant (p-value = .058)

There was no association between any infection severity indicator and bradycardia. Although an underlying cardiovascular disease and Tocilizumab had a correlation with bradycardia in multivariable analysis, it was not confirmed in the multivariate logistic model (Table 3). The covariates of age above 60, female sex, c-reactive protein (CRP) > 50 mg/L, O2 saturation <90%, underlying cardiovascular disease, hypertension (HTN) and diabetes mellitus, and beta-blockers were used as inputs in the multivariable regression analysis model. The results showed that none of these factors were associated with bradycardia in COVID-19 patients receiving Remdesivir (Table 3)

3.2 Electrocardiographic characteristics

The characteristics of baseline and final ECGs are summarized in Table 3. The baseline and final ECG characteristics were compared using the Wilcoxon rank test (p-value = .05), and the changes in ventricular rate, QT interval, and QTc interval were statistically significant (Table 4). There was also a statistically significant reduction in heart rate after five doses of Remdesivir compared to the baseline heart rates (87.43 ± 15.52 at baseline vs. 67.62 ± 14.81) ($p < .001$). Mean heart rate changes (±95% confidence interval (CI)) after each Remdesivir administrations are shown in Figure 1. As shown in Table 5, ECG parameters like ventricular rate, QT, and QTc interval durations changed significantly after Remdesivir administration. In the present study, the mean QTc interval duration shortened significantly after the fifth dose of Remdesivir compared to baseline (reduced 6 ms, p-value = .026). Of all patients, 9.1% had QTc interval prolongation prior to Remdesivir administration, and 6.7% developed QTc interval prolongation afterward. Three patients (two women and a man) aged 37 to 42 developed extreme QTc prolongation (QTc>500 ms), but none of the patients developed an arrhythmia, including torsades de pointes and atrial fibrillation.

TABLE 3 Univariable and multivariable logistic regression model of predictor variables for bradycardia.

Characteristics	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P	OR (95% CI)	P
Age>60	.994 (.972–1.016)	.598	2.237 (.679–7.369)	.185
Female gender	.791 (.403–1.553)	.495	.825 (.359–1.895)	.650
D-dimer	1.000 (.998–1.001)	.498		
Lymphocyte	1.000 (.999–1.001)	.850		
O2 sat<90%	.965 (.903–1.032)	.296	.459 (.147–1.435)	.181
CRP>50	1.007 (.987–1.027)	.525	2.022 (.364–11.235)	.421
Beta-blocker	.609 (.238–1.559)	.301	.115 (.010–1.321)	.083
Temperature	.890 (.509–1.555)	.683		
CVD	.279 (.095–.821)	.020	.589 (.097–3.571)	.564
Cr	.817 (.329–2.034)	.665		
BUN	1.000 (.985–1.015)	.960		
Diabetes	.810 (.310–2.117)	.668	.986 (.238–4.085)	.985
Hypertension	1.456 (.510–4.156)	.483	4.349 (.486–38.895)	.188
Tocilizumab	2.583 (1.106–6.034)	.028		

CVD, cardiovascular diseases; CRP, C-reactive protein; Cr, creatinine; BUN, blood urea nitrogen.

4 Discussion

The present study evaluated the incidence of sinus bradycardia, the most frequent cardiovascular adverse drug reaction of Remdesivir (Lucijanic and Bistrovic, 2022), in patients infected with COVID-19 and also the association between patients' demographic characteristics, clinical conditions, and the severity of COVID-19 and developing bradycardia.

The prevalence of bradycardia following Remdesivir administration varies widely based on the literature review, from only 3.6% to up to 60%. This wide diversity may be due to the differences in patients' demographic characteristics, comorbidities and risk factors, medication history, and severity of COVID-19 infection, different study designs and selection bias. Based on the data in the present study, the overall incidence of bradycardia was 27% with 19% HR < 50 bpm, which further supports previous findings on the association between Remdesivir and bradycardia. Palloto et al. found 60% bradycardia incidence after Remdesivir administration *versus* 23% in the control group. However, their sample size was small (46 patients) and included only 20 patients in the Remdesivir group. They found that the age >65 years and Remdesivir were associated with bradycardia. (Pallotto et al., 2021a). In another retrospective study on 141 patients, the incidence of bradycardia in Remdesivir group was significantly higher (46.8% compared to 27.8% in the control group (OR = 2.15)). (Pallotto et al., 2021b). In a recent study of 180 patients with COVID-19 infection who received Remdesivir, 28.7% developed bradycardia, similar to the incidence of bradycardia in this study. (Kumar et al., 2021a). In the study by Toufchia et al. based on the VigiBase reports, there were only 94 reports of bradycardia among 2,603 patients who received Remdesivir (3.6%), and 17% developed fatal bradycardia. (Touafchia et al., 2021). The low overall

TABLE 4 Ventricular rate changes before and after Remdesivir administration.

Rate	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
					25th	50th (Median)	75th
Basal	87.43	15.522	60	130	75.00	88.00	100.00
Final	67.62	14.814	25	115	60.00	65.00	75.00

TABLE 5 ECG characteristics before and after Remdesivir administration.

Characteristics	Basal ECG	Final ECG	*p*-Value
Ventricular rate (bpm)	87.43 ± 15.522	67.62 ± 14.814	.000
PR segment duration (ms)	144.17 ± 31.059	149.59 ± 35.469	.080
QT interval duration (ms)	335.78 ± 44.891	381.98 ± 82.232	.000
QTc interval duration (ms)	400.35 ± 39.429	394.52 ± 54.413	.026
QRS width (ms)	79.94 ± 39.209	78.65 ± 29.545	.281

Abbreviations: bpm, beats per minute; ECG, electrocardiographic; ms, millisecond*$p < .05$ considered statistically significant, using Wilcoxon signed test for paired samples.

incidence of bradycardia in this study could be due to the indirect investigation of Remdesivir complication reports possibly leading to underestimation and selection bias. (Pallotto et al., 2021b; Touafchia et al., 2021). The present study has a large sample size estimatedprioribased on the literature review, and the patients were selected randomly to limit the selection bias and maintain the external validity. Also, the prospective design of this study maintained the direct evaluation of patients in a real-life setting. These, along with controlling potential cofounders by takingcomplete history and concise methods formeasurements to maintain the internal validity, are the strong points of the present study.

The results of the present study showed that the incidence of bradycardia increased over continuous exposure to Remdesivir. The highest incidence of bradycardia occurred within the five sessions of drug administration (21%), with the most HR reduction compared to baseline. Accordingly, no case of bradycardia was observed after the first session of Remdesivir administration. The mean HR decreased significantly with each drug administration. This can be explained by the accumulative toxicity effect of Remdesivir observed by Choi et al. (Choi et al., 2020; Kumar et al., 2021a) who revealed that Remdesivir cell toxicity increases over time. They observed that the viability of cardiomyocytes considerably decreased by a longer treatment with Remdesivir (48 vs. 24 h). (Choi et al., 2020). This observation is also consistent with the study of Bistrovic et al., who found the frequency of bradycardia consistently increased with every further dose of Remdesivir administration, indicating the causal relation between Remdesivir and bradycardia. (Bistrovic et al., 2022). They also observed that the increased level of Remdesivir above the estimated level of peak plasma concentration was potentially associated with QT interval prolongation. The spontaneous beating was almost completely blocked at higher doses of Remdesivir in their experiment. (Choi et al., 2020). Jung et al. also found that the risk of developing serious cardiac complications increases with drug accumulation or overdose. (Jung et al., 2022). They suggested ECG monitoring during Remdesivir administration, especially for severe COVID-19 infection cases as well as those with structural heart diseases. (Choi et al., 2020; Nabati and Parsaee, 2022). we found that cardiovascular disease and Tocilizumab administration associated with bradycardia in multivariable regression model but it was not confirmed in the multivariable model.

In the present study, the mean QTc interval duration shortened significantly after the fifth dose of Remdesivir compared to baseline. This was in contrast with the hypothesis about Remdesivir induced QTc prolongation. Remdesivir has the potential to inhibit the potassium channel encoded by the human ether-a-go-go gene (hERG) and prolongs the ventricular repolarization, causing QT prolongation and torsades de pointes. (Haghjoo et al., 2021; Michaud et al., 2021; Touafchia et al., 2021).In contrast to the present results, the study of Haghjoo et al. on 67 COVID-19 patients treated with Remdesivir showed a significant increase in QTc interval duration but no arrhythmic event such as torsades de pointes (Tdp) was observed. Their only case with critical QTc prolongation was under treatment with Azithromycin and Remdesivir. (Haghjoo et al., 2021). Gupta et al. (Gupta et al., 2020) reported a case with COVID-19 who developed critical QTc prolongation on the third dose of Remdesivir (>555). However, this patient had received Azithromycin as well, which is known to cause QTc prolongation. (Gupta et al., 2020). In a prospective study, Bistrovic et al. investigated 14 patients with COVID-19 infection and found no significant difference in QTc interval and HR after Remdesivir administration. (Bistrovic and Lucijanic, 2021). Even though the mean QTc duration was reduced in the present study, three patients (1.6%) developed extreme QTc prolongation (QTc>500) after Remdesivir administration. It is noteworthy that none of these patients had a history of prior cardiovascular structural diseases and other

comorbidities and risk factors for QTc prolongation or a clinically severe COVID-19 infection. They all remained asymptomatic, and none developed consequent arrhythmia related to QTc prolongation. It appears that Remdesivir has a low potential risk of inducing torsades de pointes, as no case of this and other arrhythmias related to QTc prolongation were observed in this neither study nor previous studies. (Gupta et al., 2020; Haghjoo et al., 2021).

While no association was found between COVID-19 severity indicators and bradycardia, previous studies have shown that SARS-CoV-2 can itself induce bradycardia and arrhythmias in severely infected patients. (Oliva et al., 2021). One possible mechanism for this clinical observation is the cardiotoxicity caused by the inflammation and cytokine release during COVID-19 infection, which may increase the vagal tonicity in the heart. Interleukin 6 (IL6), as an important component of cytokine storm, can increase vagal tonicity. Other mechanisms include the impairment of sinus node normal activity due to direct viral inhibition and defects in the autonomic system function due to direct SARS-CoV-2 toxic effects on the nervous system. (Ye et al., 2018; Hu et al., 2020; Oliva et al., 2021). According to these potential mechanisms, the bradycardia development in the context of severe COVID-19 infection regardless of Remdesivir treatment, may be suggestive of the unfavorable infection course as was observed in Kumar et al. study in which developing bradycardia was associated with a higher mortality rate (OR = 6.59). (Kumar et al., 2021b). Nevertheless, the CRP levels were not suggestive of developing bradycardia in patients in the present study (p-value = .41). All studied patients had a favorable prognosis regardless of developing bradycardia and no case of mortality or intubation during hospitalization was observed. The reason could be that most patients in this study had less severe COVID-19 infections and received Remdesivir in an outpatient setting. 19.

Two recent studies revealed that the possibility of developing bradycardia is even higher in less severe COVID-19 cases. (Brunetti et al., 2021; Bistrovic et al., 2022). In a study on 52 patients, The highest HR reduction after Remdesivir treatment was observed in patients with a less clinically severe COVID-19 infection. No association was observed between age, underlying cardiovascular diseases, drugs, and other comorbidities with HR reduction in their multivariate logistic regression analysis. The only significant correlation of bradycardia was observed in less severe COVID-19 infection cases. (Brunetti et al., 2021).

This was similar to the result of this study as to no association between age, comorbidities and risk factors, drug history and developing bradycardia in the multivariate regression model observed. It is noteworthy that in their study, 76% of the subjects were older than 50, 53% had an underlying cardiovascular disease, and 77% had severe COVID-19 presentation. These observations suggest the absence of contraindication when administrating Remdesivir to even critical patients and those with cardiovascular diseases and risk factors despite what was generally hypothesized. (Brunetti et al., 2021). Bistrovic et al. conducted a retrospective investigation on 455 patients who received Remdesivir for the COVID-19 infection and found that the prevalence of bradycardia was significantly higher among survived patients compared to those who died (19% vs. 7%). They observed that developing bradycardia caused by Remdesivir had a significant relationship with a favorable disease course and prognosis. (Bistrovic et al., 2022). The reason may be the intensified sympathetic-adrenergic simulation in patients with severe infections and respiratory failure or that the higher concentrations of Remdesivir metabolites lead to higher simultaneous antiviral and chronotropic effects. So, developing bradycardia following Remdesivir administration should encourage clinicians to continue rather than discontinue the treatment. However, close monitoring is suggested, especially for patients with comorbidities who need synchronous medications for their underlying clinical conditions. (Bistrovic et al., 2022; Lucijanic and Bistrovic, 2022).

In the present study, almost all cases with bradycardia were asymptomatic, and for all patients, sinus bradycardia was transient and returned to normal HR after Remdesivir discontinuation. This is consistent with other case reports about Remdesivir-induced bradycardia being a transitory phenomenon. Developing bradycardia did not affect the clinical outcome of patients in the present study and did not impede the continued drug courses. Only one patient experienced presyncope symptoms at the fifth Remdesivir dose with an extreme decrease in the HR to 25bpm. The drug administration stopped, and the patient received Atropine. The HR returned to normal, and the patient could receive Remdesivir in the following days. None of the studied patients developed an arrhythmia, including atrial fibrillation and cardiac arrest. This finding may be due to the scarcity of these complications, and further investigation of these complications with larger sample sizes is required.

5 Limitations

The sample size was small for rare cardiac complications of Remdesivir, such as atrial fibrillation, cardiac arrest, and other rarely reported cardiac arrhythmias.

6 Conclusion

Sinus bradycardia is a prevalent adverse cardiac effect of Remdesivir. It is recommended that all COVID-19 patients receiving Remdesivir, be evaluated for heart rate based on examination; and in the case of bradyarrhythmia, cardiac monitoring should be performed during administration to prevent adverse events.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The study obtained the approval of the Iran National Committee for Ethics in Biomedical Research and followed the national standards for performing Medical Research in Iran (Ethic

code: IR. SBMU.NRITLD.REC.1400.050, approval date: 2021-09-26), and the ethical guidelines outlined in the 1975 Helsinki Declaration. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Study concept and design: BS, FD, MH, AA, FN, AM, SL, and SS. Acquisition of data: SA, MAS, MT, MH, and YA. Analysis and interpretation of data: SS and MH. Drafting of the manuscript: MH and SN. Critical revision of the manuscript for important intellectual content: SS, MH, and MK. Statistical analysis: NA and MH. Administrative, technical, and material support: SN, MS, MH, and ZR. Study supervision: BK.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Baghaei, P., Nadji, S. A., Marjani, M., Moniri, A., Hashemian, S. M., Sheikhzade, H., et al. (2020). Clinical manifestations of patients with coronavirus disease 2019 (COVID-19) in a referral center in Iran. *Tanaffos* 19 (2), 122.

Barkas, F., Styla, C-P., Bechlioulis, A., Milionis, H., and Liberopoulos, E. (2021). Sinus bradycardia associated with remdesivir treatment in COVID-19: A case report and literature review. *J. Cardiovasc. Dev. Dis.* 8, 18. doi:10.3390/jcdd8020018

Bazett, H. C. (1997). AN analysis of the time-relations of electrocardiograms. *Ann. Noninvasive Electrocardiol.* 2 (2), 177–194. doi:10.1111/j.1542-474x.1997.tb00325.x

Beigel, J. H., Tomashek, K. M., Dodd, L. E., Mehta, A. K., Zingman, B. S., Kalil, A. C., et al. (2020). Remdesivir for the treatment of covid-19 - final report. *N. Engl. J. Med.* 383 (19), 1813–1826. doi:10.1056/NEJMoa2007764

Bistrovic, P., and Lucijanic, M. (2021). Remdesivir might induce changes in electrocardiogram beyond bradycardia in patients with coronavirus disease 2019-The pilot study. *J. Med. virology* 93, 5724–5725. doi:10.1002/jmv.27177

Bistrovic, P., Manola, S., and Lucijanic, M. (2022). Bradycardia during remdesivir treatment might be associated with improved survival in patients with COVID-19: A retrospective cohort study on 473 patients from a tertiary centre. *Postgrad. Med. J.* 98 (1161), 501–502. doi:10.1136/postgradmedj-2021-141079

Brunetti, N. D., Poliseno, M., Bottalico, I. F., Centola, A., Montemurro, L., Sica, S., et al. (2021). Safety and heart rate changes in Covid-19 patients treated with Remdesivir. *Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis.* 112, 254–257. doi:10.1016/j.ijid.2021.09.036

Chen, J., Zhang, Z. Z., Chen, Y. K., Long, Q. X., Tian, W. G., Deng, H. J., et al. (2020). The clinical and immunological features of pediatric COVID-19 patients in China. *Genes. Dis.* 7 (4), 535–541. doi:10.1016/j.gendis.2020.03.008

Ching, P. R., and Lee, C. (2021). Remdesivir-associated bradycardia. *BMJ Case Rep.* 14 (9), e245289. doi:10.1136/bcr-2021-245289

Choi, S. W., Shin, J. S., Park, S-J., Jung, E., Park, Y-G., Lee, J., et al. (2020). Antiviral activity and safety of remdesivir against SARS-CoV-2 infection in human pluripotent stem cell-derived cardiomyocytes. *Antivir. Res.* 184, 104955. doi:10.1016/j.antiviral.2020.104955

Coronavirus Disease 2019 (2021). *(COVID-19) treatment guidelines*. Bethesda (MD): National Institutes of Health US.

Day, L. B., Abdel-Qadir, H., and Fralick, M. (2021). Bradycardia associated with remdesivir therapy for COVID-19 in a 59-year-old man. *C Can. Med. Assoc.* 193 (17), E612–E615. doi:10.1503/cmaj.210300

Drumheller, B. C., Tuffy, E., Gibney, F., Stallard, S., Siewers, C., and Korvek, S. (2022). Severe bradycardia from severe hyperkalemia: Patient characteristics, outcomes and factors associated with hemodynamic support. *Am. J. Emerg. Med.* 55, 117–125. doi:10.1016/j.ajem.2022.03.007

Ganesh, B., Rajakumar, T., Malathi, M., Manikandan, N., Nagaraj, J., Santhakumar, A., et al. (2021). Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: An updated overview of current knowledge and future perspectives. *Clin. Epidemiol. Glob. Heal* 10, 100694. doi:10.1016/j.cegh.2020.100694

Goldman, J. D., Lye, D. C. B., Hui, D. S., Marks, K. M., Bruno, R., Montejano, R., et al. (2020). Remdesivir for 5 or 10 Days in patients with severe covid-19. *N. Engl. J. Med.* 383 (19), 1827–1837. doi:10.1056/NEJMoa2015301

Gordon, C. J., Tchesnokov, E. P., Woolner, E., Perry, J. K., Feng, J. Y., Porter, D. P., et al. (2020). Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem.* 295 (20), 6785–6797. doi:10.1074/jbc.RA120.013679

Gottlieb, R. L., Vaca, C. E., Paredes, R., Mera, J., Webb, B. J., Perez, G., et al. (2022). Early remdesivir to prevent progression to severe covid-19 in outpatients. *N. Engl. J. Med.* 386 (4), 305–315. doi:10.1056/NEJMoa2116846

Grein, J., Ohmagari, N., Shin, D., Diaz, G., Asperges, E., Castagna, A., et al. (2020). Compassionate use of remdesivir for patients with severe covid-19. *N. Engl. J. Med.* 382 (24), 2327–2336. doi:10.1056/NEJMoa2007016

Gubitosa, J. C., Kakar, P., Gerula, C., Nossa, H., Finkel, D., Wong, K., et al. (2020). Marked sinus bradycardia associated with remdesivir in COVID-19: A case and literature review. *JACC. Case Rep.* 2, 2260–2264. doi:10.1016/j.jaccas.2020.08.025

Gupta, A. K., Parker, B. M., Priyadarshi, V., and Parker, J. (2020). Cardiac adverse events with remdesivir in COVID-19 infection. *Cureus* 12, e11132. doi:10.7759/cureus.11132

Haghjoo, M., Golipra, R., Kheirkhah, J., Golabchi, A., Shahabi, J., Oni-Heris, S., et al. (2021). Effect of COVID-19 medications on corrected QT interval and induction of torsade de pointes: Results of a multicenter national survey. *Int. J. Clin. Pract.* 75 (7), e14182. doi:10.1111/ijcp.14182

Hu, L., Gong, L., Jiang, Z., Wang, Q., Zou, Y., and Zhu, L. (2020). Clinical analysis of sinus bradycardia in patients with severe COVID-19 pneumonia. *Crit. care* 24, 257. doi:10.1186/s13054-020-02933-3

Jacinto, J. P., Patel, M., Goh, J., and Yamamura, K. (2021). Remdesivir-induced symptomatic bradycardia in the treatment of COVID-19 disease. *Hear. case Rep.* 7, 514–517. doi:10.1016/j.hrcr.2021.05.004

Jung, S. Y., Kim, M. S., Li, H., Lee, K. H., Koyanagi, A., Solmi, M., et al. (2022). Cardiovascular events and safety outcomes associated with remdesivir using a World Health Organization international pharmacovigilance database. *Clin. Transl. Sci.* 15 (2), 501–513. doi:10.1111/cts.13168

Kow, C. S., Aldeyab, M., and Hasan, S. S. (2021). Quality of adverse event reporting in clinical trials of remdesivir in patients with COVID-19. *Eur. J. Clin. Pharmacol.* 77, 435–437. doi:10.1007/s00228-020-03008-6

Kumar, S., Arcuri, C., Chaudhuri, S., Gupta, R., Aseri, M., Barve, P., et al. (2021). A novel study on SARS-COV-2 virus associated bradycardia as a predictor of mortality-retrospective multicenter analysis. *Clin. Cardiol.* 44 (6), 857–862. doi:10.1002/clc.23622

Kumar, S., Arcuri, C., Chaudhuri, S., Gupta, R., Aseri, M., Barve, P., et al. (2021). Remdesivir therapy associated with Bradycardia in SARS-CoV2. *Clin. Cardiol.* 44, 1190–1191. doi:10.1002/clc.23700

Lucijanic, M., and Bistrovic, P. (2022). Remdesivir-associated bradycardia might be a sign of good prognosis in COVID-19 patients. *Clin. Microbiol. Infect.* 28, 619. doi:10.1016/j.cmi.2021.12.017

Michaud, V., Dow, P., Al Rihani, S. B., Deodhar, M., Arwood, M., Cicali, B., et al. (2021). Risk assessment of drug-induced long QT syndrome for some COVID-19 repurposed drugs. *Clin. Transl. Sci.* 14 (1), 20–28. doi:10.1111/cts.12882

Mirenayat, M. S., Abedini, A., Kiani, A., Eslaminejad, A., Malekmohammad, M., Heshmatnia, J., et al. (2022). National research institute of tuberculosis and lung disease (NRITLD) protocol for the treatment of patients with COVID-19. *Iran. J. Pharm. Res.* 21 (1).

Nabati, M., and Parsaee, H. (2022). Potential cardiotoxic effects of remdesivir on cardiovascular system: A literature review. *Cardiovasc Toxicol.* 22 (3), 268–272. doi:10.1007/s12012-021-09703-9

Oliva, A., Franchi, C., Gatto, M. C., Galardo, G., Pugliese, F., and Mastroianni, C. (2021). Prevalence and clinical significance of relative bradycardia at hospital admission in patients with coronavirus disease 2019 (COVID-19). *Clin. Microbiol. Infect.* 27, 1185–1187. doi:10.1016/j.cmi.2021.04.013

Pallotto, C., Blanc, P., Esperti, S., Suardi, L. R., Gabbuti, A., Vichi, F., et al. (2021). Remdesivir treatment and transient bradycardia in patients with coronavirus diseases 2019 (COVID-19). *J. Infect.* 83, 237–279. doi:10.1016/j.jinf.2021.05.025

Pallotto, C., Suardi, L. R., Gabbuti, A., Esperti, S., Mecocci, L., and Blanc, P. (2021). Potential remdesivir-related transient bradycardia in patients with coronavirus disease 2019 (COVID-19). *J. Med. virology* 93, 2631–2634. doi:10.1002/jmv.26898

Pasquini, Z., Montalti, R., Temperoni, C., Canovari, B., Mancini, M., Tempesta, M., et al. (2020). Effectiveness of remdesivir in patients with COVID-19 under mechanical ventilation in an Italian ICU. *J. Antimicrob. Chemother.* 75 (11), 3359–3365. doi:10.1093/jac/dkaa321

Rezaei, M., Jamaati, H., Tabarsi, P., Moniri, A., Marjani, M., and Velayati, A. A. (2021). NRITLD protocol for the management of outpatient cases of COVID-19. *TANAFFOS (Respiration)* 20 (3), 192–196.

Russo, V., Carbone, A., Mottola, F. F., Mocerino, R., Verde, R., Attena, E., et al. (2020). Effect of triple combination therapy with lopinavir-ritonavir, Azithromycin, and hydroxychloroquine on QT interval and arrhythmic risk in hospitalized COVID-19 patients. *Front. Pharmacol.* 11, 582348. doi:10.3389/fphar.2020.582348

Sanchez-Codez, M. I., Rodriguez-Gonzalez, M., and Gutierrez-Rosa, I. (2021). Severe sinus bradycardia associated with Remdesivir in a child with severe SARS-CoV-2 infection. *Eur. J. Pediatr.* 180, 1627. doi:10.1007/s00431-021-03940-4

Sarkar, C., Mondal, M., Torequl Islam, M., Martorell, M., Docea, A. O., Maroyi, A., et al. (2020). Potential therapeutic options for COVID-19: Current status, challenges, and future perspectives. *Front. Pharmacol.* 11, 572870. doi:10.3389/fphar.2020.572870

Selvaraj, V., Bavishi, C., Patel, S., and Dapaah-Afriyie, K. (2021). Complete heart block associated with remdesivir in COVID-19: A case report. *Eur. heart J. Case Rep.* 5, ytab200. doi:10.1093/ehjcr/ytab200

Touafchia, A., Bagheri, H., Carrié, D., Durrieu, G., Sommet, A., Chouchana, L., et al. (2021). Serious bradycardia and remdesivir for coronavirus 2019 (COVID-19): A new safety concerns. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* 27 (5), 791–798. doi:10.1016/j.cmi.2021.02.013

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., et al. (2020). Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *in vitro*. *Cell. Res.* 30, 269–271. doi:10.1038/s41422-020-0282-0

Ye, F., Winchester, D., Stalvey, C., Jansen, M., Lee, A., Khuddus, M., et al. (2018). Proposed mechanisms of relative bradycardia. *Med. Hypotheses* 119, 63–67. doi:10.1016/j.mehy.2018.07.014

Zeng, H., Ma, Y., Zhou, Z., Liu, W., Huang, P., Jiang, M., et al. (2021). Spectrum and clinical characteristics of symptomatic and asymptomatic coronavirus disease 2019 (COVID-19) with and without pneumonia. *Front. Med.* 8, 645651. doi:10.3389/fmed.2021.645651

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Evaluation of noscapine-licorice combination effects on cough relieving in COVID-19 outpatients: A randomized controlled trial

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Background: As February 2023, SARS-CoV-2 is still infecting people and children worldwide. Cough and dyspnea are annoying symptoms almost present in a large proportion of COVID-19 outpatients, and the duration of these symptoms might be long enough to affect the patients' quality of life. Studies have shown positive effects for noscapine plus licorice in the previous COVID-19 trials. This study aimed to assess the effects of the combination of noscapine and licorice-for relieving cough in outpatients with COVID-19.

Methods: This randomized controlled trial was conducted on 124 patients at the Dr. Masih Daneshvari Hospital. Participants over 18 years of age with confirmed COVID-19 and cough were allowed to enter the study if the onset of symptoms was less than 5 days. The primary outcome was to assess the response to treatment over 5 days using the visual analogue scale. Secondary outcomes included the assessment of cough severity after 5 days using Cough Symptom Score, as well as the cough-related quality of life and dyspnea relieving. Patients in the noscapine plus licorice group received Noscough® syrup 20 mL every 6 h for 5 days. The control group received diphenhydramine elixir 7 mL every 8 h.

Results: By day five, 53 (85.48%) patients in the Noscough® group and 49 (79.03%) patients in the diphenhydramine group had response to treatment. This difference was not statistically significant (p-value = 0.34). The presence of dyspnea was significantly lower in the Noscough® group *versus* diphenhydramine at day five (1.61% in the Noscough® group vs. 12.9% in the diphenhydramine group; p-value = 0.03). The cough-related quality of life and severity also significantly favored Noscough® syrup (p-values <0.001).

Conclusion: Noscapine plus licorice syrup was slightly superior to diphenhydramine in relieving cough symptoms and dyspnea in the COVID-19 outpatients. The severity of cough and cough-related quality of life were also significantly better in the noscapine plus licorice syrup. Noscapine plus licorice may be a valuable treatment in relieving cough in COVID-19 outpatients.

KEYWORDS

COVID-19, cough, glycyrrhiza, noscapine, outpatients

Introduction

As of December 2022, seroprevalence studies suggest that up to 80%–90% of the global population has already had an infection with SARS-CoV-2 (Huang et al., 2020). The key strategies to battle the infection are crucial to reducing disease spread—including physical distancing, wearing masks, and avoiding crowds (Dhand and Li, 2020). The cough is a key symptom of COVID-19 comparable to the more common but less severe respiratory infections, including common cold or flu in the acute and post-infective phases of the infection. Moreover, cough increases the risk of community transmission by respiratory droplets, distressing patients and leading to social isolation (Song et al., 2021). Many mechanisms have been suggested for COVID-19- induced cough including neuroinflammation or neuroimmunomodulation *via* the sensory nerves (Song et al., 2021). Another potential mechanism of cough in COVID-19 is bradykinin syndrome which is defined as the reduced degradation of bradykinin in the body that may further lead to dry cough not responding to regular treatments (Alkotaji and Al-Zidan, 2021). Identifying ways to relieve COVID-19-associated cough could help to prevent community transmission and disease spread, along with removing the stigma of this symptom (Dhand and Li, 2020). As well, the exact mechanisms of COVID-19-associated cough are unclear. Accordingly, evidence-based treatment options for COVID-19 cough are needed (Song et al., 2021). The current antitussive medication used for the treatment of cough includes diphenhydramine which has much anticholinergic activity leading to blurred vision, constipation, urinary retention and xerostomia. Most elderly patients will not tolerate these adverse events. Moreover, opioid antitussives including dextromethorphan may induce central nervous system (CNS) depression and they also may be associated with respiratory issues (Enna and Bylund 2008).

Different measures have existed in viral respiratory infections to treat cough. Among them, natural and herbal products are common (Shergis et al., 2015). Products containing noscapine or licorice are used widely to relieve cough (Shergis et al., 2015; Kuang et al., 2018). Noscapine is a naturally occurring opium-isoquinoline alkaloid that is related to papaverine. It acts centrally as a cough suppressant and has actions and uses similar to dextromethorphan (Enna and Bylund 2008). Unlike opioid antitussives, respiratory and CNS depression, as well as addiction, have not been reported with noscapine (Enna and Bylund 2008). Moreover, noscapine can help decrease bradykinin-mediated cytokine release due to Angiotensin converting 2 enzyme inhibition by SARS-CoV-2. In turn, it can reduce tissue damage, especially in the lungs (Ebrahimi, 2020). As the previous studies have shown that the accumulation of bradykinin along with cytokine storms may be the culprit for the pathogenesis of SARS-CoV-2 (Wilczynski et al., 2021). A cytokine storm is a hyperinflammatory state that can lead to excessive production of cytokines by a deregulated immune system (Zanza et al., 2022).

The licorice is a popular traditional Chinese medicine (TCM) used to treat respiratory diseases, including cough, sore throat, asthma, and bronchitis (Kuang et al., 2018).

Noscough® is a natural syrup (containing noscapine and licorice extract) used as an antitussive medication. Based on these reasons, the use of Noscough® may be a safe and effective option for the treatment of COVID-19 cough. Hence, this study aimed to investigate the effects of Noscough® syrup in relieving cough in the outpatients with COVID-19.

Materials and methods

Study design

This randomized controlled trial was conducted at Dr. Masih Daneshvari Hospital—a tertiary referral center in Tehran, Iran, affiliated with Shahid Beheshti University of Medical Sciences (SBMU).

The study was approved by the Ethics Committee of SBMU, Tehran, Iran (Ethics code: IR.SBMU.PHARMACY.REC.1400.252) with registry code of IRCT20151227025726N31 in the Iranian registry of clinical trials (IRCT). Informed written consent was obtained from all patients before allocation.

Participants

Patients over 18 years of age with cough and positive reverse transcriptase polymerase chain reaction (RT-PCR) test for COVID-19 with the onset time of less than 5 days were included in the study.

Exclusion criteria were as follows: pregnancy or breastfeeding, history of allergy to noscapine, licorice, diphenhydramine, morphine or other excipients of the study medications, history of seizure, diarrhea or diabetes, consumption of warfarin, benzodiazepines, opioid agonists, and other antitussive medications.

Randomization and blinding

The block balanced randomization method (twenty-five blocks, including four patients in each block) was used to allocate patients to the Noscough® and control groups. In each block, two patients were assigned to the Noscough® group and two to the control group. The participants were not blinded to the study due to the differences in administration schedules.

Outcomes

The primary outcome of the study was to assess the response to treatment during 5 days. The visual analogue scale (VAS) score was assessed by patients to evaluate treatment response. The VAS employs a linear scoring method with a straight line with calibration of 0, 1, 2–10 cm (scale lines marked from 0 to 100 mm can also be used); 0 indicates asymptomatic, and 10 represents the most serious (Spinou and Birring, 2014). Treatment response was defined as a decrease of ≥50% in the average VAS score.

Secondary outcomes included the assessment of cough severity after 5 days using cough symptom score (CSS). The CSS is a two-part questionnaire referring to symptoms during the day and night. Based on the frequency, intensity, and influence of cough on daily activities and sleep, cough symptoms are scored from 0 to 5, with 0 indicating no cough and five indicating the most severe cough (Wang et al., 2019). VAS and CSS scores were measured at baseline and day five after treatment.

Another secondary outcome included the assessment of the cough-related quality of life *via* a cough-specific quality of life questionnaire (CQLQ). The CQLQ comprises 28 questions regarding cough and its effects on life. This questionnaire is scored with a 4-point Likert scale,

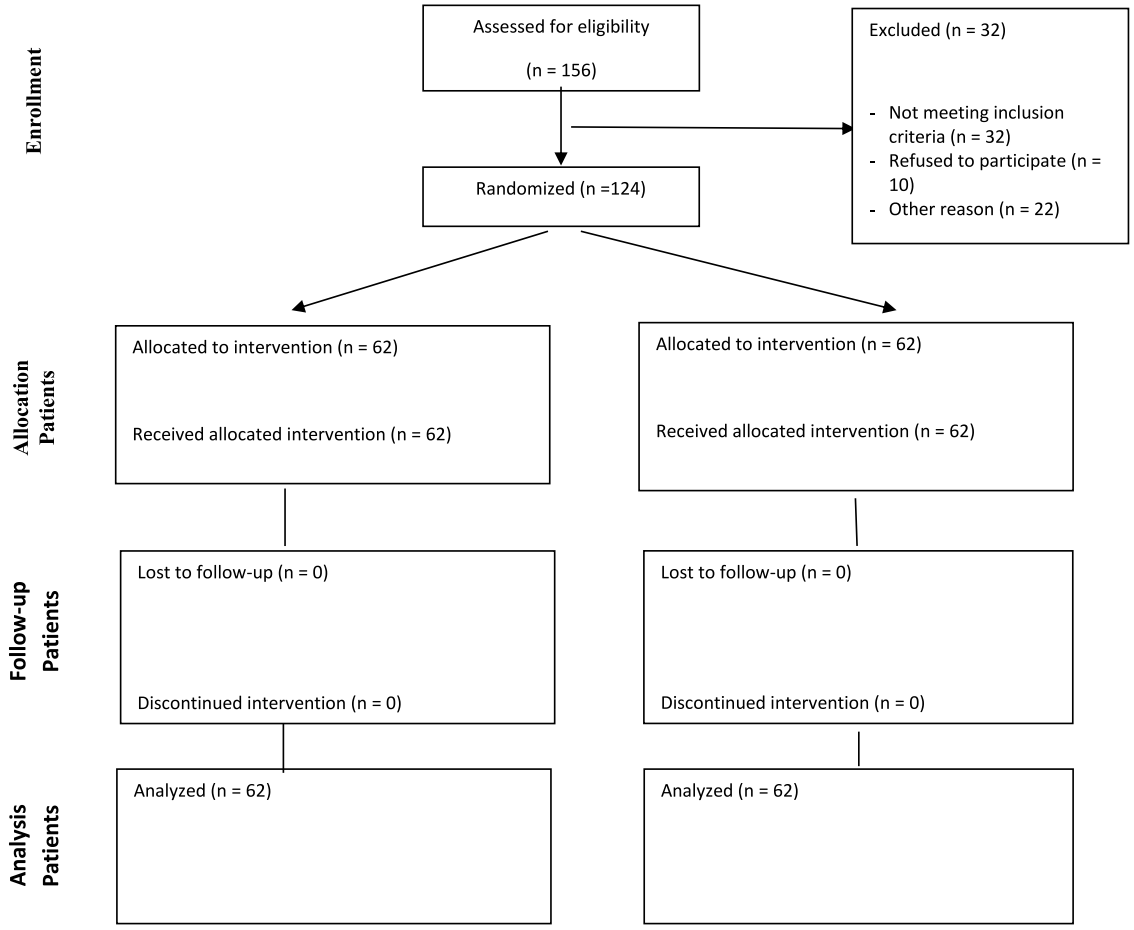

FIGURE 1
CONSORT diagram showing screening, randomization, and analysis of the participants. The whole participants were included in the analysis.

with lower scores indicating less impact of cough on health-related quality of life. The CQLQ total score can range from 28 to 112 (Lechtzin et al., 2013). The CQLQ questionnaire was measured and recorded on day one (baseline) and 5 days after treatment.

Intervention

The patients in the Noscough® group received Noscough® syrup (Faran Shimi, Iran, each 5 mL contain 7 mg noscapine and 5 mg licorice extract), 20 mL every 6 h for 5 days. The control group received diphenhydramine (Pursina, Iran, each 5 mL contain 12.5 mg diphenhydramine) 7 mL every 8 h. No other medications were received. Patients in both groups received cetirizine 10 mg once daily for the relief of coryzal symptoms. Patients were allowed to leave the study at any time. Their demographic characteristics, underlying diseases, and medication histories were recorded at baseline.

Statistical analysis

The sample size was calculated based on assuming 55% response to treatment for the diphenhydramine group and 80% response for the Noscough® syrup. This difference was estimated based on the investigators' opinion and evidence-based useful theoretical mechanisms for the antitussive effects of Noscough®. Considering 80% power, error type 1 of 0.05 and a 12% drop-out rate, 62 patients were calculated in each group.

The statistical analyses were performed using SPSS software for Windows (Version 23.0; SPSS Inc., Chicago, IL, United States) and STATA 17. Categorical and nominal variables were expressed as frequency (%) and were compared using the Chi-Square test. The risk difference was calculated as a proper effect size for the primary outcome. Continuous variables were expressed as means ± standard deviations or 95% confidence intervals. An ANCOVA model was employed to assess the differences between the patient-reported outcomes using the baseline values as covariates. p-values <0.05 were considered significant.

Results

Study participants

In total, 124 patients were randomized to diphenhydramine and Noscough® groups equally. The screening and randomization

TABLE 1 Demographics and past medical histories of the patients.

Characteristics	Diphenhydramine (*N* = 62)	Noscough® (*N* = 62)
Sex — n (%)		
Male	38 (61.29)	34 (54.83)
Age (y) — mean ± SD	43.88 ± 11.17	44.98 ± 15.3
Medical and drug history — n (%)		
Receiving corticosteroids	36 (58.06)	37 (59.67)
Receiving Remdesivir	36 (58.06)	36 (58.06)
Hypertension	8 (12.09)	9 (14.5)
Diabetes mellitus	7 (11.29)	8 (12.09)
Lung Diseases	0 (0)	4 (6.45)
Chronic kidney diseases	4 (6.45)	1 (1.61)
Immunodeficient	3 (4.83)	2 (3.22)
Baseline cough related characteristics		
CSS — mean ± SD	3.06 ± 1.09	3.2 ± 1.04
VAS — mean ± SD	5.53 ± 2.4	5.98 ± 2.45
CQLQ — mean ± SD	60.95 ± 9.06	63.09 ± 10.35
Dyspnea symptom— n (%)	29 (46.77%)	24 (38.7%)

CSS, cough symptom score; VAS, visual analogue scale; CQLQ, cough-specific quality of life questionnaire.

TABLE 2 Results of the primary and secondary outcomes.

Characteristics	Diphenhydramin (*N* = 62)	Noscough® (*N* = 62)	*p*-value
Patients with response to treatment during 5 days — n (%) §	49 (79.03%)	53 (85.48%)	0.34
Dyspnea at day 5	8 (12.9%)	1 (1.61%)	0.03
CSS day 5 — mean (95% CI) ¥	1.53 (1.31—1.76)	1.25 (1.02—1.47)	<0.001
VAS day 5 — mean (95% CI) ¥	2.1 (1.69—2.5)	1.42 (1.02—1.83)	<0.001
CQLQ day 5 — mean (95% CI) ¥	43.6 (41.38—45.81)	39.91 (37.69—42.12)	<0.001

§: based on the chi-square test.
¥: adjusted based on baseline values (ANCOVA, model).

process of the patients are provided in the CONSORT diagram in Figure 1.

Table 1 shows the demographics and past medical histories of the patients. It also demonstrates the baseline values of the patient reported outcomes. There were no meaningful differences regarding the baseline parameters among the two groups.

The results of the primary and secondary outcomes are provided in Table 2. By day five, the incidence of response to treatment in the Noscough® group was 85.48% *versus* 79.03% in the diphenhydramine group. However, this difference was not statistically significant (*p*-value = 0.34). The effect size of this difference was calculated to be 0.06.

As the table shows, by day five, dyspnea was still present in 12.9% of the patients in the diphenhydramine group. Whereas only 1.61% of the patients in the Noscough® group still had dyspnea by the end of the treatment period (*p*-value = 0.03).

As Table 2 shows, considering the baseline values, the results of the quality of life and cough severity were significantly in favor of the Noscough® group (*p*-values <0.001).

Discussion

The results of our trial confirmed that Noscough® syrup which consists of noscapine and licorice has similar effects with diphenhydramine in terms of response to treatment. However, Noscough® syrup was superior to diphenhydramine in terms of quality of life, dyspnea relief, and cough severity.

Notably, the effect size of the response to treatment as the primary outcome of the study was 0.06 in favor of Noscough® syrup, which is considered a small effect size (Rahlfs and Zimmermann, 2019). Hence, it can be concluded that noscapine may be slightly superior to diphenhydramine in terms of response to treatment, and the study was underpowered to detect this small difference. Moreover, the mean scores of CSS, VAS, and CQLQ, were all in favor of the Noscough® group with significant results with adjustment of their baseline values. The presence of dyspnea was also significantly lower in the Noscough® group by the end of day five. The number needed to treat (NNT) was calculated to be 17, which is also considered a small effect.

Noscough® syrup consists of noscapine and licorice. Noscapine is an opioid antitussive that has been shown to reduce bradykinin-induced cough in humans (Ebrahimi, 2020). Moreover, studies have shown many symptoms related to COVID-19 can be justified with the development of bradykinin (Garvin et al., 2020). Hence, bradykinin and cytokine storms might be associated with worse outcomes in COVID-19. Therefore, mediating these pathways may lead to better symptom-relieving (Rex et al., 2022). Noscapine is considered a safe candidate with the potential benefits of modulating both pathways (Luo et al., 2020; Kumar et al., 2022). The advantages of this medication to diphenhydramine are lack of sedative effects and unlike diphenhydramine it has no anticholinergic effects which is an important advantage in the elderly. In Addition, unlike other opioids, no addiction or respiratory issues have been reported with this medication. Noscugh® side effects only include potential sedative effects in non-pharmacologic doses based on the manufacturer label.

Licorice also has been shown to have antiviral effects against various families, including SARS coronaviruses (Diomede et al., 2021). Moreover, a study using bioinformatics analysis and molecular dynamic stimulation has shown that phaseol in licorice may have beneficial effects in reducing the inflammatory response to COVID-19 by inhibiting the activation of CXCL8 and IL2RA (Cao et al., 2022). An *in silico* analysis performed by Neeraj kumar et al., showed that the combination of noscapine and hydroxychloroquine conjugates has much binding affinity for main protease of SARS-CoV-2 which has critical role in pathogenesis of COVID-19 (Kumar et al., 2022). Other potential effects against COVID-19 symptoms have also been shown for other ingredients of licorice, including Glycerol and Glyasperin F (Cao et al., 2022). Another *in vitro* study has revealed that licorice may block SARS-CoV-2 replication by inhibiting the viral main protease (van de Sand et al., 2021).

The main limitation of the study was that the patients were not blinded to the study interventions. Due to the differences in dosing and interval of administrations blinding was not feasible. Hence, their own believes of the antitussive medication might have influenced the study results. Another limitation is that the investigators had considered a significant effect for Noscough® syrup based on the potential literature-based mechanisms while powering the study. Due to this fact, the study did not meet its primary endpoint as the sample size was not large enough to detect smaller effects. It is suggested to perform the study with larger sample size and also with different reference products as control group including the other opioids.

Conclusion

Noscough® syrup was slightly superior to diphenhydramine in relieving the symptoms of cough and dyspnea in COVID-19 outpatients. The severity of cough and cough-related quality of life were also in favor of Noscough® syrup significantly. Considering the favorable safety profile of this syrup, Noscough® may be a valuable treatment in relieving cough in COVID-19 outpatients.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee of Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran (Ethics code: IR.SBMU.PHARMACY.REC.1400.252). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SB, FD, and FF contributed to conception and design of the study. HJ organized the database. SB performed the statistical analysis. FF wrote the first draft of the manuscript. HK, HS, HF, and SB wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Alkotaji, M., and Al-Zidan, R. N. (2021). Indomethacin: Can it counteract bradykinin effects in COVID-19 patients? *Curr. Pharmacol. Rep.* 7 (3), 102–106. doi:10.1007/s40495-021-00257-6

Cao, J. F., Gong, Y., Wu, M., Yang, X., Xiong, L., Chen, S., et al. (2022). Exploring the mechanism of action of licorice in the treatment of COVID-19 through bioinformatics analysis and molecular dynamics simulation. *Front. Pharmacol.* 13, 1003310. doi:10.3389/fphar.2022.1003310

Dhand, R., and Li, J. (2020). Coughs and sneezes: Their role in transmission of respiratory viral infections, including SARS-CoV-2. *Am. J. Respir. Crit. Care Med.* 202 (5), 651–659. doi:10.1164/rccm.202004-1263PP

Diomede, L., Beeg, M., Gamba, A., Fumagalli, O., Gobbi, M., and Salmona, M. (2021). Can antiviral activity of licorice help fight COVID-19 infection? *Biomolecules* 11 (6), 855. doi:10.3390/biom11060855

Ebrahimi, S. A. (2020). Noscapine, a possible drug candidate for attenuation of cytokine release associated with SARS-CoV-2. *Drug Dev. Res.* 81 (7), 765–767. doi:10.1002/ddr.21676

Enna, S. J., and Bylund, D. B. (2008). xPharm: the comprehensive pharmacology reference. Amsterdam: Elsevier Science (Firm).

Garvin, M. R., Alvarez, C., Miller, J. I., Prates, E. T., Walker, A. M., Amos, B. K., et al. (2020). A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm. *Elife* 9, e59177. doi:10.7554/eLife.59177

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395 (10223), 497–506. doi:10.1016/S0140-6736(20)30183-5

Kuang, Y., Li, B., Fan, J., Qiao, X., and Ye, M. (2018). Antitussive and expectorant activities of licorice and its major compounds. *Bioorg Med. Chem.* 26 (1), 278–284. doi:10.1016/j.bmc.2017.11.046

Kumar, N., Awasthi, A., Kumari, A., Sood, D., Jain, P., Singh, T., et al. (2022). Antitussive noscapine and antiviral drug conjugates as arsenal against COVID-19: A comprehensive chemoinformatics analysis. *J. Biomol. Struct. Dyn.* 40 (1), 101–116. doi:10.1080/07391102.2020.1808072

Lechtzin, N., Hilliard, M. E., and Horton, M. R. (2013). Validation of the Cough Quality-of-Life Questionnaire in patients with idiopathic pulmonary fibrosis. *Chest* 143 (6), 1745–1749. doi:10.1378/chest.12-2870

Luo, P., Liu, D., and Li, J. (2020). Pharmacological perspective: Glycyrrhizin may be an efficacious therapeutic agent for COVID-19. *Int. J. Antimicrob. Agents* 55 (6), 105995. doi:10.1016/j.ijantimicag.2020.105995

Rahlfs, V., and Zimmermann, H. (2019). Effect size measures and their benchmark values for quantifying benefit or risk of medicinal products. *Biom J.* 61 (4), 973–982. doi:10.1002/bimj.201800107

Rex, D. A. B., Vaid, N., Deepak, K., Dagamajalu, S., and Prasad, T. S. K. (2022). A comprehensive review on current understanding of bradykinin in COVID-19 and inflammatory diseases. *Mol. Biol. Rep.* 49 (10), 9915–9927. doi:10.1007/s11033-022-07539-2

Shergis, J. L., Wu, L., May, B. H., Zhang, A. L., Guo, X., Lu, C., et al. (2015). Natural products for chronic cough: Text mining the East Asian historical literature for future therapeutics. *Chron. Respir. Dis.* 12 (3), 204–211. doi:10.1177/1479972315583043

Song, W. J., Hui, C. K. M., Hull, J. H., Birring, S. S., McGarvey, L., Mazzone, S. B., et al. (2021). Confronting COVID-19-associated cough and the post-COVID syndrome: Role of viral neurotropism, neuroinflammation, and neuroimmune responses. *Lancet Respir. Med.* 9 (5), 533–544. doi:10.1016/S2213-2600(21)00125-9

Spinou, A., and Birring, S. S. (2014). An update on measurement and monitoring of cough: What are the important study endpoints? *J. Thorac. Dis.* 6 (Suppl 7), S728–S734. doi:10.3978/j.issn.2072-1439.2014.10.08

van de Sand, L., Bormann, M., Alt, M., Schipper, L., Heilingloh, C. S., Steinmann, E., et al. (2021). Glycyrrhizin effectively inhibits SARS-CoV-2 replication by inhibiting the viral main protease. *Viruses* 13 (4), 609. doi:10.3390/v13040609

Wang, Z., Wang, M., Wen, S., Yu, L., and Xu, X. (2019). Types and applications of cough-related questionnaires. *J. Thorac. Dis.* 11 (10), 4379–4388. doi:10.21037/jtd.2019.09.62

Wilczynski, S. A., Wenceslau, C. F., McCarthy, C. G., and Webb, R. C. (2021). A cytokine/bradykinin storm comparison: What is the relationship between hypertension and COVID-19? *Am. J. Hypertens.* 34 (4), 304–306. doi:10.1093/ajh/hpaa217

Zanza, C., Romenskaya, T., Manetti, A. C., Franceschi, F., La Russa, R., Bertozzi, G., et al. (2022). Cytokine storm in COVID-19: Immunopathogenesis and therapy. *Med. Kaunas.* 58 (2), 144. doi:10.3390/medicina58020144

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Evaluating the efficacy of inhaled amikacin as an adjunct to intravenous combination therapy (ceftazidime and amikacin) in pediatric cystic fibrosis pulmonary exacerbation

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Background: *Pseudomonas aeruginosa* is the most common microorganism found in the sputum culture of Cystic fibrosis (CF) patients causing the pulmonary destruction. Aminoglycosides have a low diffuse rate from lipid membranes, and respiratory system secretions. Regarding the burden of pulmonary exacerbation caused by the *pseudomonas aeruginosa* in cystic fibrosis patients in the long term and the limited number of clinical trials focused on appropriate treatment strategies, the present study evaluated the concurrent inhaled and intravenous aminoglycoside antibiotics for pulmonary exacerbation caused by the *pseudomonas aeruginosa* as a safe and effective treatment in children.

Method: This study was a blinded, randomized clinical trial phase conducted in a tertiary referral pediatric teaching hospital from May 2021 to May 2022. The patients were randomly allocated to receive intravenously administered ceftazidime and Amikacin alone or with inhaled Amikacin. Forced expiratory volume (FEV1), Amikacin *via* the level, kidney function tests, audiometry, inflammatory markers (erythrocyte sedimentation rate and C-reactive protein), hospital stay, and bacterial eradication rate were compared in two therapy groups.

Results: the average FEV_1 has increased by 47% in Neb + group compared to Neb– group following treatment. Hospital stay was lower in Neb + group. No renal toxicity or ototoxicity was observed in both therapy groups. *Pseudomonas aeruginosa* eradication rate Neb– and Neb + groups were 44% and 69%, respectively (p-value = 0.15).

Conclusion: Concurrent inhaled and intravenous Amikacin is safe and effective to treat *Pseudomonas aeruginosa* exacerbation in CF patients. Moreover, co-delivery antibiotics' route treatment increased the eradication rate. Although not statistically significant, never the less, it is clinically relevant. The intervention reduced the length of hospitalization in this group.

Clinical Trial Registration: clinicaltrials.gov, identifier [IRCT20120415009475N10].

KEYWORDS

aminoglycosides, cystic fibrosis-CF, nebulizer, psedudomonas aeruginosa, toxicicity

1 Introduction

Cystic fibrosis (CF) is a hereditary illness involving various human systems, especially respiratory system. CF is an autosomal recessive disease caused by a defect in the CFTR gene located in the 7q31.2 chromosome, producing cystic fibrosis transmembrane conductance regulator (CFTR protein). Because it affects ion channels, CFTR protein disruption plays a crucial part in the pathophysiology of the trans-membranous epithelium of the pulmonary, sweat gland, digestive, and reproductive issues in CF patients. CF patients cannot appropriately secret salt and water across epithelia resulting in the viscous, thickened secretions. Therefore, maladapted airway clearance induced bacterial colonization and inflammatory responses, leading to the pulmonary exacerbation (Radlović, 2012). Among various CF respiratory complications, many patients present problems, such as cough, bronchiolitis, pulmonary atelectasis, and pneumonia. Considering the lower effectivity of the innate immune system of the patient with CF in respiratory systems compared to others, infections in this system are more common by microorganisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Burkholderia cepacia (Blanchard and Waters, 2019).

Since *Pseudomonas aeruginosa* is the most prevalent bacteria isolated from the sputum of cystic fibrosis (CF) patients and is responsible for the deterioration in pulmonary function, it is crucial to choose the correct antibiotic therapy in order to eradicate this pathogen. (Taccetti et al., 2012). According to different studies, double antibiotics treatment should be selected for the coverage of *Pseudomonas aeruginosa*; primarily, beta-lactam and aminoglycoside combination is recommended for their synergistic effect (Flume et al., 2009; Ratjen et al., 2009). Despite the marketing of newer antibiotics, aminoglycoside antibiotics are still widely used as an effective treatment for the cystic fibrosis pulmonary exacerbations against *Pseudomonas aeruginosa* (Flume et al., 2009; den Hollander et al., 1997). Aminoglycoside antibiotics are effective against numerous Gram-negative aerobic bacilli by irreversibly binding to 30 s subunit of ribosomes, causing codon reading errors and leading to cell death (Young et al., 2013). Considering the renal toxicity may occur when aminoglycoside antibiotics are taken, most guidelines recommend that a once-daily dose is preferred to divided dosages (Flume et al., 2009). Although using two different drug delivery routes may increase antimicrobial effects by helping improve drug delivery to the respiratory tract, an increasing risk of aminoglycoside side effects is expected. Therefore, the pharmacokinetic studies and therapeutic drug monitoring while using inhalation and intravenous medication can help achieve the most effective treatment regime for microbial eradication and reduce the risk of side effects. Thus, based on CFF (Cystic Fibrosis Foundation) recommendation, the decision to use inhaled aminoglycoside concomitantly with intravenous aminoglycoside should be individually made for each patient until further studies are conducted in this regard (Flume et al., 2009; Ratjen et al., 2009; Hewer et al., 2020).

Several studies showed that dual antibiotic therapy is required to treat *Pseudomonas aeruginosa*. The systemic administration of aminoglycosides alone is not particularly effective to eradicate CF exacerbation phase in terms of low diffuse rate of aminoglycosides from the lipid membrane to respiratory system secretion. For a reason mentioned above, some studies recommended adding inhaled antibiotics to increase the aminoglycoside concentration in CF patients' respiratory tracts and bronchial tract (Langton Hewer and Smyth, 2017; Blanchard and Waters, 2019; Taccetti et al., 2021).

Considering the burden of pulmonary exacerbation caused by *pseudomonas aeruginosa* in cystic fibrosis patients in long term and the limited number of clinical trials focused on appropriate treatment strategies, the present study evaluated the concurrent inhaled, and intravenous aminoglycoside antibiotics for the pulmonary exacerbation caused by *pseudomonas aeruginosa* as a safe and effective treatment in children.

2 Materials and Method

2.1 Study design and patients

This study was a blinded randomized clinical trial phase three conducted in a tertiary referral pediatric teaching hospital from May 2021 to May 2022. Inclusion criteria were patients with CF admitted in terms of CF exacerbation to the pulmonary ward at Mofid children's Hospital, Shahid Beheshti University of medical science. The patients should be between 6–18 years old and have a sputum culture positive with *pseudomonas aeruginosa*. A pulmonary exacerbation was described by the Cystic Fibrosis Foundation as having an increased cough, more sputum being produced, shortness of breath, chest discomfort, reduced appetite, weight loss, and decreased pulmonary function tests (Flume et al., 2009). Patients who had a history of allergy to aminoglycosides or beta-lactam antibiotics, chronic renal failure from stage 3 to end-stage stage according to KDIGO (Kidney Disease Improving Global Outcomes) guidelines, Liver failure (acute or chronic) defined as Child-Pugh class B or C or a liver enzyme test above five times the normal upper limit, congenital metabolic disorders, myopathies, hearing impairment (>15 dB hearing level at a *range* of 125 *Hz*–6000 *Hz* on standard Audiogram), neuromuscular diseases (such as Amyotrophic

lateral sclerosis (ALS), Cramp-fasciculation syndrome, *etc.*), severe electrolyte imbalance (including potassium less than 2.5 or more Than 5.5; Sodium less than 125 or more than 155; Magnesium less than 1.8 or more than 2.2) and had a positive COVID-19 Polymerase Chain Reaction (PCR) at admission were excluded.

University Ethics Committee approved the trial (with code IR.SBMU.PHARMACY.REC.1400.039), and informed consent was obtained from patients or their legal guardians. The trial was registered at Iranian registry of clinical trials by the code IRCT20120415009475N10. The sample size was estimated with a confidence level of 95 percent and power of 80 percent with data from previous studies of 40 Patients divided into two main groups of 20; Patients in Neb− Group received intravenous Ceftazidime, intravenous Amikacin and inhaled NaCl 0.9%. Patients in the Neb + group received intravenous Ceftazidime, intravenous Amikacin, and inhaled Amikacin.

Amikacin 250 mg (for patients under 50 kg) or 500 mg (for patients over 50 kg) were added to 3 ccs 0.9% sodium chloride and was instilled into a vibrating mesh nebulizer chamber for use. The nebulizer used was a vibrating mesh nebulizer, PARI eFlow®rapid. All patients utilized the identical kind of device. 5 ml of NaCl 0.9% was utilized as a placebo in the nebulizer chamber for participants in the Neb-group. Patients in both groups received standard-of-practice care according to Cystic Fibrosis Foundation Guideline (Flume et al., 2009), including an intravenous infusion of Ceftazidime (250 mg/kg/day divided every 8 h) and Amikacin (25 mg/kg/day every 24 h), a set anti-pseudomonas combination. Other supportive treatments, including chest physiotherapy, adequate hydration, supplemental oxygen, oral pancreatic enzymes supplement, strict COVID-19 isolation, and a high-calorie diet, were considered equally for the patients in both groups.

2.2 Randomization

Block Randomization method was used for the randomization of samples. The patients were allocated based on quadruple blocks using the sealedenvelope.com website. The encoded normal saline *versus* amikacin-containing syringes were prepared in identical packaging, and non-sequential alphanumerical codes provided by sealedenvelope.com were used for blinding. The patient, caregiver, researcher, and statistical analyzer were blinded.

2.3 Clinical outcome parameters

After recording the demographic information for each patient on admission, the medical staff requested a complete laboratory profile. Spirometry was performed on days 0 and 14th to evaluate baseline and final FEV_1. Changes in the FEV_1 parameter were considered the main finding and as the primary outcome to determine the effectiveness of treatment. The renal function was calculated every other day by eGFR was calculated according to the revised Schwartz formula as follows: eGFR (ml/min × 1.73 m^2) = 0.413 × Ht (cm)/SCreat (mg/dl). Liver function tests (aminotransferases, bilirubin, INR), Albumin level, and inflammatory parameters (ESR (erythrocyte sedimentation rate) and CRP (C-reactive protein)) were assessed at the baseline, 7th day, and 14th day of treatment. O_2 saturation, Temperature, and Respiratory rate were measured and recorded daily. The hospital stays for each patient and the need for intensive care unit (ICU) admission -if needed-were recorded.

2.4 Audiometry

All patients had pure tone auditory testing at baseline and after the conclusion of therapy. This test is performed by presenting a pure sound to the ear through the earphone and measuring the lowest intensity in decibels (dB), and the standard Audiogram was used to measure hearing levels between 125 Hz and 6000 Hz. Results of the audiometry tests were provided in both quantitative and qualitative ways.

2.5 Microbiology

Microbiological cultures and antibiotic susceptibility testing were performed on sputum samples at baseline and the end of the treatment (day 14th) for all patients. Sputum samples were taken from patients with the help of bronchoalveolar lavage and transferred to the laboratory under appropriate conditions for examination and culture.

2.6 Pharmacokinetic assessment

Blood samples were collected from all patients on 4th day of treatment 30 min before receiving the 4th dose of Amikacin. The blood samples were collected in the anticoagulated tubes. The serum of blood samples was separated by centrifuging at 1100–1300 rpm for 15 min. The amikacin trough level was measured with Roche COBAS INTEGRA 400 (Produced in Roche Diagnostics GmbH Company Mannheim, Germany) access auto analyzer by fluorescence polarization immunoassay (FPIA) test.

2.7 Statistical analysis

SPSS statistical software was used for statistical analysis. First, by defining the variables, the data were entered into SPSS software, and then the descriptive results of the quantitative variables were reported.

All analysis were performed based on the same pattern of initial randomization of patients and intention-to-treat (ITT).

Multiple linear regression modeling was used to adjust confounding variables.

3 Results

3.1 Study population

The summary of process carried out in the study is shown in Figure 1. Patients' baseline characteristics are shown in Table 1. There was no significant difference between two groups regarding age, sex, ESR, CRP, and pulmonary functions at baseline. Two patients from Neb+ and one from Neb-groups were lost to follow-up because they left in terms of the hospital's strict COVID-19 isolation policy and continued treatment in other centers.

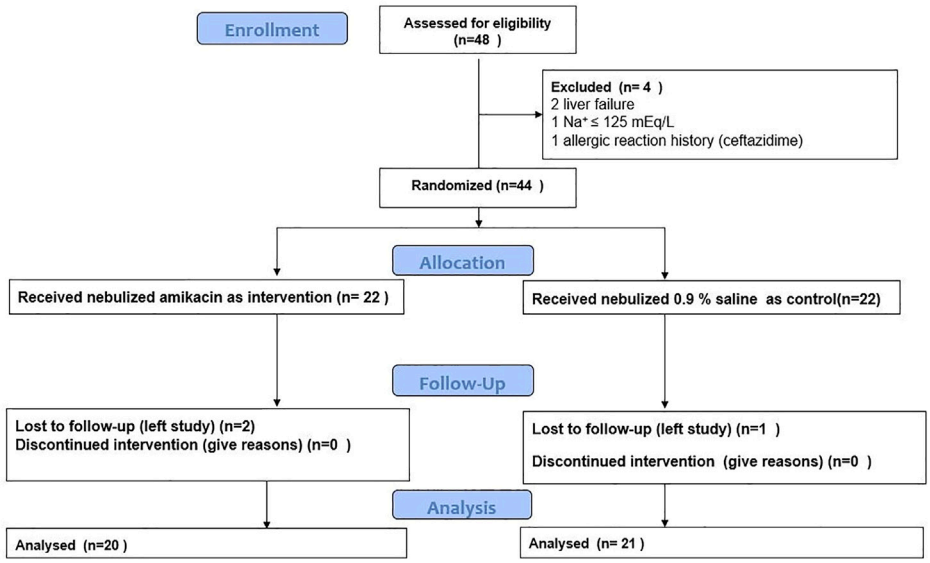

FIGURE 1
Study CONSORT diagram.

TABLE 1 Baseline characteristics of the study patients

	Total	Neb− group	Neb+ group	*p* value
Age (y)	13.61±4.13	13.73±3.49	13.50±4.69	N.S
Number of patients	41	21	20	
Male (%)	17(41.5%)	5(23.9%)	12(60.0%)	N.S
Female (%)	24(58.5%)	16(76.1%)	8(40.0%)	N.S
Pulmonary function FEV_1 (L)	1.18±0.62	0.98±0.47	1.31±0.66	N.S
ESR (mm/h)	36.3±29.9	42.52±33.14	26.95±19.43	N.S
CRP (mg/L)	22.4±17.0	24.79±17.81	25.17±17.71	N.S

Abbreviations: FEV1: Forced expiratory volume in second 1; N.S: Not significant; ESR: erythrocyte sedimentation rate; CRP: C reactive protein.

3.2 Clinical outcome

The changes in clinical outcome for Neb- and Neb + groups are shown in Table 2.

FEV_1 at the baseline and end of the treatment was compared in two groups by paired sample t-test. There was a significant difference between baseline and final FEV_1 (p-value <0.001 for Neb + group and p-value = 0.02 for Neb-group) in both groups. The differences in the FEV_1 at baseline and the end of the treatment were compared between two groups. In the Neb-group, the average FEV_1 at baseline was 0.98 L; at the end of treatment, it was 1.14 L, which increased by 0.16 L. In contrast, in the Neb + group, the average FEV_1 at baseline was 1.31 L and at the end of treatment was 1.67 L which had an increase of 0.36 L. The coefficient of determination (R^2), according to linear regression modeling was 52% (R squared = 0.52) and The average FEV1 increased by 0.47 in the Neb + group compared to the Neb-group after therapy (ß = 0.47). The improvement in FEV1 level is significantly correlated with getting additional amikacin nebulizer therapy, according to this research. The changes in the level of inflammatory markers (ESR and CRP) are shown in Table 2. Using the openepi.com website, considering the mean and standard deviation of the FEV1 level at the end of the study, the power of the study was higher than 80%.

Microbial outcome (eradication rate), length of hospital stay, and need for ICU admission in both groups are shown in Figure 2.

3.3 Adverse effects

The risk of hearing loss as an adverse effect associated with the aminoglycoside antibiotics was assessed by pure tone audiometry at the baseline and end of the treatment. Based on quantitative data from Audiogram, covering frequencies between 125 and 6000 Hz, none of the patients had a qualitative decline in hearing level, and all patients had normal hearing at the conclusion of treatment. Kidney function was monitored every other day by measuring the serum creatinine and calculating the Schwartz Formula equation. None of

TABLE 2 Changes in lung function and inflammatory markers after 14 days of treatment in Neb+ group vs. Neb- group.

Parameters:	Neb– group			Neb+ group		
	Before	**After**	***p* value**	**Before**	**After**	***p* value**
FEV_1(L)	0.98±0.47	1.14±0.60	<0.05	1.31±0.66	1.67±0.71	< 0.001
ESR (mm/h)	42.52±33.14	26.95±21.18	< 0.001	26.95±19.43	9.50±10.33	< 0.001
CRP (mg/L)	24.79±17.81	7.58±6.88	< 0.001	25.17±17.71	5.61±4.14	< 0.001

Abbreviations: FEV1: Forced expiratory volume in second 1; ESR: erythrocyte sedimentation rate; CRP: C reactive protein.

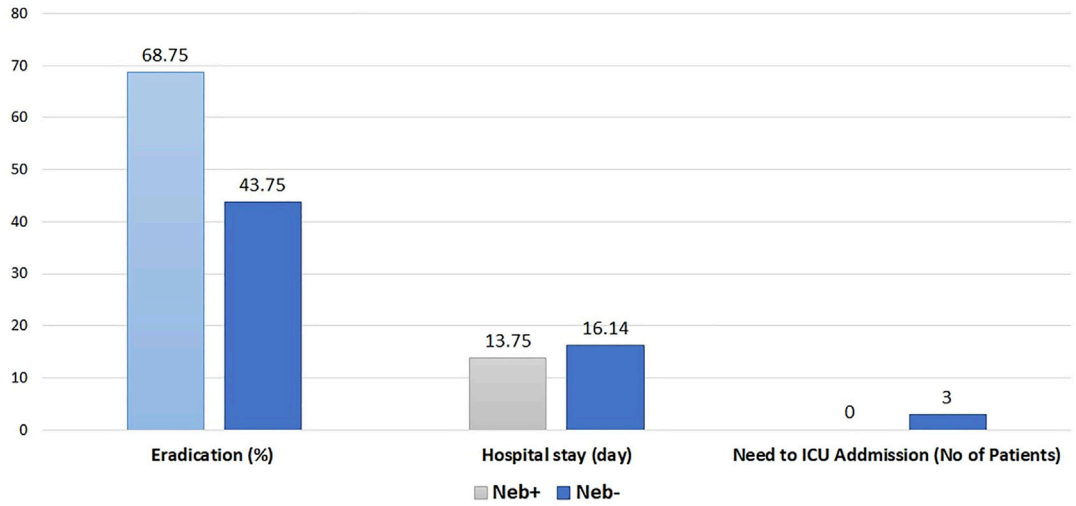

FIGURE 2
Rate of eradication (*p* value = 0.15), Hospital stay (*p* value = 0.3), Need to ICU admission (*p* value < 0.05).

the patients developed renal impairment during the study period. Electrolyte levels of the patients were in the normal range during the study.

3.4 Microbiological outcome

At baseline, per inclusion criteria, *Pseudomonas aeruginosa* was confirmed in all Patients' sputum samples. McNemar's and chi-square test was performed to check the difference in the eradication rate between the two groups in the analysis. In the Neb + group, at the end of treatment, eradication occurred in 68.75%; While in the Neb-group, eradication occurred in 43.75% of patients. These results are shown in Figure 2.

3.5 Amikacin serum concentrations

Amikacin trough level for all patients before receiving the fourth dose was measured. The average trough in Neb- and Neb + groups were 2.01 and 1.61, respectively. There was no significant difference in the amikacin trough level between the two groups.

4 Discussion

The present randomized clinical trial compares two treatments for *Pseudomonas aeruginosa* exacerbation in CF patients, between intravenous Amikacin and Ceftazidime and adding inhaled Amikacin to the same treatment. Our investigation proved the benefit of supplementing the conventional therapy with inhaled Amikacin. This research is interesting since it was conducted during the exacerbation phase, whereas most prior studies were conducted on stable CF patients. This study was designed to address the concern for high aminoglycoside plasma levels regarding the concomitant use of inhaled and intravenous aminoglycoside. The ototoxicity and renal toxicity related to aminoglycoside accumulation were explicitly monitored. There was no significant difference among plasma levels in the two groups, and no ototoxicity or renal toxicity was monitored.

FEV_1 is one of the best objective parameters to monitor lung function in CF patients. By calculating the amount of air forced in the first second of breathing time from the patient's lungs, FEV_1 was measured as a ratio (Taccetti et al., 2012). The difference between FEV_1 in both groups was none significant initially. We collected final FEV_1 at the end of the study (after exacerbation recovered) to compare the effect of the intervention. Even though both groups'

final FEV1 levels increased just slightly, the Neb + group, which got supplemental inhaled Amikacin, had a much higher FEV1 percentage than the control group. Schaad et al. conducted a similar study on 87 CF patients during the exacerbation phase and demonstrated pulmonary lung function by plethysmography and not by spirometry; therefore, the parameters they measured were forced vital capacity and airway resistance, which improved after treatment. They did not show significant improvement in lung function in patients who received additional inhaled Amikacin (Schaad et al., 1987).

ELITE study and other similar studies showed that inhaled aminoglycosides, such as tobramycin or gentamicin (it has the same effect as Amikacin in Gram-negative germs) were effective in long-term eradication of *Pseudomonas* (Schaad et al., 1987; Ratjen et al., 2010; Taccetti et al., 2012; Van Stormbroek et al., 2019). In line with the ELITE trial, but at the exacerbation stage, the current study demonstrated the effects of inhaled Amikacin in CF patients. The EPIC study, on the other hand, found no discernible differences between IV and inhaled antibiotic therapy as compared to IV administration alone (Treggiari et al., 2011). In Schaad et al. study, the eradication rate of *Pseudomonas* was 41% and 70% in Neb- and Neb + groups, comparable to our study (Schaad et al., 1987).

It is perceived that the pharmacokinetics of antibiotics in the CF patients differ from others. It is essential to conduct pharmacokinetic studies to maximize clinical effects, select optimal treatment regimens, and minimize the side effects of antibiotics (Caceres Guido et al., 2019). When using Amikacin, ototoxicity and renal toxicity are possible side effects (Mulheran et al., 2001; Young et al., 2013). When using aminoglycosides, a daily dose rather than many doses per day is advised to lower the risk of renal problems. Although the concurrent inhalation and IV antibiotic delivery routes can increase the aminoglycoside effectiveness, they may increase the possibility of systemic side effects (Akkerman-Nijland et al., 2021). It ought to be noted that studies in children showed that, generally, aminoglycosides are cleared from the blood faster than adults. Compared to the adults and normal populations, the pharmacokinetic parameters in children and CF patients are fundamentally different, which justifies the need for independent studies on this particular group of patients (Caceres Guido et al., 2019).

Both groups were screened for ototoxicity and renal toxicity, and no one appeared with these adverse effects. A similar result was seen in Schaad et al. study (Schaad et al., 1987). We compared the drug toxicity of both groups by measuring the serum trough levels, and no significant differences between the groups were observed. These data show adding inhaled aminoglycosides to standard treatment does not have substantial systemic absorption or side effects. In other words, both groups of our trial have the same plasma levels, but Neb + group that received concurrent IV and inhaled Amikacin had better clinical outcomes with no extra side effects.

Strombroek et al. study describes that co-treatment with nebulized and IV routes of antibiotics *versus* only the IV route has reduced the length of hospitalization, and it is appropriate to use both instead of just the IV route. Therefore, by spending less on treating CF patients, this strategy has helped nations' health systems by lowering overall treatment costs (Van Stormbroek et al., 2019). Patients in this trial who received IV and inhalation antibiotics were released sooner than those in the control group. The cost-benefit of earlier discharge may be interesting to study the reduction in both the marginal and opportunity cost of disease.

Pseudomonas colonization creates a significant disease burden for CF patients in the long term. The residing bacteria, directly and indirectly, destroys lung function and any intervention to eradicate *pseudomonas* infection is crucial for the patients, even though the infection would return in time. The eradication rate was not significantly different among groups, but it was noticeably higher in Neb + group; this finding is valuable for clinical practice and helpful in designing future studies.

5 Conclusion

Concurrent inhaled, intravenous Amikacin is safe and effective to treat the *Pseudomonas aeruginosa* exacerbation in CF patients. Moreover, co-delivery antibiotics' route treatment increased the eradication rate. Although not statistically significant, never the less, it is clinically meaningful. The intervention reduced the length of hospitalization in this group.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional ethics committee for Pharmacy, Nursing and Midwifery Schools, Shahid Beheshti University of medical sciences, Tehran, Iran. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AR, BM, MS, and NF participate in the research design, the acquisition of data, the writing of the manuscript, and the performance of the research. GK, SS, ST, FD, and MH contributed to the acquisition of data interpretations of data. AR and NF participate in the preparation of the manuscript and final revision. BM proofread and finalized the manuscript. All authors read and approved the last revision of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer LM declared a shared affiliation with the authors ARB to the handling editor at the time of review.

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References

Akkerman-Nijland, A. M., Akkerman, O. W., Grasmeijer, F., Hagedoorn, P., Frijlink, H. W., Rottier, B. L., et al. (2021). The pharmacokinetics of antibiotics in cystic fibrosis. *Expert Opin. Drug Metab. Toxicol.* 17 (1), 53–68. doi:10.1080/17425255.2021.1836157

Blanchard, A. C., and Waters, V. J. (2019). Microbiology of cystic fibrosis airway disease. *Semin. Respir. Crit. Care Med.* 40 (6), 727–736. doi:10.1055/s-0039-1698464

Caceres Guido, P., Perez, M., Halac, A., Ferrari, M., Ibarra, M., Licciardone, N., et al. (2019). Population pharmacokinetics of Amikacin in patients with pediatric cystic fibrosis. *Pediatr. Pulmonol.* 54 (11), 1801–1810. doi:10.1002/ppul.24468

den Hollander, J. G., Horrevorts, A. M., van Goor, M. L., Verbrugh, H. A., and Mouton, J. W. (1997). Synergism between tobramycin and Ceftazidime against a resistant *Pseudomonas aeruginosa* strain, tested in an *in vitro* pharmacokinetic model. *Antimicrob. Agents Chemother.* 41 (1), 95–100. doi:10.1128/AAC.41.1.95

Flume, P. A., Mogayzel, P. J. J., Robinson, K. A., Goss, C. H., Rosenblatt, R. L., Kuhn, R. J., et al. (2009). Cystic fibrosis pulmonary guidelines: Treatment of pulmonary exacerbations. *Am. J. Respir. Crit. Care Med.* 180 (9), 802–808. doi:10.1164/rccm.200812-1845PP

Hewer, S. C. L., Smyth, A. R., Brown, M., Jones, A. P., Hickey, H., Kenna, D., et al. (2020). Intravenous versus oral antibiotics for eradication of *Pseudomonas aeruginosa* in cystic fibrosis (TORPEDO-CF): A randomised controlled trial. *Lancet Respir. Med.* 8 (10), 975–986. doi:10.1016/S2213-2600(20)30331-3

Langton Hewer, S. C., and Smyth, A. R. (2017). Antibiotic strategies for eradicating *Pseudomonas aeruginosa* in people with cystic fibrosis. *Cochrane database Syst. Rev.* 4, CD004197. doi:10.1002/14651858.CD004197.pub5

Mulheran, M., Degg, C., Burr, S., Morgan, D. W., and Stableforth, D. E. (2001). Occurrence and risk of cochleotoxicity in cystic fibrosis patients receiving repeated high-dose aminoglycoside therapy. *Antimicrob. Agents Chemother.* 45 (9), 2502–2509. doi:10.1128/aac.45.9.2502-2509.2001

Radlović, N. (2012). Cystic fibrosis. *Srp. Arh. Celok. Lek.* 140 (3–4), 244–249. doi:10.2298/sarh1204244r

Ratjen, F., Brockhaus, F., and Angyalosi, G. (2009). Aminoglycoside therapy against *Pseudomonas aeruginosa* in cystic fibrosis: A review. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* 8 (6), 361–369. doi:10.1016/j.jcf.2009.08.004

Ratjen, F., Munck, A., Kho, P., and Angyalosi, G.ELITE Study Group (2010). Treatment of early *Pseudomonas aeruginosa* infection in patients with cystic fibrosis: The ELITE trial. *Thorax* 65 (4), 286–291. doi:10.1136/thx.2009.121657

Schaad, U. B., Wedgwood-Krucko, J., Suter, S., and Kraemer, R. (1987). Efficacy of inhaled Amikacin as adjunct to intravenous combination therapy (Ceftazidime and Amikacin) in cystic fibrosis. *J. Pediatr.* 111 (4), 599–605. doi:10.1016/s0022-3476(87)80130-0

Taccetti, G., Bianchini, E., Cariani, L., Buzzetti, R., Costantini, D., Trevisan, F., et al. (2012). Early antibiotic treatment for *Pseudomonas aeruginosa* eradication in patients with cystic fibrosis: A randomised multicentre study comparing two different protocols. *Thorax* 67 (10), 853–859. doi:10.1136/thoraxjnl-2011-200832

Taccetti, G., Francalanci, M., Pizzamiglio, G., Messore, B., Carnovale, V., Cimino, G., et al. (2021). Cystic fibrosis: Recent insights into inhaled antibiotic treatment and future perspectives. *Antibiot. (Basel, Switz.* 10, 338. doi:10.3390/antibiotics10030338

Treggiari, M. M., Retsch-Bogart, G., Mayer-Hamblett, N., Khan, U., Kulich, M., Kronmal, R., et al. (2011). Comparative efficacy and safety of 4 randomized regimens to treat early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Arch. Pediatr. Adolesc. Med.* 165 (9), 847–856. doi:10.1001/archpediatrics.2011.136

Van Stormbroek, B., Zampoli, M., and Morrow, B. M. (2019). Nebulized gentamicin in combination with systemic antibiotics for eradicating early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Pediatr. Pulmonol.* 54 (4), 393–398. doi:10.1002/ppul.24254

Young, D. C., Zobell, J. T., Stockmann, C., Waters, C. D., Ampofo, K., Sherwin, C. M. T., et al. (2013). Optimization of anti-pseudomonal antibiotics for cystic fibrosis pulmonary exacerbations: V. Aminoglycosides. *Pediatr. Pulmonol.* 48 (11), 1047–1061. doi:10.1002/ppul.22813

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Navigating drug repurposing for Chagas disease: advances, challenges, and opportunities

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Chagas disease is a vector-borne illness caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). It poses a significant public health burden, particularly in the poorest regions of Latin America. Currently, there is no available vaccine, and chemotherapy has been the traditional treatment for Chagas disease. However, the treatment options are limited to just two outdated medicines, nifurtimox and benznidazole, which have serious side effects and low efficacy, especially during the chronic phase of the disease. Collectively, this has led the World Health Organization to classify it as a neglected disease. To address this problem, new drug regimens are urgently needed. Drug repurposing, which involves the use of existing drugs already approved for the treatment of other diseases, represents an increasingly important option. This approach offers potential cost reduction in new drug discovery processes and can address pharmaceutical bottlenecks in the development of drugs for Chagas disease. In this review, we discuss the state-of-the-art of drug repurposing approaches, including combination therapy with existing drugs, to overcome the formidable challenges associated with treating Chagas disease. Organized by original therapeutic area, we describe significant recent advances, as well as the challenges in this field. In particular, we identify candidates that exhibit potential for heightened efficacy and reduced toxicity profiles with the ultimate objective of accelerating the development of new, safe, and effective treatments for Chagas disease.

KEYWORDS

Chagas disease, combination therapy, drug discovery, drug repositioning, drug repurposing, neglected tropical diseases, parasitic disease, *Trypanosoma cruzi*

1 Introduction

Chagas disease (CD), also known as American trypanosomiasis, is a potentially life-threatening Neglected Tropical Disease (NTD) caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). An estimated 6 to 7 million people worldwide are infected with *T. cruzi* (WHO, 2023). The disease is found mainly in endemic areas of 21 Latin American countries but with climate change and population movement the impact is spreading to other regions of the globe (Figure 1). CD places a significant direct financial burden on healthcare system and society that has been estimated to exceed $625 million in healthcare costs and 806,170 DALYs (Gómez-Ochoa et al., 2022). However, the full economic cost to endemic communities is much more significant, resulting in a loss of 752,000 workdays annually due to premature deaths, which carries an average financial burden of 1.2 billion dollars per year in the southern countries of Latin America (Ramsey

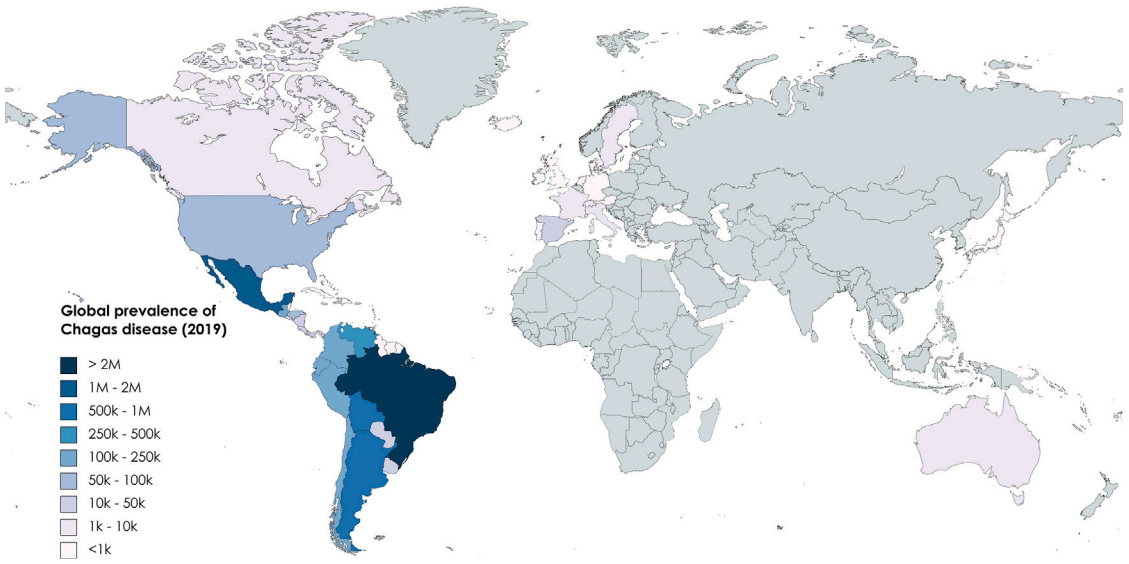
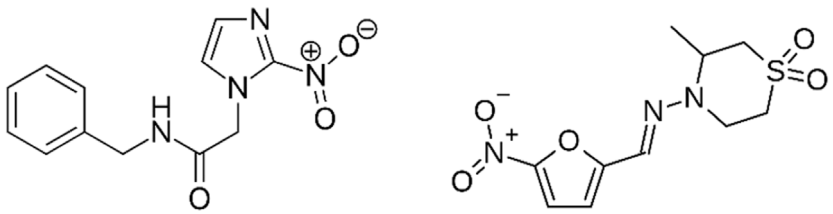


FIGURE 1
Global prevalence of CD based on data from Global Burden of Disease (2019).



Benznidazole **1**

Nifurtimox **2**

FIGURE 2
Structure of benznidazole **1** and nifurtimox **2**.

et al., 2014). Additionally, it is currently estimated that <1% of those infected with CD have access to care and treatment (Cucunubá et al., 2017).

There is no vaccine for CD and there are only two drugs' treatments approved for this illness. These are benznidazole **1** (BZN), in use since 1971, and nifurtimox **2** (NFX), which was first approved in 1965 (Figure 2). While these old medications remain effective in preventing or curbing disease progression in infected adults, especially those with no symptoms and during the acute infection, they are not without challenges (Crespillo-Andújar et al., 2022). The duration of treatment is long (up to 2 months) and possible adverse reactions can occur in up to 40% of treated adult patients (Jackson et al., 2020), potentially requiring additional treatment for cardiac, digestive, or neurological manifestations. The drugs are contraindicated for certain populations, such as pregnant women and people with kidney or liver failure (Meymandi et al., 2018). Collectively, these factors can contribute to poor patient adherence, which may result in relapse and the development of resistance.

Given this, there is an urgent need for the development of new cost-effective, efficacious drugs. This is challenged by the global resources dedicated to ameliorating the burden of the NTDs. Moreover, even within this limited space, research into CD received only 0.67% of the overall funding allocated to all NTDs over a span of 10 years (2010–2020) (Miranda-Arboleda et al., 2021) reinforcing its reputation as the "most neglected of the neglected tropical diseases" (Zaidel and Sosa Liprandi, 2021).

Drug repurposing (the process of finding new indications for existing drugs) presents a promising approach to addressing this challenge. In this review, we discuss the use of drug repositioning as a cost-effective strategy for the development of new solutions to this devastating disease. We not only explore the latest advances in this approach but also shed light on the challenges that remain for *T. cruzi* chemotherapies.

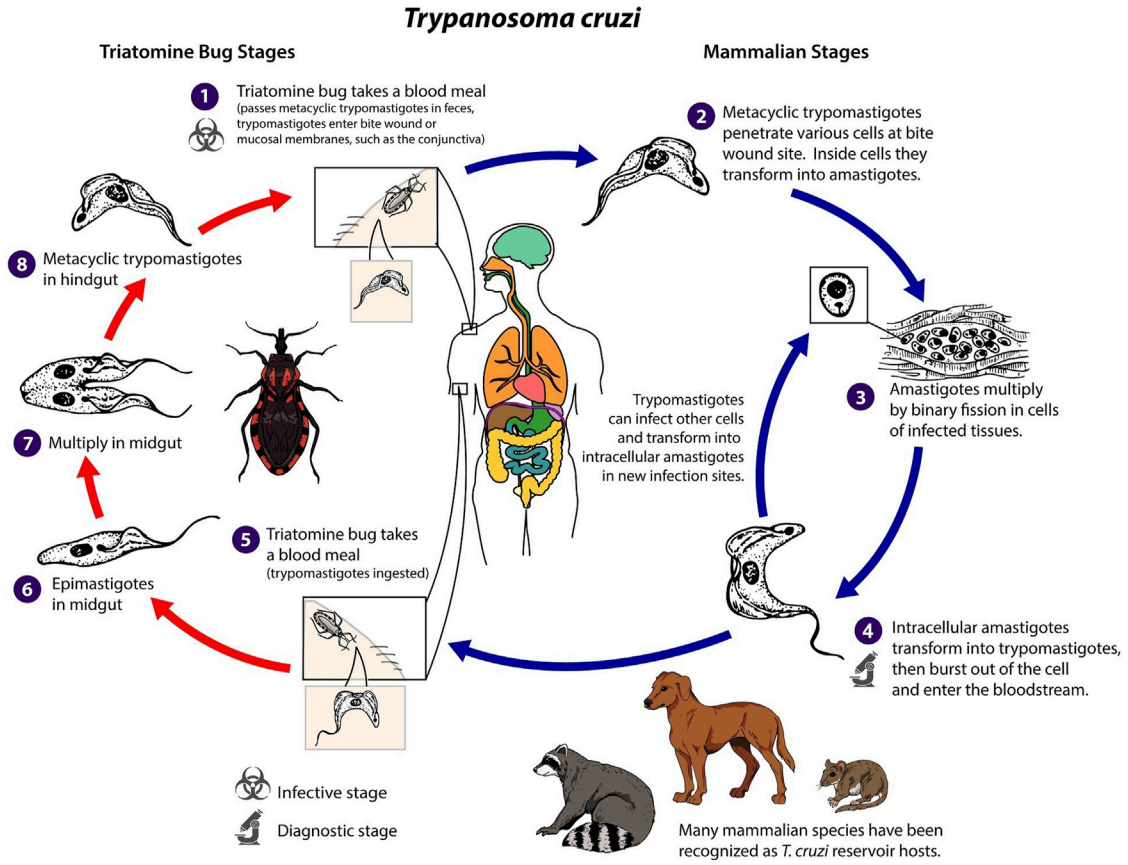


FIGURE 3
Trypanosoma cruzi life cycle adapted from Centers for Diseases Control and Prevention (CDC, 2023).

2 Chagas disease

Identifying and developing new drugs against *T. cruzi* is complicated by the parasite's complex life cycle (Figure 3). The life cycle has four developmental stages, involving, metacyclic trypomastigotes, epimastigotes, blood-form trypomastigotes, and intracellular amastigotes, which span two host organisms; a triatomine insect, commonly known as a kissing bug, and the mammalian host (Nagajyothi et al., 2012). In humans and other mammals, transmission primarily occurs through insect vectors. This happens when an infected triatomine insect feeds on the blood of a host. Trypomastigotes are released in its faeces near the site of the bite wound. The trypomastigotes can then enter the host's body through the wound or through intact mucosal membranes, such as the conjunctiva. Alternative modes of transmission can occur through ingestion of contaminated food, mother-to-child transmission and infected blood transfusions and organ transplantation.

Once inside the host, the trypomastigotes invade nearby cells and differentiate into intracellular amastigotes. These amastigotes multiply by binary fission and differentiate back into trypomastigotes, which are then released into the bloodstream. The trypomastigotes can infect cells from various tissues and transform into intracellular amastigotes in new infection sites. Unlike African trypanosomes (*T. brucei*), bloodstream trypomastigotes of *T. cruzi* do not replicate. Replication only resumes when the parasites enter another cell or are ingested by another vector. The cycle is propagated when an uninfected kissing bug feeds on human or animal blood that contains circulating parasites. The ingested trypomastigotes transform into epimastigotes in the vector's midgut, where they multiply and differentiate. The parasites then differentiate into infective metacyclic trypomastigotes in the hindgut, ready to be transmitted to another host. This life cycle leads to a disease with two distinct phases (PAHO, 2023). Following first infection there is an initial acute phase, which can last for several months, during which a high number of parasites circulate in the blood. This can lead to a range of visible signs, initially a skin lesion or swelling of the eye lids but may involve fever, headache, enlarged lymph glands, muscle pain, breathing difficulties, and abdominal and chest discomfort. However, challenging early diagnosis for the majority of individuals, these symptoms are often mild and not easily attributed to a *T. cruzi* infection. During the chronic phase, the blood population of the parasite falls dramatically, and the parasite reside mainly in the heart and digestive muscles. One to three decades later, up to a third of patients suffer from cardiac disorders (CD is the second leading cause of chronic heart failure in Latin America) and up to 1 in 10 suffer from digestive (typically

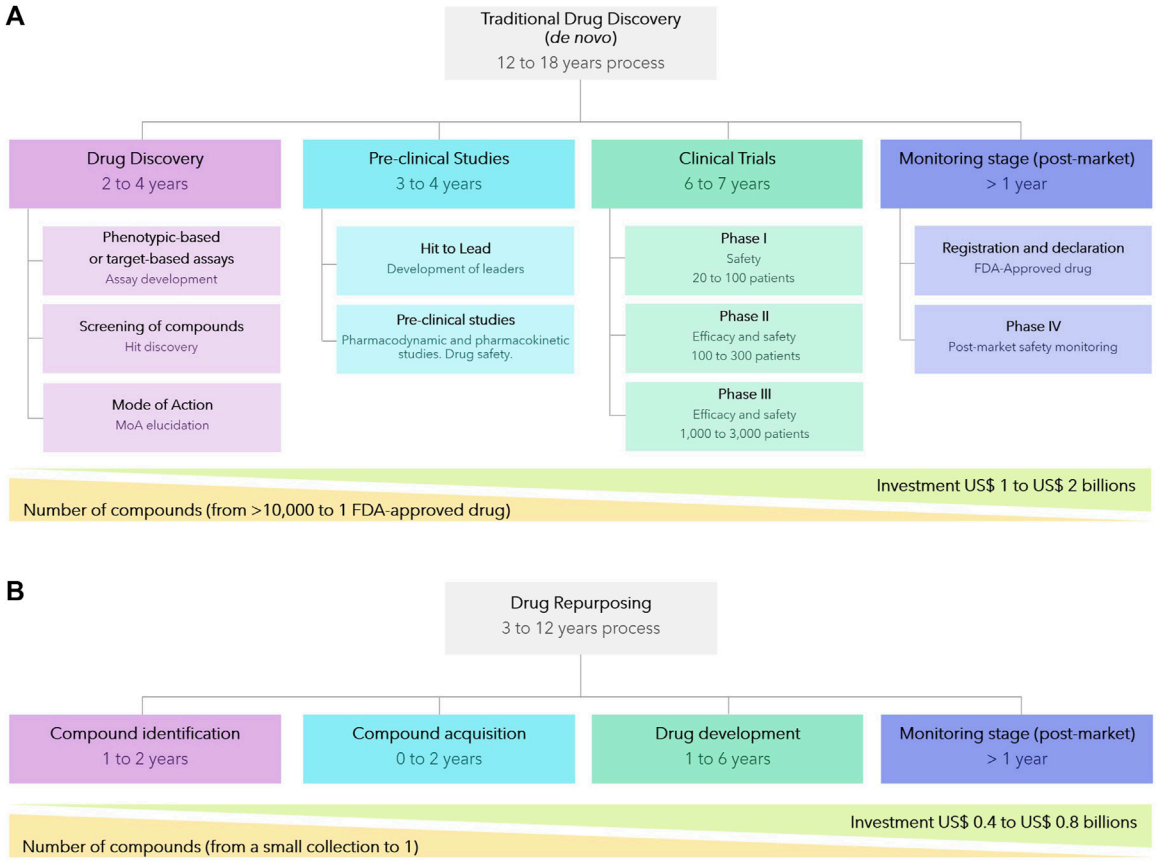


FIGURE 4
Comparison between *de novo* traditional drug discovery **(A)** and drug repurposing strategies **(B)**.

enlargement of the esophagus or colon), neurological or mixed alterations. Then, the infection can lead to progressive damage to the nervous system and heart muscle, resulting in cardiac arrhythmias, heart failure, and sudden death. This unusually slow progression, often without noticeable symptoms has led CD to be labelled as a "silent and silenced disease" and challenges diagnosis and a recognition of the severity of the problem (WHO, 2023).

3 Drug discovery vs. drug repurposing

De novo drug discovery, spanning from biochemical concept to the clinic, is an intricate, time-consuming, and expensive journey encompassing several crucial stages. These stages include compound identification, formulation, verification of efficacy and safety in animal models and human volunteers, numerous rounds of pre-clinical and clinical trials, and, ultimately, registration and regulatory approval. Collectively, this is estimated to take between 12–18 years and cost $1–2B to develop a new drug (Figure 4A). Moreover, the probability of failures, most commonly at later more expensive clinical trial stages, are high and this is reflected in the high cost of patented drugs. Consequently, the discovery of new drugs for CD, and other NTDs, for which the average patient exists on an income of less than $2 per day (Clark et al., 2014), is simply not viable for pharmaceutical companies without significant public cross-subsidy.

Drug repurposing, also known as drug repositioning or drug reprofiling, in which compounds developed for one indication are then utilized to provide solutions to an alternative disease, offers various advantages over developing an entirely new drug for a given indication (Pushpakom et al., 2019; Jourdan et al., 2020). Firstly, the risk of failure is lower because the repurposed drug has, if early-stage trials have been completed, already been found to be sufficiently safe in preclinical models and humans. Secondly, the drug development time frame is commonly both shorter and cheaper (Figure 4B) since some preclinical testing, safety assessments, and sometimes even formulation development, will have been completed. Given the reduced costs, drug repurposing is a particularly attractive approach for NTD (Krishnamurthy et al., 2022). Whilst repurposing approaches has been successful applied to afford approved drugs for other diseases, including NTDs such as Human African Trypanosomiasis and Leishmaniasis (where the majority of the current chemotherapy are repurposed drug (Charlton et al., 2018; Braga, 2019), there has yet not been a successful outcome for CD.

4 Approaches and techniques for drug repurposing

Strategies for drug repurposing follow traditional models for other drug discovery processes and encompass a combination of

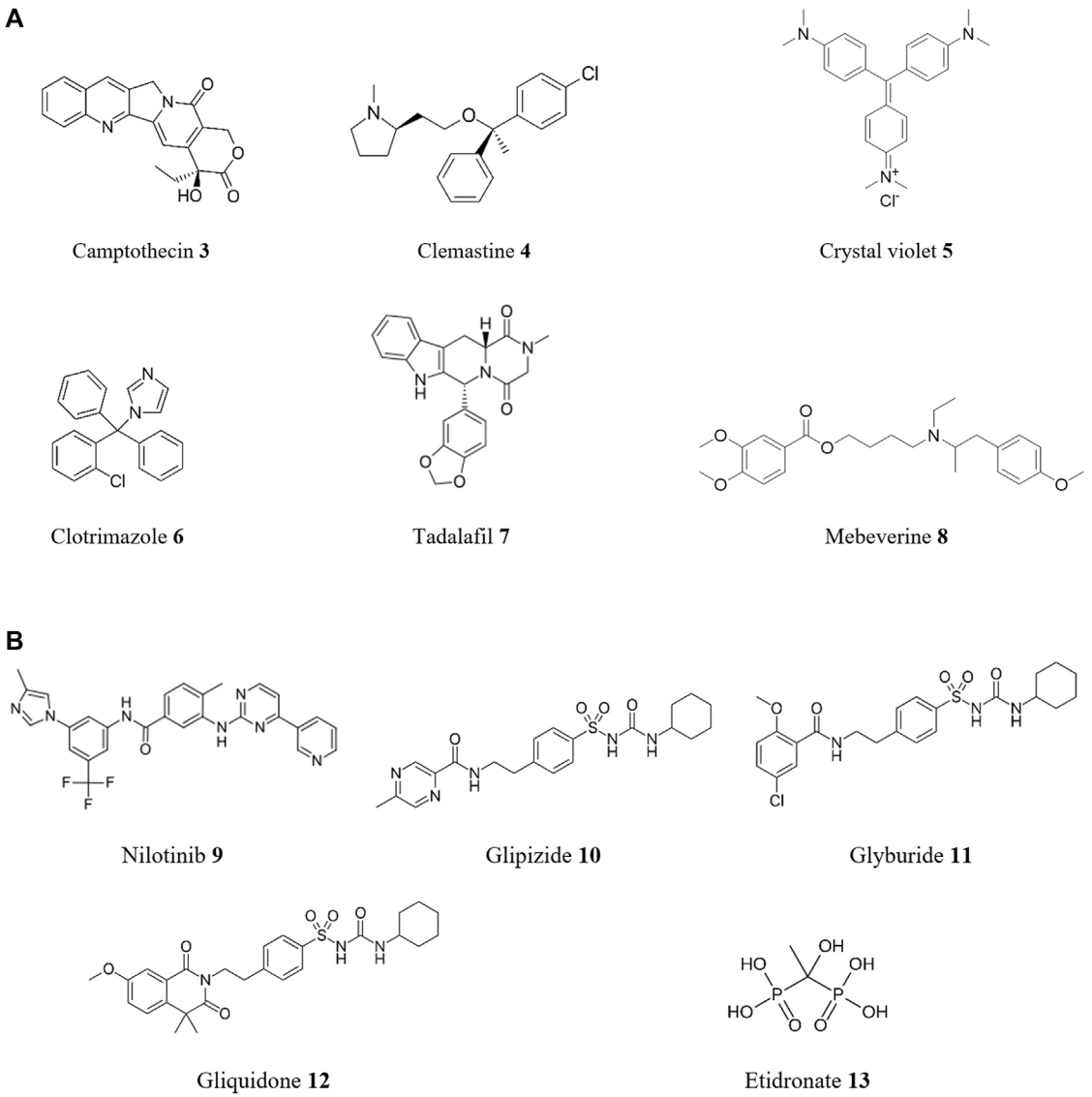


FIGURE 5
Structures of reported repurposed drugs for CD that have been identified using phenotypic-based approaches [**(A)**, compounds **3** to **8**] or by *in silico* target-based approaches [**(B)**, compounds **9** to **13**].

molecular and empirical approaches to enhance effectiveness and efficiency. Molecular approaches, commonly known as target-based strategies, primarily rely on hypothesis-driven methods at the single protein level. On the other hand, empirical approaches, known as phenotypic strategies, are centered around evaluating observable measures of response in a whole cell, organism, or system (Schenone et al., 2013; Swinney and Lee, 2020). Both strategies have their advantages and disadvantages that have been discussed in numerous review articles (Swinney, 2013; Moffat et al., 2017).

The process of discovering drugs through phenotypic screening is also referred to as "forward (or classical) pharmacology" or "forward chemical biology". For instance, a sensitive assay to identify compound activity against *T. cruzi* was developed by Sykes and Avery (2015). This used an image-based assay to estimate the effect of compound treatment on *T. cruzi* amastigotes in 3T3 fibroblasts and host cells. This assay identified active compounds from an in-house FDA-approved drug library and the MMV Malaria Box collection. Prominent compounds include camptothecin **3**, clemastine **4**, crystal violet **5**, and clotrimazole **6** (Figure 5A), all with sub-micromolar activities against this protozoan. In another phenotypic-based assay using a library of 100 registered drugs with drug repositioning potential for NTDs, two compounds with *in vitro* activity against *T. cruzi* were discovered (Kaiser et al., 2015). These compounds were tadalafil **7**, a phosphodiesterase type 5 inhibitor used to treat erectile dysfunction, with an EC_{50} of 8.6 μM and a selectivity index >26 (selectivity index or SI is defined as the ratio of the EC_{50} of host cell versus the EC_{50} of *T. cruzi*), and the antispasmodic mebeverine **8** with an EC_{50} of 3.89 μM and a SI = 18.

In contrast, target-based drug discovery, also known as 'reverse pharmacology,' begins with the formulation of a hypothesis that modulation of a specific protein target, believed to be crucial in disease modification, will yield favorable therapeutic effects (Swinney, 2013). The initial step involves the identification and validation of a molecular target, such as an enzyme or receptor, that plays a significant role in the disease process. Subsequently, a range of strategies is employed to discover and develop drugs capable of modulating the target's activity. These strategies encompass diverse techniques including simple biochemical and biophysical assays, in both single and high-throughput formats (*vide infra*), as well as virtual screening and other *in silico* methods. In the spirit of reducing cost, *in silico* methods which can screen large numbers of drug candidate compounds in a virtual manner using computer generated models of targets or known ligands are ideally suited to repurposing previously reported structures. By employing these techniques, researchers strive to identify innovative therapeutic interventions for CD, aiming to tackle the disease at its molecular roots. Over the past decade, numerous successful cases of drug repurposing for the treatment of CD have been reported using *in silico* approaches (Figure 5B). As an example, a novel virtual screening approach was developed by Juárez-Saldivar et al. (2020) to identify drug repositioning opportunities against *T. cruzi* infection by targeting the bifunctional enzyme dihydrofolate reductase-thymidylate synthase (DHFR-TS). Ten putative *Tc*DHFR-TS inhibitors were identified, including nilotinib **9**, glipizide **10**, glyburide **11**, and gliquidone **12**. These compounds showed growth inhibitory activity against *T. cruzi* epimastigotes. In other study (Valera-Vera et al., 2020), a virtual screening strategy was employed to identify potential inhibitors of enolase, a key enzyme and potential drug target for CD. This *in silico* study proposed etidronate **13**, a bisphosphonate inhibitor of bone resorption, as a potential inhibitor of this molecular target. Due to its safety profile, etidronate **13** provides valuable insights for the development of new drugs targeting *T. cruzi* enolase (*Tc*ENO). However, further exploration of its potential through *in vitro* and *in vivo* studies is necessary.

An important technique in both approaches is high-throughput screening (HTS), which allows researchers to rapidly screen thousands to millions of compounds in a relatively short period of time, greatly accelerating the drug discovery process. In a typical HTS experiment, a library of compounds is screened against a particular molecular target (target-based) or whole cell (phenotypic-based) assay (Blay et al., 2020). For instance, in the search for drug targets against *T. cruzi*, a HTS campaign focused on *Tc*GlcK, a glucokinase enzyme was performed by Mercaldi et al. (2019). Glucokinase and hexokinase are crucial in *T. cruzi*'s metabolic pathways, making their inhibition a promising strategy for drug discovery. Out of 13,040 compounds screened, the HTS campaign found 25 enzyme inhibitors from nine chemical classes. Thirteen compounds displayed low micromolar IC_{50} values for enzyme inhibition, with four showing low toxicity to NIH-3T3 murine host cells and notable *in vitro* trypanocidal activity. These four compounds, belonging to three chemical classes (3-nitro-2-phenyl-2H-chromene, N-phenyl-benzenesulfonamide, and gossypol scaffolds), include two potential hit-to-lead candidates (Figure 6A) from the 3-nitro-2-phenyl-2H-chromene class (namely, GLK2-003 **14** and GLK2-004 **15**), holding promise for further exploration in drug discovery.

However, it is important to note that drug repurposing strategies often incorporate both molecular and empirical approaches in complementary ways. This integrative approach maximizes the chances of success by leveraging the strengths of each strategy. By combining target-based and phenotypic methods, researchers can gain a more comprehensive understanding of the underlying biology, discover new targets or pathways, and optimize drug candidates for efficacy and safety. For instance, cysteine proteases are a class of enzymes that have been validated as drug targets for the development of safe and effective pharmacological agents for CD. One such cysteine protease is cruzain, the major cysteine protease of *T. cruzi*. It has been shown to be essential in various stages of the *T. cruzi* life cycle and is a target of rational drug design for chemotherapy of CD (Ferreira et al., 2022). A study screened the ZINC database for compounds with lead-like properties to find new inhibitors of the cruzain protease (Wiggers et al., 2013). Using consensus scoring, target-based molecular docking, and ligand-based similarity searching, 8,600 lead structures were identified from an initial library of 8.5 million compounds. These were then screened through Glide XP and HQSAR, and the top 5% were visually inspected. 23 compounds were selected for *in vitro* phenotypic-based testing against *T. cruzi*-infected cells, with the top two hits (NEQ176 **16** with an EC_{50} of 108 μM and NEQ42 **17** with an EC_{50} of 10.6 μM—Figure 6B) being structurally characterized by X-ray crystallography to understand their binding modes with cruzain. In a second virtual screening campaign, 163 FDA-approved and investigational drugs identified from the DrugBank database, were screened against cruzipain, another major cysteine protease and validated molecular target of *T. cruzi* (Bellera et al., 2014). In this case, using a combination of target-based and phenotypic-based approaches, levothyroxine **18** (Figure 6B), a drug used in hormone replacement therapy for hypothyroidism, had dose-dependent inhibition of cruzipain (IC_{50} of 38.43 μM) and antiproliferative activity on the *T. cruzi* epimastigotes (EC_{50} of 121 mM).

An intriguing strategy for drug repositioning involves the use of combination drug therapy. Combination therapy or polytherapy is a therapeutic intervention in which more than one medication or modality is used. This approach has become increasingly common in medicine, particularly in the treatment of cancer (Bayat Mokhtari et al., 2017) and infectious diseases (Shyr et al., 2021), as it can offer several benefits over single-drug therapies including enhanced efficacy, commonly greater than simple additive effects (synergistic effect), delaying the development of drug resistance, and reducing the risk of side effects and toxicity. Combining two or more drugs with different mechanisms of action can increase the success rate of drug repositioning by providing an alternative approach to treatment (Sun et al., 2016). For example, disulfiram **19**, a medicine used to treat chronic alcoholism, is currently undergoing Phase I/II clinical trials as combined chemotherapy with BZN **1** for CD (Saraiva et al., 2021). *In vitro* and *in vivo* experiments of BZN **1** with disulfiram **19** and/or its metabolite diethyldithiocarbamate **20** (Figure 6C) demonstrated synergistic effects, inhibiting parasite proliferation in infected macrophages and improved survival rates in infected mice compared to BZN **1** alone (Almeida-Silva et al., 2022). Microscopic analysis revealed

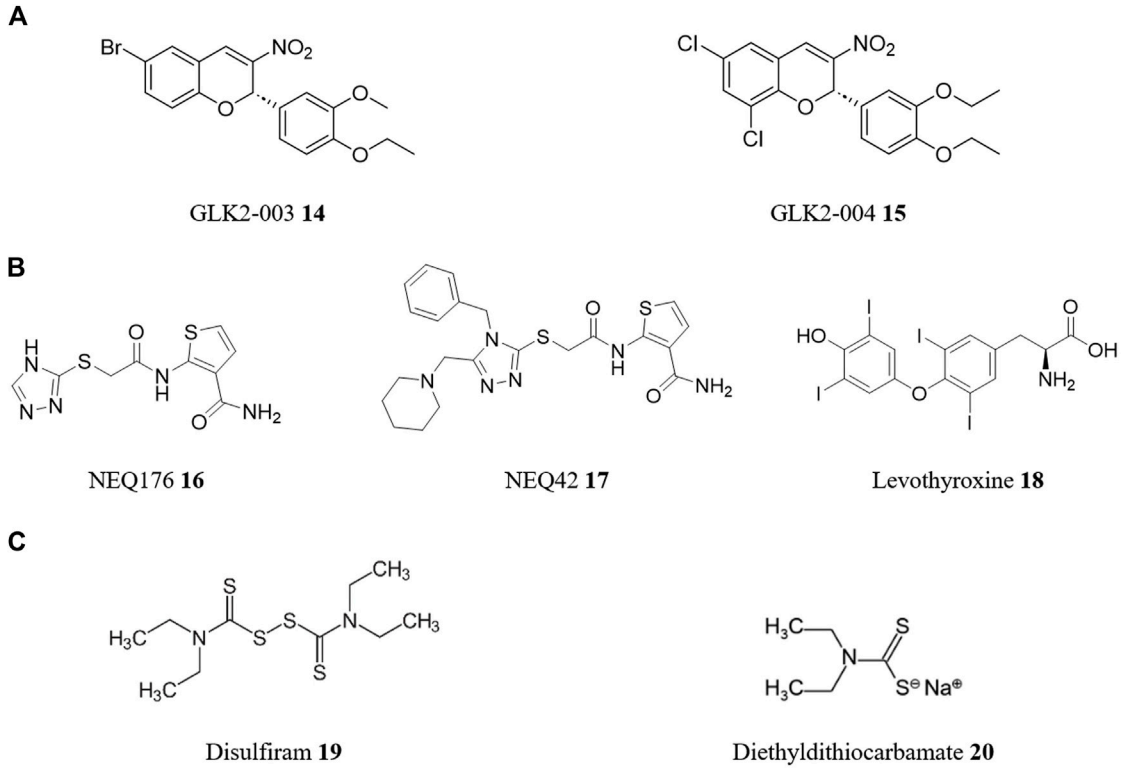


FIGURE 6
Structures of reported repurposed drugs for CD that have been identified using HTS [**(A)**, compounds **14** and **15**] or by a combination of both target-based and phenotypic-based approaches [**(B)**, compounds **16** to **18**]. Structures of repurposed drugs for combinatory therapy for CD treatment [**(C)**, compounds **19** and **20**].

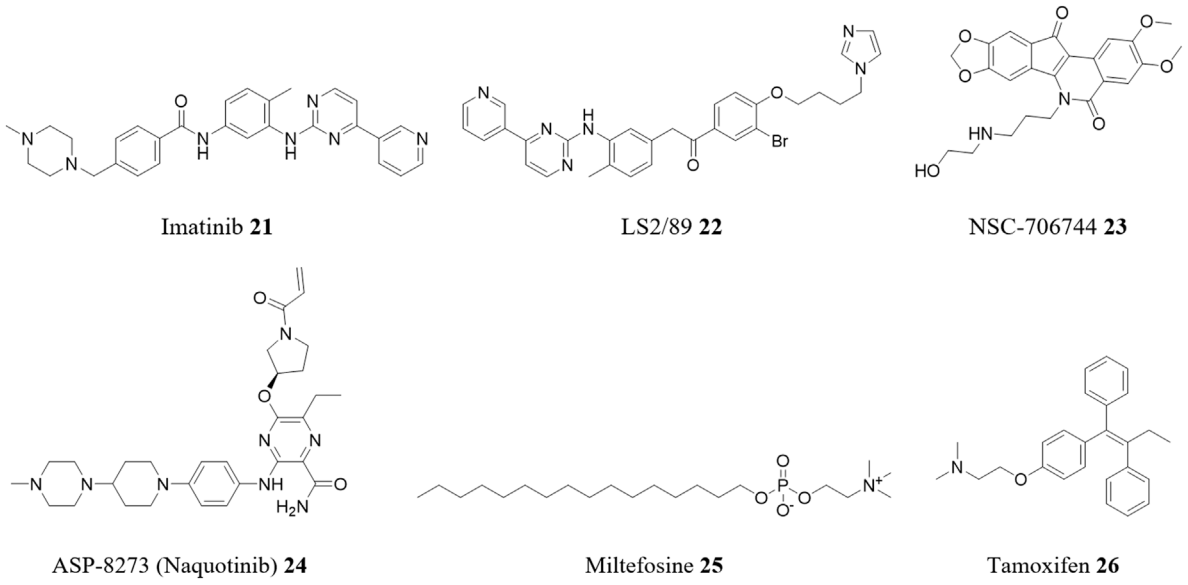


FIGURE 7
Structures of repurposed anticancer drugs for CD.

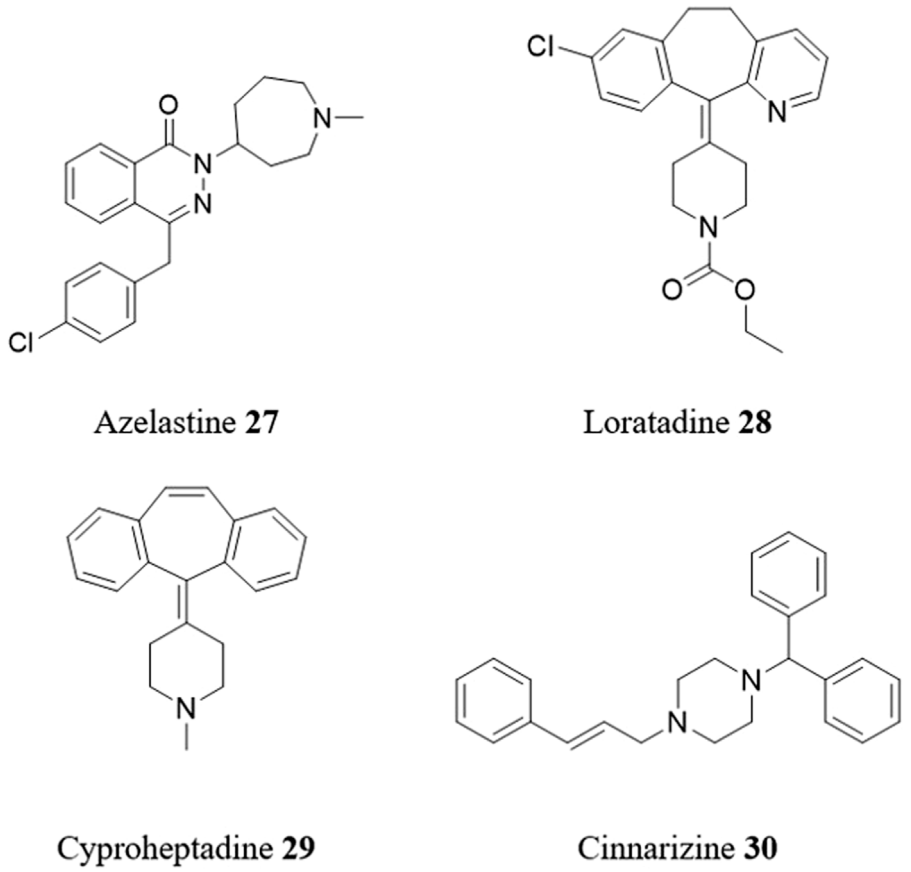


FIGURE 8
Structures of repurposed antihistaminic drugs for CD.

structural alterations in the parasites, whilst diethyldithiocarbamate **20** treatment increased reactive oxygen species production.

5 Advances on drug repurposing for CD

Given the pressing need for new treatments for CD, significant progress has been made over the past years through drug repositioning efforts. In this review, we present an overview of these works based on the therapeutic origin of the drugs used (for a comprehensive list of the compounds discussed and their biological activities, please refer to Supplementary Table S1; Supplementary Figures S1–S3, in Supplementary Material).

Many natural products exhibit significant therapeutic potential and possess a diverse array of biological properties, including antibacterial, antiprotozoal, antimycobacterial, antileishmanial, antitumor, and anti-human immunodeficiency virus (HIV) activities and represent a valuable and fruitful resource for the discovery and development of potent drugs against a variety of diseases (Álvarez-Bardón et al., 2020; Tempone et al., 2021; Lazarin-Bidóia et al., 2022). As such, there are numerous review studies focusing on the potential use of natural products with effects on *T. cruzi*, providing valuable insights for further investigation (Scotti et al., 2016; Nekoei et al., 2022; Barbosa et al., 2023). For instance, antibiotics such as echinomycin derived from *Streptomyces echinatus*, exhibit notable anti-*T. cruzi* activity at nanomolar concentrations (EC_{50} of 1.1 nM) (Annang et al., 2015). However, reflecting the complexities associated with the use of natural products as repurposing therapies, notably the lack of comprehensive *in vivo* studies and limited progress through clinical phases that hinder their immediate application, they will not be further explored in this review.

In these next sections, we delve into the exciting frontier of FDA-approved drug repositioning towards CD, investigating the rationale behind this approach, the challenges encountered, and the successful examples that have paved the way for the identification of novel therapeutic options. Although the majority of drugs discussed in this review are FDA-approved, we have also included, in certain instances, noteworthy molecules that are currently or have been undergoing clinical assessments, expanding the scope of potential therapeutic options for CD. The ultimate target product profile for CD is a multifactorial issue, but for simplicity in this review is described in terms of enhance efficacy and selectivity, safety and tolerability, and is realized by comparison to BZN **1** (DNDi, 2023). Additionally, we classified the selected examples of drug repurposing for CD in two ways: by approach (target-based or phenotypic-based strategies) and by technique. Within the technique category, we further subcategorize our findings into single drug trials (or small libraries), high-throughput screening, virtual screening, and combination drug therapy.

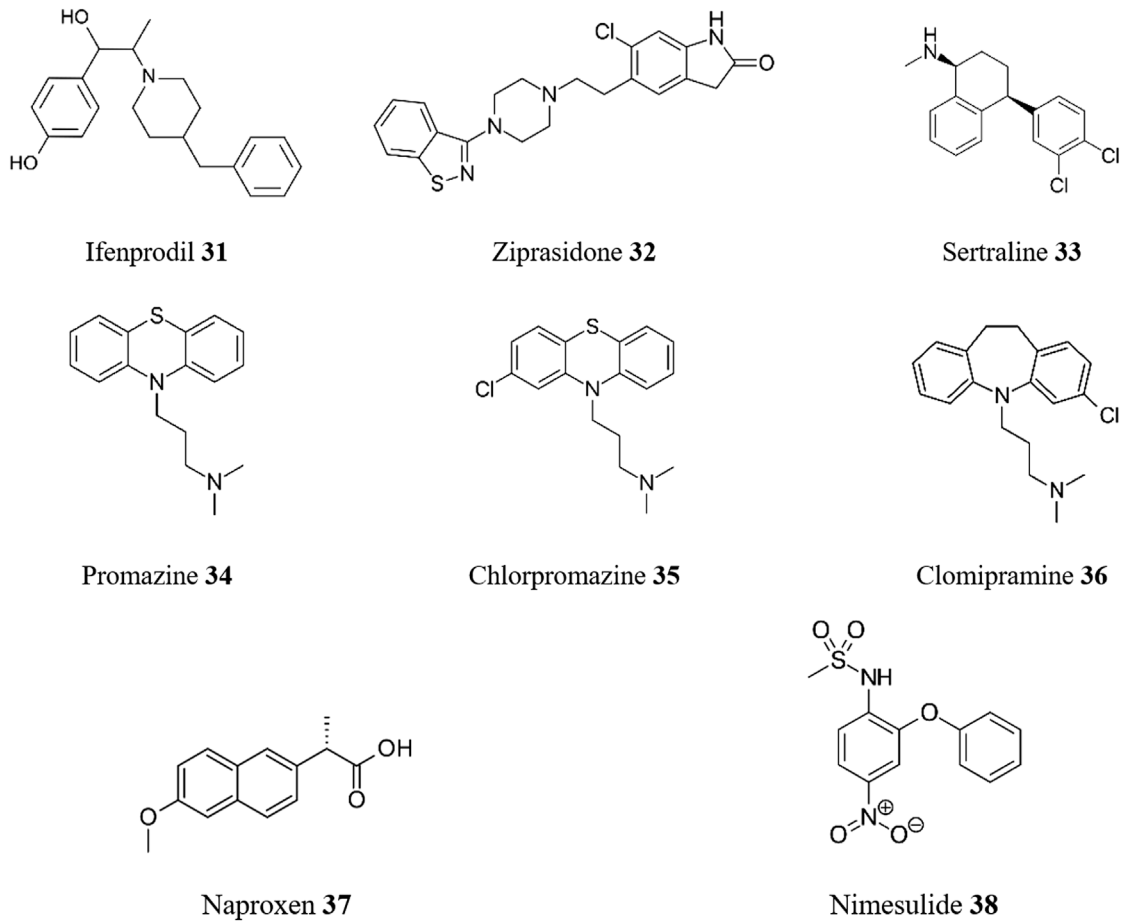


FIGURE 9
Structures of repurposed CNS drugs for CD.

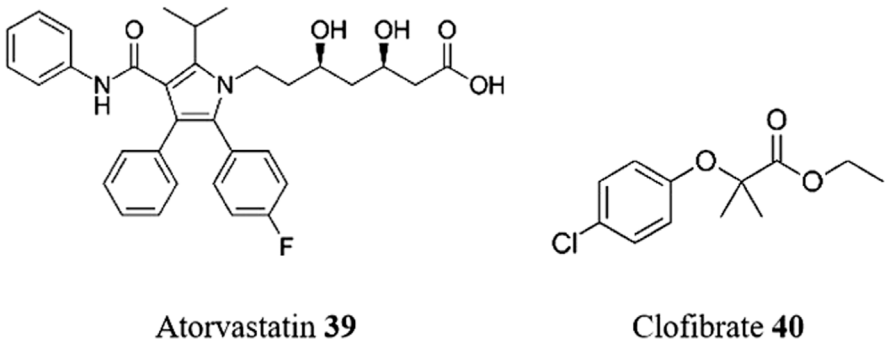


FIGURE 10
Structures of repurposed cholesterol-lowering medicines for CD.

5.1 Repurposing anticancer drugs

Anticancer agents, targeting a wide range of proteins such as kinases, epigenetic regulators, DNA repair enzymes, and proteasomes (Zhong et al., 2021), have emerged as a compelling avenue for repurposing towards CD, attracting considerable attention due to their diverse mechanisms of action and potential for multi-targeted effects. For these reasons, there are many instances of anticancer drugs being repurposed to other diseases, including Alzheimer's disease (Ancidoni et al., 2021),

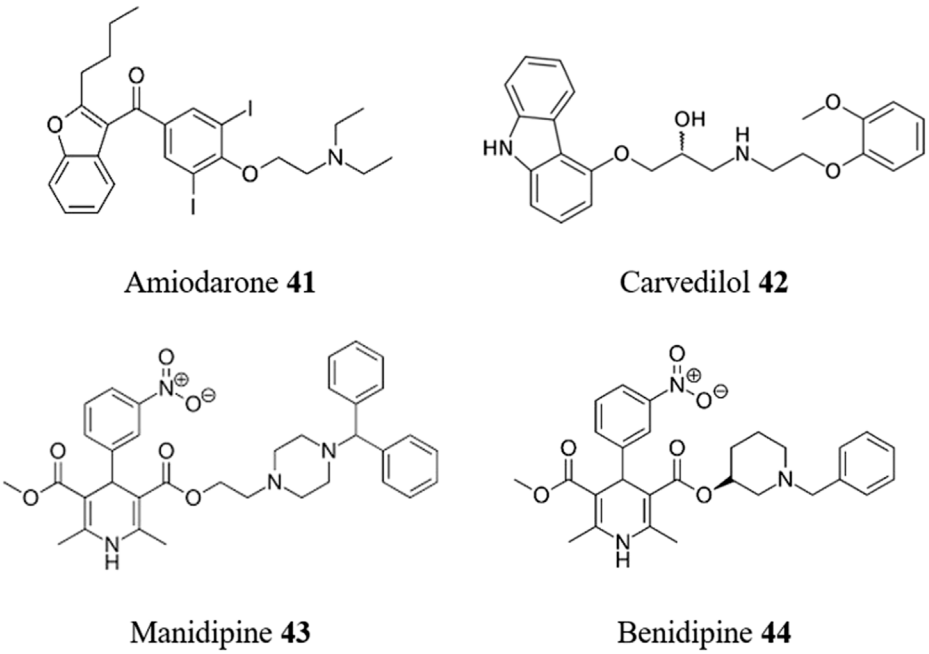


FIGURE 11
Structures of repurposed medicines for heart conditions toward CD.

malaria (Porta et al., 2019), COVID-19 (El Bairi et al., 2020), among others.

The tyrosine kinase inhibitor imatinib **21** (Figure 7), the first small-molecule tyrosine kinase inhibitor (TKI) approved for clinical use by the US Food and Drug Administration (FDA) (Savage and Antman, 2002), was screened for activity against *T. cruzi* (Simões-Silva et al., 2019a). In this phenotypic-based approach, imatinib **21** showed an *in vitro* trypanocidal activity (EC_{50}) of 24.8 μM against the intracellular forms of the parasite (SI = 1.5, Y strain) and an EC_{50} of 30.0 μM against the extracellular forms. An additive effect was observed in the combination of imatinib **21** + BZN **1**, in fixed-ratio proportions. Similarly, by using this drug as a pharmacophore in a hit-to-lead strategy, a promising synthetic analogue was developed: LS2/89 **22** (Nesic de Freitas et al., 2023). Although not a repurposed compound, this analogue demonstrated significantly improved potency, with antiparasitic activities more than 100-times lower against the intracellular form (EC_{50} of 0.19 μM) and 10-times lower against the trypomastigote form (EC_{50} of 2.67 μM), compared to imatinib **21**.

In another phenotypic assay, this time in HTS format, screening 7680 compounds from the Repurposing, Focused Rescue, and Accelerated Medchem (ReFRAME) library against *T. cruzi* (CA-I/72) identified seven compounds with potent *in vitro* activity (Bernatchez et al., 2020). These included the DNA topoisomerase inhibitor NSC-706744 **23** with a EC_{50} of 0.44 nM (SI = 214) and the EGFR inhibitor ASP-8273 **24** (naquotinib) with a EC_{50} of 2.7 nM (SI = 191).

Miltefosine **25**, an anti-breast cancer drug already repositioned for leishmaniasis, was evaluated as a monotherapy and in combination with BZN **1** against *T. cruzi* (Gulin et al., 2022). Miltefosine **25** alone showed efficacy against the parasite (VD strain, DTU TcVI) in both *in vitro* (amastigotes and trypomastigotes) and *in vivo* models, with no observed cytotoxic effects on host cells. When combined with BZN **1**, miltefosine **25** exhibited enhanced effectiveness, preventing parasitemia rebound. Given that miltefosine **25** is currently the sole approved oral antileishmanial drug and has demonstrated success in its repositioning for leishmaniasis treatment, it emerges as a highly promising and attractive candidate for further exploration in clinical phases to address CD.

Many drug repositioning studies for CD have shown promising *in vitro* activity profiles. However, there is often a lack of data on subsequent *in vivo* studies, which is crucial as many repositioned drugs fail during this stage. Tamoxifen **26** is an example of this. This antineoplastic drug was tested for its activity against *T. cruzi* and showed promising results *in vitro* against the epimastigote, trypomastigote, and amastigote forms of the CL14, Y, and Y benznidazole-resistant *T. cruzi* strains (Miguel et al., 2010). Tamoxifen **26** demonstrated activity against all life-cycle stages of the parasite, with EC_{50} ranging from 0.7 to 17.9 μM across all strains. However, *in vivo* tests using two experimental models of acute CD showed no significant differences in parasitemia or mortality between tamoxifen-treated and control mice.

5.2 Repurposing antihistaminic compounds

De Rycker et al. (2016) has described a screening cascade for the identification of compounds with anti-*T. cruzi* activity from 963 clinically tested compounds (from NIH Clinical Collection and the SelleckChem FDA-approved drug library). The cascade includes a primary assay that allows the determination of

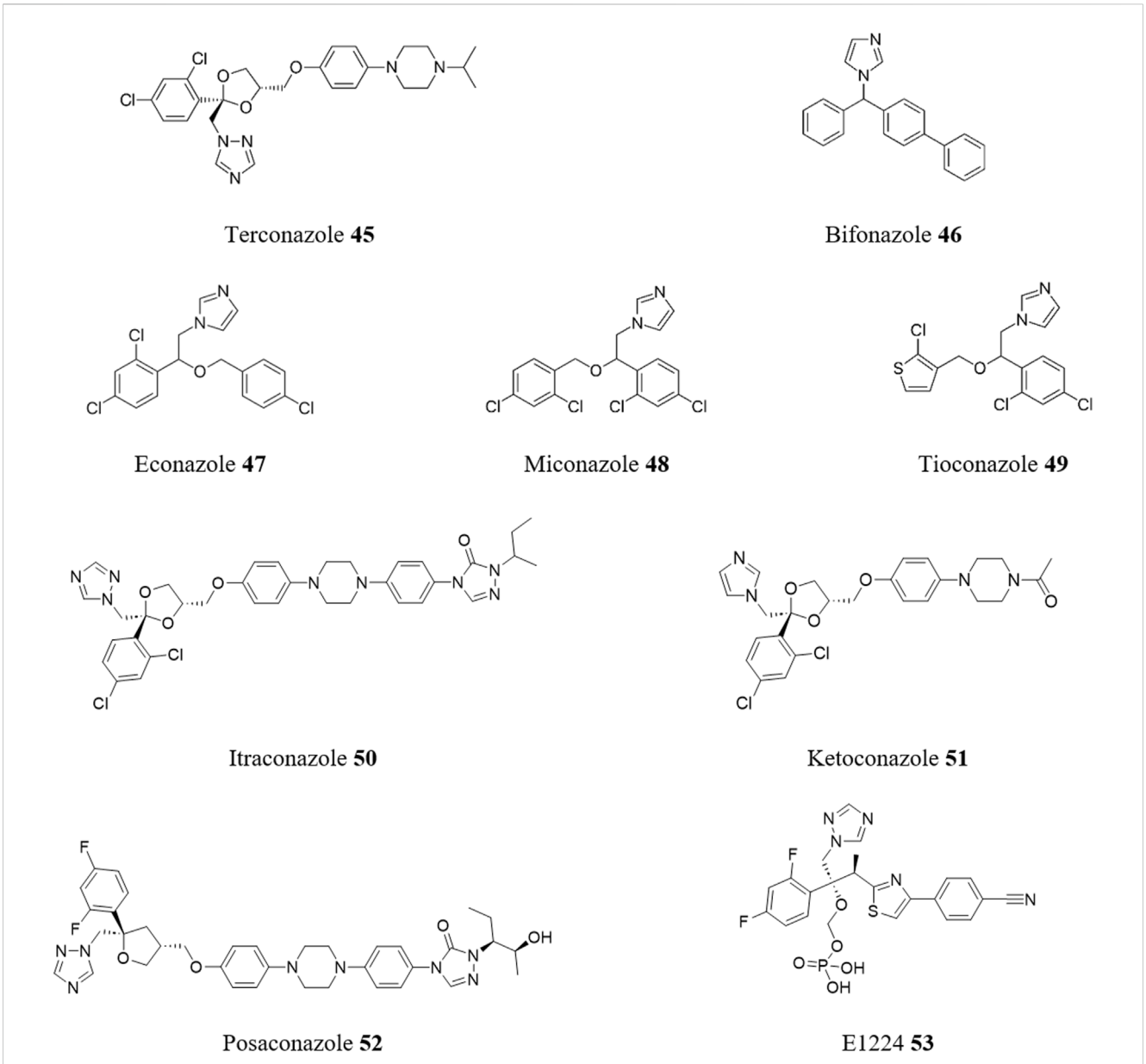


FIGURE 12
Structures of repurposed antifungal drugs for CD.

14,080 single point measurements (for library screening) or up to 1,280 potency determinations in a single run, and secondary assays to assess static-cidal, rate-of-kill, and cytochrome P450 CYP51 inhibition. A number of promising targets were identified including the first-generation H1 histamine antagonists clemastine **4** and azelastine **27** (Figure 8). Although further optimization is needed due to limitations in their pharmacokinetic and toxicity profiles, these compounds could offer valuable starting points for the development of effective treatments for CD.

The dye crystal violet **5** is known for its ability to eliminate *T. cruzi* in blood banks (Docampo et al., 1983) by inhibiting proline uptake through the proline permease *Tc*AAAP069. However, due to its high cytotoxicity and low selectivity, it is not considered a suitable candidate for drug repositioning. Using ligand based *in silico* drug repurposing, the FDA-approved antihistamines loratadine **28** and cyproheptadine **29** were identified as structurally related compounds to crystal violet **5** (Sayé et al., 2020). These drugs inhibited *Tc*AAAP069 activity and displayed trypanocidal action against all *T. cruzi* life stages in different strains. When combined with BZN **1**, a synergistic effect was observed with loratadine **28** or cyproheptadine **29**.

Alberca et al. (2018) reported a combined ligand- and structure-based virtual screening campaign to find inhibitors of putrescine (a polyamine) uptake in *T. cruzi*. Using an ensemble of linear ligand-based classifiers as an initial screening filter and then docking the results into a homology model of the putrescine permease *Tc*PAT12, the DrugBank and Sweetlead databases were screened for drug repositioning opportunities. As a result, cinnarizine **30**, an

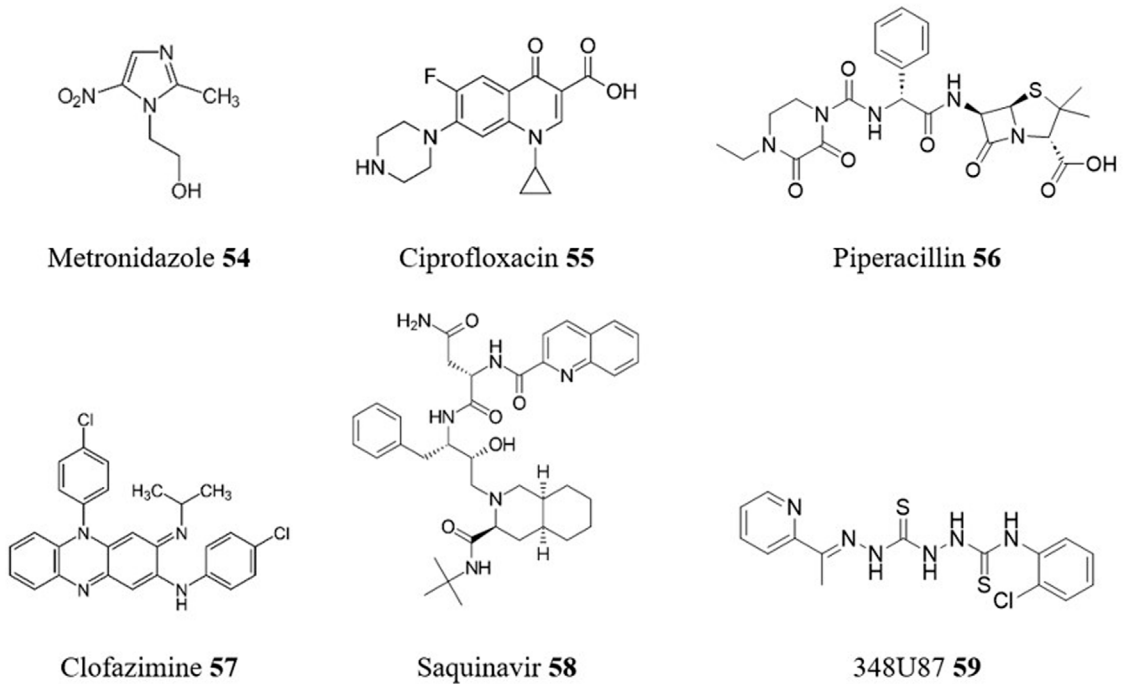


FIGURE 13
Structures of repurposed antibacterial and antiviral drugs for CD.

antihistamine drug for motion sickness and balance disorder, was selected and tested against *T. cruzi*. Its trypanocidal effects and inhibitory effects on putrescine uptake were confirmed by *in vitro* studies.

5.3 Repurposing CNS drugs

CNS drugs are medications that affect the central nervous system (CNS). There are many different types of drugs that work on the CNS, including anesthetics, anticonvulsants, antiemetics, antiparkinsonian agents, CNS stimulants, muscle relaxants, narcotic analgesics (pain relievers), nonnarcotic analgesics (such as acetaminophen and NSAIDs), and sedatives. Additionally, pathological studies in CD have confirmed that nodular encephalitis in multiple foci is a key finding in the acute nervous form of the disease (Pittella, 2009). CNS involvement is uncommon in mild cases but can occur in immunosuppressed patients. Ischemic cerebral changes associated with chronic Chagas cardiomyopathy are also common. Given these findings, it is not surprising that the repurposing of CNS medications for the treatment of CD shows promise. For example, ifenprodil **31** (Figure 9), an inhibitor of the NMDA receptor that is currently undergoing clinical trials as a potential repurposed treatment for COVID-19 (Hashimoto, 2021), and ziprasidone **32**, an atypical antipsychotic drug, were identified as promising candidates for drug repurposing through the high content phenotypic screening described above (De Rycker et al., 2016).

In a second example, sertraline **33**, an antidepressant drug, displayed effectiveness against *T. cruzi* strains *in vitro*, with EC_{50} values of 1.4 μM for intracellular amastigotes (Y strain, SI = 17.8) and 14 μM for bloodstream trypomastigotes (Ferreira et al., 2018). Sertraline **33** affected the mitochondrial integrity of *T. cruzi*, decreasing ATP levels. *In silico* approaches using chemogenomic target fishing, homology modeling, and molecular docking suggested *Tc*IDH2 (isocitrate dehydrogenase 2) as a potential target for this drug.

It is well-established that *T. cruzi* relies on the uptake of polyamines from the extracellular medium for survival, therefore, polyamine transporters are promising targets for trypanosomatids (Talevi et al., 2019), as well as other protozoan parasites (Panozzo-Zénere et al., 2018). A ligand-based virtual screening using Ant4, an anthracene-putrescine conjugate inhibitor of the polyamine transport system, as a reference molecule identified three CNS drugs as possible inhibitors of polyamine transport (Reigada et al., 2019a). The antipsychotics promazine **34** and chlorpromazine **35**, and the antidepressant clomipramine **36** were effective inhibitors of putrescine uptake and showed good trypanocidal activity against *T. cruzi* (EC_{50} < 10 μM in all cases). Molecular docking simulations suggest good interactions between the *T. cruzi* polyamine transporter *Tc*PAT12 and these drugs. Additionally, these phenothiazine derivatives have been reported as specific inhibitors of parasite-trypanothione reductase (Iribarne et al., 2009). A study explored combining clomipramine **36** with BZN **1** (García et al., 2016). *In vitro* and *in vivo* tests showed a synergistic effect against *T. cruzi*. During the acute phase, BZN **1** reduced parasitemia, but combining it with clomipramine **36** completely suppressed it. Importantly in the chronic phase, mice treated with both drugs had lower heart damage and inflammation when compared to BZN **1** alone suggesting that this combination shows potential to enable lower BZN **1** doses and concomitant improved safety.

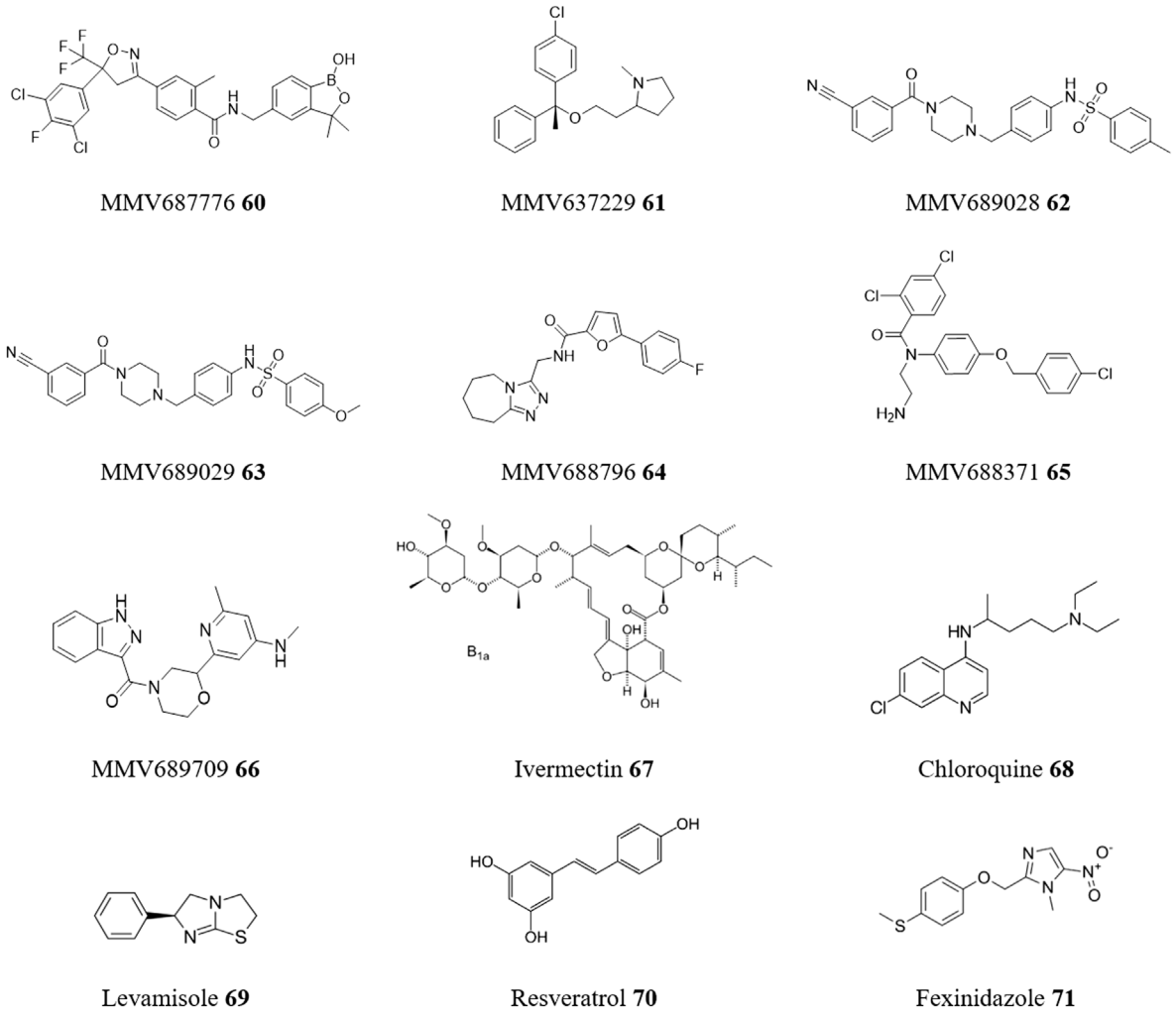


FIGURE 14
Structures of repurposed antiparasitic drugs for CD.

In another case, a structure-based drug repositioning approach was conducted over a set of 20 *T. cruzi* targets to find new treatments for CD (Adasme et al., 2020). The screening yielded over 500 molecules as hits, out of which 38 drugs were prioritized. Compounds showing growth inhibitory activity (<100 μM) when tested on *T. cruzi* trypomastigotes and epimastigotes were selected for further *in vivo* investigation. In mice, a single dose of 100 mg/kg body weight of the nonsteroidal anti-inflammatory naproxen **37** demonstrated the highest inhibition of parasitemia (85.8%) of this collection. For reference, a single dose of at 100 mg/kg body weight of NFX **2** exhibited parasitemia inhibition of 77.8%. Another non-steroidal anti-inflammatory drug nimesulide **38** was identified by Trindade et al. (2021) as a potential candidate for repositioning as a treatment for CD. Treatment of *T. cruzi* epimastigotes with nimesulide **38** resulted in dose-dependent cell death. Nimesulide **38** also inhibited the replication of intracellular amastigotes in *T. cruzi*-infected macrophages. The study suggested that nimesulide **38** affects the parasite's cell redox balance, leading to cell death primarily through oxidative stress. Ultrastructural changes and a mixed mechanism of cell death involving both apoptosis and necrosis were observed in nimesulide-treated epimastigotes.

5.4 Repurposing cholesterol-lowering medicines

Trypanosoma cruzi has a high affinity for host lipoproteins and uses the low-density lipoprotein receptor to invade cells. Moreover, Johndrow et al. (2014) has shown that *T. cruzi* infection is associated with an accumulation of low-density lipoprotein and cholesterol in tissues during both the acute and chronic stages of murine CD. This has led to the suggestion that drugs that help lower cholesterol levels in the blood, may have positive effects for CD. The most common anti-cholesterol drugs are the statins, for which the primary target is HMG-CoA reductase. Interestingly, this enzyme is also relevant in the case of *T. cruzi* (Peña-Diaz et al., 2004). However, statins also been shown to possess additional roles including antioxidative, anti-inflammatory, antiatherogenic, and chemotherapeutic activities (Liao and Laufs, 2005; Zhang et al., 2020). Considering these factors together, there have been numerous reports on the repurposing of statins in the context of *T. cruzi*. In this respect, it is noteworthy that the antiparasitic effect of the antifungal ketoconazole (*vide infra*) was improved by lovastatin in a murine

model of CD (Urbina et al., 1993). This is proposed to arise through statin mediated reduction of heart inflammation that is commonly observed in chronic *T. cruzi* infection (Guzmán-Rivera et al., 2020).

The activity and selectivity of atorvastatin **39** (Figure 10), an inhibitor of cholesterol synthesis, against *T. cruzi* were evaluated by Araujo-Lima et al. (2018). Atorvastatin **39** showed activity against different strains (Y and Tulahuen) and forms of the parasite (amastigotes and trypomastigotes) with good selectivity in all cases (SI > 20). Combinatory approaches using atorvastatin **39** and BZN **1** in fixed ratio gave synergistic interactions against both trypomastigotes and intracellular forms. Recently, a phase II, multicenter trial to evaluate the impact of atorvastatin **39** on inflammation and cardiac function in patients with chronic CD has been described (Campos-Estrada et al., 2023).

Clofibrate **40**, a lipid-lowering agent commonly used to control high cholesterol and triglyceride levels in the blood, belongs to the class of fibrates. Two groups independently explored its therapeutic potential using an initial virtual screening approach. On one hand, a computer-guided drug repositioning method was employed to identify potential FDA-approved drugs as inhibitors of cruzain, the major cysteine protease of *T. cruzi* (Palos et al., 2017). Through virtual screening of 3180 FDA drugs, clofibrate **40** was selected for *in vitro* and *in vivo* testing. Interestingly, clofibrate **40** emerged as one of the highlighted and selected drugs in the cascade screening conducted by De Rycker et al. (2016), as discussed previously. However, whilst clofibrate **40** exhibited superior activity profiles compared to commercially available drugs (EC_{50} in amastigotes of 6.31 μM), BZN **1** and NFX **2**, in *in vitro* studies, it demonstrated inferior results in short-term *in vivo* studies to reduce parasitemia in infected mice, using a single dose of 100 mg/kg of each drug (reduction to 50% of parasitemia at 6 h for BZN **1**, and a range of 60%–90% for clofibrate **40**).

5.5 Repurposing medicines for heart conditions

Given that CD frequently manifest itself in cardiac failure it is not surprising that repurposing drugs targeted toward other heart conditions has been a popular avenue to explore. The combination of BZN **1** with amiodarone **41** (Figure 11), an antiarrhythmic drug used to treat chronic cardiac CD and also previously recognized as a trypanocidal agent (Adesse et al., 2011), has been investigated (Barbosa et al., 2022). The combined treatment did not improve the direct trypanocidal effect of amiodarone **41** but attenuated the infection-induced cytoskeleton damage of host cells and cytotoxic effects of amiodarone **41**. Therefore, this combination treatment may favor parasite control and limit tissue damage. An alternative coupling of amiodarone **41** with the antifungal itraconazole **51** (*vide infra*) provides an effective treatment of *T. cruzi*-infected dogs and supports the potential therapeutic application of this combination against trypanosomatid infections in humans (Benaim et al., 2021).

Carvedilol **42**, a beta-blocker, selected through virtual screening on the cysteine protease cruzipain of the SWEETLEAD library of approved drugs, promotes the accumulation of immature autophagosomes with decreased acidity and hydrolytic properties by inhibiting the autophagy flux (Rivero et al., 2021). As a result, the viability of trypomastigotes is compromised, and the replication of epimastigotes and amastigotes at 10 μM is hindered, leading to a significant reduction in infection and parasite load. Significantly this effect is maintained in *in vivo* studies with carvedilol diminishing the peak whole-body parasite burden in infected mice.

The antihypertensive drug manidipine **43** was evaluated *in vitro* against *T. cruzi* (Correa et al., 2021) showing potent antiparasitic activity against multiple life cycle stages (EC_{50} of 0.1 μM in amastigotes and 3 μM in trypomastigotes), with promising selectivity against intracellular amastigotes versus the host cell (SI > 1459). Fluorometric analysis showed that manidipine **43** caused depolarization of the plasma membrane and decreased ATP levels, suggesting mitochondrial bioenergetic alteration of the parasite as a potential mode of action.

Finally, benidipine **44**, a calcium channel blocker, has emerged as another promising candidate for repurposing as a trypanocidal drug (Bellera et al., 2015). Discovered by computer-aided screening as a cruzipain inhibitor, this compound underwent comprehensive evaluation, including biochemical and cellular studies, as well as biopharmaceutical, toxicological, physiopathological, and preclinical experiments utilizing an acute model of infection. Remarkably, benidipine **44** demonstrated potent efficacy in reducing parasitemia in an experimental preclinical acute murine infection model at significantly lower doses compared to the positive control, BZN **1** (10 mg/kg/day versus 100 mg/kg/day, respectively). The same research group later found that chronically infected mice treated with this compound showed a reduction in both quantitative and qualitative measures of inflammation compared to untreated mice (Sbaraglini et al., 2016). This was particularly significant in cardiac and skeletal muscle. The reduction in tissue damage can be attributed to the parasiticidal properties of cruzipain inhibitors.

5.6 Repurposing antifungal drugs

Unlike mammalian cells, trypanosomatids primarily produce ergosterol rather than cholesterol, which is similar to the sterol metabolism found in fungi. As such, the sterol biosynthesis pathway presents promising targets for the development of new drugs to treat kinetoplastid infections (Porta et al., 2014; Porta et al., 2023). These targets are sensitive to azoles (Leaver, 2018). These well-known antifungal drugs are already recognized for their activity against *T. cruzi* and act, generally, via inhibition of 14-alpha-sterol demethylase (Buckner and Urbina, 2012). For instance, using a phenotypic-based repurposing strategy, the trypanocidal activity of terconazole **45** (Figure 12), a triazole antifungal drug, was evaluated (Reigada et al., 2019b), revealing trypanocidal activity *in vitro* against epimastigotes of different parasite strains and clinically relevant life-stages of *T. cruzi* (EC_{50} between 4 and 25 μM). Consistent with the above premise, molecular docking simulations suggested that terconazole inhibits *T. cruzi* cytochrome P450 14-alpha-demethylase.

In another case, a collection of 100 registered drugs with repositioning potential for NTDs was gathered and assessed *in vitro* (Kaiser et al., 2015). From a dose-response phenotypic-based screen seven azoles and triazoles were identified as the most potent class of inhibitors from this set of compounds. These active compounds displayed EC_{50} values in the range of 0.003–0.3 μM and SI > 100, and include the imidazoles bifonazole **46**, clotrimazole **6**, econazole nitrate **47**, miconazole **48** and tioconazole **49**, and the triazoles itraconazole **50** and ketoconazole **51**.

However, the failure of two triazole antifungals, posaconazole **52** and E1224 **53**, to demonstrate sustained clearance of *T. cruzi* parasitemia in chronically infected patients in phase II clinical trials (Molina et al., 2014) has challenged the sole use of azoles as a therapeutic class for the treatment of CD. Given this, coupled with the limited efficacy of treatment for CD in the chronic phase, a study was conducted in dogs infected with a benznidazole-resistant strain of *T. cruzi* to test the effectiveness of combining BZN **1** with the antifungal azole itraconazole **50** (Cunha et al., 2022). The dogs were divided into four groups and treated with BZN **1**, itraconazole **50**, a combination of BZN **1** and itraconazole **50**, or left untreated. Over a period of 24 months, the BZN **1** and BZN **1** + itraconazole **50** groups showed negative results in PCR and hemoculture tests, while BZN **1** alone showed partial success. Reactive immunoassay results persisted in all treated animals. Echocardiography and histopathological analysis indicated improved cardiac conditions in the BZN **1** + itraconazole **50** group compared to itraconazole **50** alone. Inflammation and fibrosis were significantly reduced in the BZN **1** + itraconazole **50** group, suggesting an improvement or stabilization of the dogs' clinical condition.

5.7 Repurposing antibacterial and antiviral drugs

Metronidazole **54** (Figure 13) is a broad-spectrum nitroimidazole antibiotic with activity against various parasites including trichomonas and giardia. In *in vitro* repurposing studies, as a monotherapy, metronidazole **54** had low potency against *T. cruzi*, but in combination with BZN **1**, it increased BZN's efficacy. *In vivo*, metronidazole **54** did not suppress parasitemia but improved survival at certain doses, and the combination therapy with BZN **1** prevented mortality and protected against electric cardiac alterations caused by the parasite (Simões-Silva et al., 2017).

A number of other antibiotics have emerged from various screening campaigns. For example, in the structure-based screening conducted by Adasme et al. (2020), the most effective *in vitro* trypanocidal compound was the fluoroquinolone antibiotic ciprofloxacin **55**. Further evaluation in an *in vivo* experiment, showed that ciprofloxacin **55** demonstrated good levels of inhibition of parasitemia in mice (66.7% in a single dose of ciprofloxacin **55** at 100 mg/kg body weight). Similarly, piperacillin **56**, a β-lactam antibiotic, emerged as the lead candidate from a virtual screening of 3180 FDA-approved drugs conducted by Palos et al. (2017) for potential inhibition of the *T. cruzi* cysteine protease cruzain. Piperacillin **56** exhibited superior *in vitro* trypanocidal activity profiles and comparable results in reducing parasitemia in infected mice during in short-term *in vivo* studies, when compared to the standard drug BZN **1**. A similar target-based virtual screening study identified the antibiotic clofazimine **57**, which finds current use for the treatment of leprosy, as a promising lead structure for the inhibition of proline uptake through the proline permease TcAAAP069 (Sayé et al., 2020).

A number of antiviral drugs have been evaluated for their potential against CD, with mixed results. Saquinavir **58**, an antiretroviral drug, showed potential as a trypanocidal compound in *in vitro* assays (Bellera et al., 2015). However, poor solubility prevented it from advancing to *in vivo* studies in mice. In contrast, the antiherpetic compound 348U87 **59** has demonstrated considerable promise against *T. cruzi* CA-I/72 strain showing a sub-nanomolar EC_{50} (0.63 nM) and a SI of 1294 (Bernatchez et al., 2020).

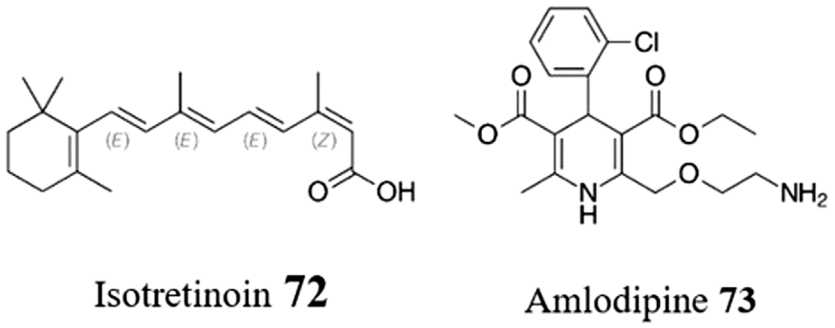
5.8 Repurposing antiparasitic drugs

It is only logical that existing antiparasitic agents are candidates for drug repositioning for treatments for CD. One resource is the open-access Pathogen Box collection provided by the Medicines for Malaria Venture (MMV), (MMV, 2023). Pathogen Box is a collection of 400 compounds, including 200 drug-like and 200 probe-like compounds. These were selected from over 20,000 antimalarial hits from corporate and academic libraries and represent a structurally diverse set. Testing the complete Pathogen Box identified many molecules with good activity (micromolar and sub-micromolar) and SI (>5) in *T. cruzi* phenotypic-based assays (Duffy et al., 2017). Among them, 7 compounds stood out (Figure 14): MMV687776 **60**, MMV637229 **61**, MMV689028 **62**, MMV689029 **63**, MMV688796 **64**, MMV688371 **65**, and MMV689709 **66**. Whilst not formally repurposing, all these structures represent strong starting points for the search for new chemical entities against CD.

In a true repurposing study, the broad-spectrum antiparasitic ivermectin **67** was investigated as a trypanocidal agent against *T. cruzi* (Fraccaroli et al., 2022). In this case, ivermectin **67** affected the proliferation of *T. cruzi* epimastigotes and amastigotes, and the viability of trypomastigotes in a dose-dependent manner, with a SI of 12 for the amastigote stage. However, drug combinations of ivermectin **67** with BZN **1** or NFX **2** showed mainly additive effects. In contrast, a combination of BZN **1** and the anti-malarial drug chloroquine **68** significantly reduced *T. cruzi* infection *in vitro* and was eight times more effective in reducing *T. cruzi* infection *in vivo* than BZN **1** monotherapy. This could enable higher treatment efficacy while mitigating the adverse effects of high doses of BZN **1** (Pandey et al., 2022).

Co-administration of the anthelminthic drug levamisole **69**, with BZN **1** partially reduced parasitemia and slightly promoted animal survival (Simões-Silva et al., 2019b). As levamisole **69** has immunomodulatory activity, and when tested alone did not decrease parasitemia or mortality rates in a murine infection model, this suggest that these effects are related to Th1-response modulation. Similarly, resveratrol **70**, an activator of type III KDACs, which has been shown to exhibit a range of anti-parasitic properties including anti-*T. cruzi* effects (Campo, 2017), also has a host cell response. For example, a resveratrol **70** dosage partially protected mammal cells from infection without activating apoptosis. However, these were *in vitro* experiments and further *in vivo* studies are needed to determine if this therapy could be used as a pre-exposure prophylactic drug (Rodriguez et al., 2022).

Finally, a notable example of drug repositioning is the translational research program by DNDi (Drugs for Neglected Diseases initiative) that repositioned fexinidazole **71** for treatment of CD. Originally developed in the late 1970s as a broad-spectrum anti-infective agent, fexinidazole **71** is a pro-drug of 5-nitroimidazole activated by NTR-1. It was later selected, among other 700 nitroheterocyclic compounds, for development by the



Isotretinoin **72** Amlodipine **73**

FIGURE 15
Structure of isotretinoin **72** and amlodipine **73**.

DNDi as a treatment for sleeping sickness (Torreele et al., 2010). It has shown superior efficacy in curing experimental *T. cruzi* infections compared to BZN **1** and NFX **2**. It also has been reported for its oral efficacy in acute and chronic experimental models of benznidazole-susceptible, partially resistant, or resistant *T. cruzi* isolates (Bahia et al., 2012). A Phase II clinical trial evaluating its effectiveness, safety, and tolerability in patients with asymptomatic chronic infections was completed at the end of 2022 (Torrico et al., 2023).

6 Perspective, challenges, and future directions

In line with the WHO's roadmap 2021–2030 for eradicating NTDs, and especially in the context of CD, there is an urgent need to expand the range of chemotherapeutic options (Casulli, 2021). The current therapy for this condition is limited, inefficient, and insufficient, consisting of only two outdated drugs. As such, finding a rapid solution is crucial. As evidenced by successes in treating other diseases (Charlton et al., 2018; Hua et al., 2022), coupled with the encouraging results achieved over the past years, summarized in this article, to develop to develop new and improved therapies for CD, drug repurposing offers hope for making progress in the fight against one of the most neglected NTDs.

Despite its promise, drug repositioning (and drug discovery in general) in *T. cruzi* faces several challenges, both technical and applied. One major challenge is the lack of comprehensive knowledge of *T. cruzi* biology and drug targets (Jansen et al., 2020). This is exemplified by the extensive morphological and metabolic transformations that occur throughout the various developmental stages of *T. cruzi*, which challenge efforts to develop effective drugs against this parasite (Teixeira et al., 2012). Most notably, the intracellular amastigote can enter in a metabolically quiescent or dormant state which can be non-responsive to otherwise effective trypanocidal drugs (Sánchez-Valdéz et al., 2018; Bhattacharya et al., 2020). As such, there is a pressing need for compounds that can address this chronic state or even restore the sensitivity of the dormant parasite. In this respect, a very recent study by Rial et al. (2023) suggest that isotretinoin **72** (Figure 15), an FDA-approved drug used for severe acne that has previously shown reduction of the trypomastigote burst from infected mammal cells at nanomolar concentrations (Reigada et al., 2017), can also reduce blood parasitemia, prevent negative chronotropic effects and lead to reduced anti-*T. cruzi* antibody levels in murine models of chronic CD.

The success of drug repurposing remains reliant on the availability of existing drugs with well-established safety profiles, which can limit the pool of potential drug candidates. Moreover, this limited pool, and the very nature of repurposing, can mean that innovation and new modes of action are less likely to be revealed. Despite this, there are multitude of libraries available for testing and it is possible to initiate large-scale screening programs that can effectively harness these resources and unlock new treatment options. However, HTS is not cheap and for repurposing it is expected that *in silico* drug-target evaluation will have a growing role. By leveraging computational tools, researchers can predict the activity of existing drugs against specific targets in *T. cruzi*, allowing for the prioritization of compounds for further testing (Trevisan et al., 2020). While this approach is well-established in other diseases, alternative rational methods such as network-based and signature-based approaches have yet to be applied in the context of CD (Bellera et al., 2020). The future application of artificial intelligence (AI) and machine learning to this field holds great promise (Villalta and Rachakonda, 2019).

CD chemotherapy is further challenged by the extensive tissue distribution of *T. cruzi* which requires a similarly wide drug distribution profile in order to clear all parasites (De Rycker et al., 2023). Furthermore, the pharmacological management of this disease is further complicated by the resistance of different parasite strains to currently available drugs, potentially leading to treatment failures (García-Huertas and Cardona-Castro, 2021). One increasingly popular solution to these issues is the use of combination drug therapy, which involves administering multiple drugs with different profiles and modes of action. This can also help address challenges due to drug toxicity. Reflecting this, most combinations that have been reported involve BZN **1** or NFX **2** as the currently approved therapies. However, other repurposing combinations have been explored (Machado et al., 2020; Rocha-Hasler et al., 2021). For instance, combinations of Posaconazole **52** with either amlodipine **73** (Figure 15) or clemastine **4** demonstrated synergistic activity and greater effectiveness in reducing parasitemia

levels in mice (Planer et al., 2014). Although attractive for these reasons, developing drug combinations does have hurdles that may not exist for a single repurposed drug, particularly in the assessment of pharmacokinetic and pharmacodynamic parameters as well as efficacy and safety within patients.

In conclusion, repurposing approved drugs presents a fast and appealing approach to address the challenge of CD (Supplementary Table S1, Supplementary Material), with a number of advantages compared to conventional bottom-up drug discovery. Firstly, it significantly reduces the time and cost associated with drug development by leveraging the known safety profiles and pharmacokinetic properties of these drugs, streamlining the regulatory approval process. Secondly, repositioning can rapidly expand the limited treatment options currently available for CD, potentially uncovering additional effective therapies. By exploring diverse mechanisms of action, repurposed drugs may address drug resistance and target different stages of the disease, leading to improved treatment outcomes. Moreover, combining repurposed drugs with existing treatments can result in synergistic effects, enhancing their efficacy in a safer manner. Lastly, utilizing existing drug supply chains facilitates broader access to medications for CD, particularly in resource-constrained regions. Despite these advantages, rigorous research and clinical trials remain essential to confirm the safety, efficacy, and optimal usage of repurposed drugs for CD. Furthermore, the regulatory landscape for drug repurposing is complex, with challenges related to intellectual property, regulatory approval, and commercialization (Halabi, 2018). Despite these minor limitations, whilst not the sole solution, drug repositioning represents a valuable, relatively fast, and cost-effective strategy for developing essential new therapies, particularly for NTDs such as CD.

Author contributions

All authors conceptualized the overall structure of the review article and contributed to writing the article. EP prepared all the figures. All listed authors have made a substantial, direct, and intellectual contribution to the article and have approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1233253/full#supplementary-material

References

Adasme, M. F., Bolz, S. N., Adelmann, L., Salentin, S., Haupt, V. J., Moreno-Rodríguez, A., et al. (2020). Repositioned drugs for Chagas disease unveiled via structure-based drug repositioning. *Int. J. Mol. Sci.* 21 (22), 8809. doi:10.3390/ijms21228809

Adesse, D., Azzam, E. M., Meirelles, M. N., Urbina, J. A., and Garzoni, L. R. (2011). Amiodarone inhibits Trypanosoma cruzi infection and promotes cardiac cell recovery with gap junction and cytoskeleton reassembly *in vitro*. *Antimicrob. Agents Chemother.* 55 (1), 203–210. doi:10.1128/AAC.01129-10

Alberca, L. N., Sbaraglini, M. L., Morales, J. F., Dietrich, R., Ruiz, M. D., Pino Martínez, A. M., et al. (2018). Cascade ligand- and structure-based virtual screening to identify new trypanocidal compounds inhibiting putrescine uptake. *Front. Cell. Infect. Microbiol.* 8, 173. doi:10.3389/fcimb.2018.00173

Almeida-Silva, J., Menezes, D. S., Fernandes, J. M. P., Almeida, M. C., Vasco-Dos-Santos, D. R., Saraiva, R. M., et al. (2022). The repositioned drugs disulfiram/diethyldithiocarbamate combined to benznidazole: Searching for Chagas disease selective therapy, preventing toxicity and drug resistance. *Front. Cell. Infect. Microbiol.* 12, 926699. doi:10.3389/fcimb.2022.926699

Álvarez-Bardón, M., Pérez-Pertejo, Y., Ordóñez, C., Sepúlveda-Crespo, D., Carballeira, N. M., Tekwani, B. L., et al. (2020). Screening marine natural products for new drug leads against trypanosomatids and malaria. *Mar. Drugs.* 18 (4), 187. doi:10.3390/md18040187

Ancidoni, A., Bacigalupo, I., Remoli, G., Lacorte, E., Piscopo, P., Sarti, G., et al. (2021). Anticancer drugs repurposed for alzheimer's disease: A systematic review. *Alzheimers Res. Ther.* 13 (1), 96. doi:10.1186/s13195-021-00831-6

Annang, F., Pérez-Moreno, G., García-Hernández, R., Cordon-Obras, C., Martín, J., Tormo, J. R., et al. (2015). High-throughput screening platform for natural product-based drug discovery against 3 neglected tropical diseases: Human african trypanosomiasis, leishmaniasis, and Chagas disease. *J. Biomol. Screen.* 20 (1), 82–91. doi:10.1177/1087057114555846

Araujo-Lima, C. F., Peres, R. B., Silva, P. B., Batista, M. M., Aiub, C. A. F., Felzenszwalb, I., et al. (2018). Repurposing strategy of atorvastatin against trypanosoma cruzi: *In vitro* monotherapy and combined therapy with benznidazole exhibit synergistic trypanocidal activity. *Antimicrob. Agents Chemother.* 62 (9), 009799–e1018. doi:10.1128/AAC.00979-18

Bahia, M. T., de Andrade, I. M., Martins, T. A., do Nascimento, Á. F., Diniz, L. de F., Caldas, I. S., et al. (2012). Fexinidazole: A potential new drug candidate for Chagas disease. *PLoS Negl. Trop. Dis.* 6 (11), e1870. doi:10.1371/journal.pntd.0001870

Barbosa, H., Thevenard, F., Quero Reimão, J., Tempone, A. G., Honorio, K. M., and Lago, J. H. G. (2023). The potential of secondary metabolites from plants as drugs or leads against trypanosoma cruzi-an update from 2012 to 2021. *Curr. Top. Med. Chem.* 23 (3), 159–213. doi:10.2174/1568026623666221212111514

Barbosa, J. M. C., Pedra-Rezende, Y., Pereira, L. D., de Melo, T. G., Barbosa, H. S., Lannes-Vieira, J., et al. (2022). Benznidazole and amiodarone combined treatment attenuates cytoskeletal damage in Trypanosoma cruzi-infected cardiac cells. *Front. Cell Infect. Microbiol.* 12, 975931. doi:10.3389/fcimb.2022.975931

Bayat Mokhtari, R., Homayouni, T. S., Baluch, N., Morgatskaya, E., Kumar, S., Das, B., et al. (2017). Combination therapy in combating cancer. *Oncotarget* 8 (23), 38022–38043. doi:10.18632/oncotarget.16723

Bellera, C. L., Alberca, L. N., Sbaraglini, M. L., and Talevi, A. (2020). *In silico* drug repositioning for Chagas disease. *Curr. Med. Chem.* 27 (5), 662–675. doi:10.2174/0929867326666191016114839

Bellera, C. L., Balcazar, D. E., Alberca, L., Labriola, C. A., Talevi, A., and Carrillo, C. (2014). Identification of levothyroxine antichagasic activity through computer-aided drug repurposing. *ScientificWorldJournal* 2014, 279618. doi:10.1155/2014/279618

Bellera, C. L., Balcazar, D. E., Vanrell, M. C., Casassa, A. F., Palestro, P. H., Gavernet, L., et al. (2015). Computer-guided drug repurposing: Identification of trypanocidal activity of clofazimine, benidipine and saquinavir. *Eur. J. Med. Chem.* 93, 338–348. doi:10.1016/j.ejmech.2015.01.065

Benaim, G., Paniz-Mondolfi, A. E., and Sordillo, E. M. (2021). The rationale for use of amiodarone and its derivatives for the treatment of Chagas' disease and leishmaniasis. *Curr. Pharm. Des.* 27 (15), 1825–1833. doi:10.2174/1381612826666200928161403

Bernatchez, J. A., Chen, E., Hull, M. V., McNamara, C. W., McKerrow, J. H., and Siqueira-Neto, J. L. (2020). High-throughput screening of the ReFRAME library identifies potential drug repurposing candidates for trypanosoma cruzi. *Microorganisms* 8 (4), 472. doi:10.3390/microorganisms8040472

Bhattacharya, A., Corbeil, A., do Monte-Neto, R. L., and Fernandez-Prada, C. (2020). Of drugs and trypanosomatids: New tools and knowledge to reduce bottlenecks in drug discovery. *Genes (Basel)* 11 (7), 722. doi:10.3390/genes11070722

Blay, V., Tolani, B., Ho, S. P., and Arkin, M. R. (2020). High-throughput screening: today's biochemical and cell-based approaches. *Drug. Discov. Today.* 25 (10), 1807–1821. doi:10.1016/j.drudis.2020.07.024

Braga, S. S. (2019). Multi-target drugs active against leishmaniasis: A paradigm of drug repurposing. *Eur. J. Med. Chem.* 183, 111660. doi:10.1016/j.ejmech.2019.111660

Buckner, F. S., and Urbina, J. A. (2012). Recent developments in sterol 14-demethylase inhibitors for Chagas disease. *Int. J. Parasitol. Drugs Drug Resist* 2, 236–242. doi:10.1016/j.ijpddr.2011.12.002

Campo, V. A. (2017). Comparative effects of histone deacetylases inhibitors and resveratrol on Trypanosoma cruzi replication, differentiation, infectivity and gene expression. *Int. J. Parasitol. Drugs Drug Resist.* 7 (1), 23–33. doi:10.1016/j.ijpddr.2016.12.003

Campos-Estrada, C., Urarte, E., Denegri, M., Villalón, L., González-Herrera, F., Kemmerling, U., et al. (2023). Effect of statins on inflammation and cardiac function in patients with chronic Chagas disease: A protocol for pathophysiological studies in a multicenter, placebo-controlled, proof-of-concept phase II trial. *PLoS One* 18 (1), e0280335. doi:10.1371/journal.pone.0280335

Casulli, A. (2021). New global targets for NTDs in the WHO roadmap 2021-2030. *PLoS Negl. Trop. Dis.* 15 (5), e0009373. doi:10.1371/journal.pntd.0009373

CDC (2023). Centers for diseases control and prevention. Available at: https://www.cdc.gov/parasites/chagas/ (Accessed May, 2023).

Charlton, R. L., Rossi-Bergmann, B., Denny, P. W., and Steel, P. G. (2018). Repurposing as a strategy for the discovery of new anti-leishmanials: The-state-of-the-art. *Parasitology* 145 (2), 219–236. doi:10.1017/S0031182017000993

Clark, E. H., Sherbuk, J., Okamoto, E., Jois, M., Galdos-Cardenas, G., Vela-Guerra, J., et al. (2014). Hyperendemic Chagas disease and the unmet need for pacemakers in the Bolivian Chaco. *PLoS Negl. Trop. Dis.* 8 (6), e2801. doi:10.1371/journal.pntd.0002801

Correa, I. T. S., da Costa-Silva, T. A., and Tempone, A. G. (2021). Bioenergetics impairment of trypanosoma cruzi by the antihypertensive manidipine: A drug repurposing strategy. *Acta Trop.* 214, 105768. doi:10.1016/j.actatropica.2020.105768

Crespillo-Andújar, C., Comeche, B., Hamer, D. H., Arevalo-Rodriguez, I., Alvarez-Díaz, N., Zamora, J., et al. (2022). Use of benznidazole to treat chronic Chagas disease: An updated systematic review with a meta-analysis. *PLoS Negl. Trop. Dis.* 16 (5), e0010386. doi:10.1371/journal.pntd.0010386

Cucunubá, Z. M., Nouvellet, P., Conteh, L., Vera, M. J., Angulo, V. M., Dib, J. C., et al. (2017). Modelling historical changes in the force-of-infection of Chagas disease to inform control and elimination programmes: Application in Colombia. *BMJ Glob. Health* 2 (3), e000345. doi:10.1136/bmjgh-2017-000345

Cunha, E. L. A., Torchelsen, F. K. V. D. S., Fonseca, K. D. S., Sousa, L. R. D., Vieira, P. M. A., Carneiro, C. M., et al. (2022). Benznidazole, itraconazole, and their combination for the treatment of chronic experimental Chagas disease in dogs. *Exp. Parasitol.* 238, 108266. doi:10.1016/j.exppara.2022.108266

De Rycker, M., Thomas, J., Riley, J., Brough, S. J., Miles, T. J., and Gray, D. W. (2016). Identification of trypanocidal activity for known clinical compounds using a new trypanosoma cruzi hit-discovery screening cascade. *PLoS Negl. Trop. Dis.* 10 (4), e0004584. doi:10.1371/journal.pntd.0004584

De Rycker, M., Wyllie, S., Horn, D., Read, K. D., and Gilbert, I. H. (2023). Anti-trypanosomatid drug discovery: Progress and challenges. *Nat. Rev. Microbiol.* 21 (1), 35–50. doi:10.1038/s41579-022-00777-y

DNDi (2023). Drugs for neglected diseases initiative. Available at: https://dndi.org/diseases/chagas/target-product-profile/ (Accessed July, 2023).

Docampo, R., Moreno, S. N., Muniz, R. P., Cruz, F. S., and Mason, R. P. (1983). Light-enhanced free radical formation and trypanocidal action of gentian violet (crystal violet). *Science* 220 (4603), 1292–1295. doi:10.1126/science.6304876

Duffy, S., Sykes, M. L., Jones, A. J., Shelper, T. B., Simpson, M., Lang, R., et al. (2017). Screening the medicines for malaria venture pathogen Box across multiple pathogens reclassifies starting points for open-source drug discovery. *Antimicrob. Agents Chemother.* 61 (9), 003799–e417. doi:10.1128/AAC.00379-17

El Bairi, K., Trapani, D., Petrillo, A., Le Page, C., Zbakh, H., Daniele, B., et al. (2020). Repurposing anticancer drugs for the management of COVID-19. *Eur. J. Cancer.* 141, 40–61. doi:10.1016/j.ejca.2020.09.014

Ferreira, D. D., Mesquita, J. T., da Costa Silva, T. A., Romanelli, M. M., da Gama Jaen Batista, D., da Silva, C. F., et al. (2018). Efficacy of sertraline against trypanosoma cruzi: An *in vitro* and *in silico* study. *J. Venom. Anim. Toxins Incl. Trop. Dis.* 24, 30. doi:10.1186/s40409-018-0165-8

Ferreira, L. L. G., de Moraes, J., and Andricopulo, A. D. (2022). Approaches to advance drug discovery for neglected tropical diseases. *Drug. Discov. Today.* 27 (8), 2278–2287. doi:10.1016/j.drudis.2022.04.004

Fraccaroli, L., Ruiz, M. D., Perdomo, V. G., Clausi, A. N., Balcazar, D. E., Larocca, L., et al. (2022). Broadening the spectrum of ivermectin: Its effect on Trypanosoma cruzi and related trypanosomatids. *Front. Cell. Infect. Microbiol.* 12, 885268. doi:10.3389/fcimb.2022.885268

García, M. C., Ponce, N. E., Sanmarco, L. M., Manzo, R. H., Jimenez-Kairuz, A. F., and Aoki, M. P. (2016). Clomipramine and benznidazole act synergistically and ameliorate the outcome of experimental Chagas disease. *Antimicrob. Agents Chemother.* 60 (6), 3700–3708. doi:10.1128/AAC.00404-16

García-Huertas, P., and Cardona-Castro, N. (2021). Advances in the treatment of Chagas disease: Promising new drugs, plants and targets. *Biomed. Pharmacother.* 142, 112020. doi:10.1016/j.biopha.2021.112020

Global Burden of Disease (2019). Global burden of disease. Available at: http://ghdx.healthdata.org/ (Accessed July, 2023).

Gómez-Ochoa, S. A., Rojas, L. Z., Echeverría, L. E., Muka, T., and Franco, O. H. (2022). Global, regional, and national trends of Chagas disease from 1990 to 2019: Comprehensive analysis of the global burden of disease study. *Glob. Heart* 17 (1), 59. doi:10.5334/gh.1150

Gulin, J. E. N., Bisio, M. M. C., Rocco, D., Altcheh, J., Solana, M. E., and García-Bournissen, F. (2022). Miltefosine and benznidazole combination improve anti-trypanosoma cruzi *in vitro* and *in vivo* efficacy. *Front. Cell. Infect. Microbiol.* 12, 855119. doi:10.3389/fcimb.2022.855119

Guzmán-Rivera, D., Liempi, A., González-Herrera, F., Fuentes-Retamal, S., Carrillo, I., Abarca, P., et al. (2020). Simvastatin improves cardiac function through notch 1 activation in BALB/c mice with chronic Chagas cardiomyopathy. *Antimicrob. Agents Chemother.* 64 (8), 021411–e2219. doi:10.1128/AAC.02141-19

Halabi, S. F. (2018). The drug repurposing ecosystem: Intellectual property incentives, market exclusivity, and the future of "new" medicines 20 yale journal of law and technology. Available at: https://scholarship.law.missouri.edu/facpubs/717.

Hashimoto, K. (2021). Repurposing of CNS drugs to treat COVID-19 infection: Targeting the sigma-1 receptor. *Eur. Arch. Psychiatry Clin. Neurosci.* 271 (2), 249–258. doi:10.1007/s00406-020-01231-x

Hua, Y., Dai, X., Xu, Y., Xing, G., Liu, H., Lu, T., et al. (2022). Drug repositioning: Progress and challenges in drug discovery for various diseases. *Eur. J. Med. Chem.* 234, 114239. doi:10.1016/j.ejmech.2022.114239

Iribarne, F., Paulino, M., Aguilera, S., and Tapia, O. (2009). Assaying phenothiazine derivatives as trypanothione reductase and glutathione reductase inhibitors by theoretical docking and molecular dynamics studies. *J. Mol. Graph. Model.* 28 (4), 371–381. doi:10.1016/j.jmgm.2009.09.003

Jackson, Y., Wyssa, B., and Chappuis, F. (2020). Tolerance to nifurtimox and benznidazole in adult patients with chronic Chagas' disease. *J. Antimicrob. Chemother.* 75 (3), 690–696. doi:10.1093/jac/dkz473

Jansen, A. M., Xavier, S. C. D. C., and Roque, A. L. R. (2020). Landmarks of the knowledge and trypanosoma cruzi biology in the wild environment. *Front. Cell. Infect. Microbiol.* 10, 10. doi:10.3389/fcimb.2020.00010

Johndrow, C., Nelson, R., Tanowitz, H., Weiss, L. M., and Nagajyothi, F. (2014). Trypanosoma cruzi infection results in an increase in intracellular cholesterol. *Microbes Infect.* 16 (4), 337–344. doi:10.1016/j.micinf.2014.01.001

Jourdan, J. P., Bureau, R., Rochais, C., and Dallemagne, P. (2020). Drug repositioning: A brief overview. *J. Pharm. Pharmacol.* 72 (9), 1145–1151. doi:10.1111/jphp.13273

Juárez-Saldivar, A., Schroeder, M., Salentin, S., Haupt, V. J., Saavedra, E., Vázquez, C., et al. (2020). Computational drug repositioning for Chagas disease using protein-ligand interaction profiling. *Int. J. Mol. Sci.* 21 (12), 4270. doi:10.3390/ijms21124270

Kaiser, M., Mäser, P., Tadoori, L. P., Ioset, J. R., and Brun, R. (2015). Antiprotozoal activity profiling of approved drugs: A starting point toward drug repositioning. *PLoS One* 10 (8), e0135556. doi:10.1371/journal.pone.0135556

Krishnamurthy, N., Grimshaw, A. A., Axson, S. A., Choe, S. H., and Miller, J. E. (2022). Drug repurposing: A systematic review on root causes, barriers and facilitators. *BMC Health. Serv. Res.* 22 (1), 970. doi:10.1186/s12913-022-08272-z

Lazarin-Bidóia, D., Garcia, F. P., Ueda-Nakamura, T., Silva, S. O., and Nakamura, C. V. (2022). Natural compounds based chemotherapeutic against Chagas disease and leishmaniasis: Mitochondrion as a strategic target. *Mem. Inst. Oswaldo Cruz.* 117, e220396. doi:10.1590/0074-02760220396

Leaver, D. J. (2018). Synthesis and biological activity of sterol 14α-demethylase and sterol C24-methyltransferase inhibitors. *Molecules* 23 (7), 1753. doi:10.3390/molecules23071753

Liao, J. K., and Laufs, U. (2005). Pleiotropic effects of statins. *Annu. Rev. Pharmacol. Toxicol.* 45, 89–118. doi:10.1146/annurev.pharmtox.45.120403.095748

Machado, Y. A., Bahia, M. T., Caldas, I. S., Mazzeti, A. L., Novaes, R. D., Vilas Boas, B. R., et al. (2020). Amlodipine increases the therapeutic potential of ravuconazole upon trypanosoma cruzi infection. *Antimicrob. Agents Chemother.* 64 (8), 024977–e2519. doi:10.1128/AAC.02497-19

Mercaldi, G. F., D'Antonio, E. L., Aguessi, A., Rodriguez, A., and Cordeiro, A. T. (2019). Discovery of antichagasic inhibitors by high-throughput screening with Trypanosoma cruzi glucokinase. *Bioorg. Med. Chem. Lett.* 29 (15), 1948–1953. doi:10.1016/j.bmcl.2019.05.037

Meymandi, S., Hernandez, S., Park, S., Sanchez, D. R., and Forsyth, C. (2018). Treatment of Chagas disease in the United States. *Curr. Treat. Options Infect. Dis.* 10 (3), 373–388. doi:10.1007/s40506-018-0170-z

Miguel, D. C., Ferraz, M. L., Alves, R. O., Yokoyama-Yasunaka, J. K., Torrecilhas, A. C., Romanha, A. J., et al. (2010). The anticancer drug tamoxifen is active against Trypanosoma cruzi *in vitro* but ineffective in the treatment of the acute phase of Chagas disease in mice. *Mem. Inst. Oswaldo Cruz.* 105 (7), 945–948. doi:10.1590/s0074-02762010000700021

Miranda-Arboleda, A. F., Zaidel, E. J., Marcus, R., Pinazo, M. J., Echeverría, L. E., Saldarriaga, C., et al. (2021). Roadblocks in Chagas disease care in endemic and nonendemic countries: Argentina, Colombia, Spain, and the United States. The NET-heart project. *PLoS. Negl. Trop. Dis.* 15 (12), e0009954. doi:10.1371/journal.pntd.0009954

MMV (2023). Medicines for malaria venture. Available at: https://www.mmv.org/mmv-open/pathogen-box/about-pathogen-box (Accessed May, 2023).

Moffat, J. G., Vincent, F., Lee, J. A., Eder, J., and Prunotto, M. (2017). Opportunities and challenges in phenotypic drug discovery: An industry perspective. *Nat. Rev. Drug. Discov.* 16 (8), 531–543. doi:10.1038/nrd.2017.111

Molina, I., Gómez i Prat, J., Salvador, F., Treviño, B., SulleiroSerre, N. E., et al. (2014). Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. *N. Engl. J. Med.* 370 (20), 1899–1908. doi:10.1056/NEJMoa1313122

Nagajyothi, F., Machado, F. S., Burleigh, B. A., Jelicks, L. A., Scherer, P. E., Mukherjee, S., et al. (2012). Mechanisms of Trypanosoma cruzi persistence in Chagas disease. *Cell Microbiol.* 14 (5), 634–643. doi:10.1111/j.1462-5822.2012.01764.x

Nekoei, S., Khamesipour, F., Habtemariam, S., de Souza, W., Mohammadi Pour, P., and Hosseini, S. R. (2022). The anti-trypanosoma activities of medicinal plants: A systematic review of the literature. *Vet. Med. Sci.* 8 (6), 2738–2772. doi:10.1002/vms3.912

Nesic de Freitas, L. S. F., da Silva, C. F., Intagliata, S., Amata, E., Salerno, L., and Soeiro, M. N. C. (2023). *In vitro* and *in silico* analysis of imatinib analogues as anti-Trypanosoma cruzi drug candidates. *Parasitology* 150 (4), 1–18. doi:10.1017/S0031182023000057

PAHO (2023). Pan American health organization. Available at: https://www.paho.org/en/topics/chagas-disease (Accessed May, 2023).

Palos, I., Lara-Ramirez, E. E., Lopez-Cedillo, J. C., Garcia-Perez, C., Kashif, M., Bocanegra-Garcia, V., et al. (2017). Repositioning FDA drugs as potential cruzain inhibitors from trypanosoma cruzi: Virtual screening, *in vitro* and *in vivo* studies. *Molecules* 22 (6), 1015. doi:10.3390/molecules22061015

Pandey, R. P., Nascimento, M. S., Franco, C. H., Bortoluci, K., Silva, M. N., Zingales, B., et al. (2022). Drug repurposing in Chagas disease: Chloroquine potentiates benznidazole activity against trypanosoma cruzi *in vitro* and *in vivo*. *Antimicrob. Agents Chemother.* 66 (11), e0028422. doi:10.1128/aac.00284-22

Panozzo-Zénere, E. A., Porta, E. O. J., Arrizabalaga, G., Fargnoli, L., Khan, S. I., Tekwani, B. L., et al. (2018). A minimalistic approach to develop new anti-apicomplexa polyamines analogs. *Eur. J. Med. Chem.* 2143, 866–880. doi:10.1016/j.ejmech.2017.11.069

Peña-Diaz, J., Montalvetti, A., Flores, C. L., Constán, A., Hurtado-Guerrero, R., De Souza, W., et al. (2004). Mitochondrial localization of the mevalonate pathway enzyme 3-Hydroxy-3-methyl-glutaryl-CoA reductase in the Trypanosomatidae. *Mol. Biol. Cell.* 15 (3), 1356–1363. doi:10.1091/mbc.e03-10-0720

Pittella, J. E. (2009). Central nervous system involvement in Chagas disease: A hundred-year-old history. *Trans. R. Soc. Trop. Med. Hyg.* 103 (10), 973–978. doi:10.1016/j.trstmh.2009.04.012

Planer, J. D., Hulverson, M. A., Arif, J. A., Ranade, R. M., Don, R., and Buckner, F. S. (2014). Synergy testing of FDA-approved drugs identifies potent drug combinations against Trypanosoma cruzi. *PLoS Negl. Trop. Dis.* 8 (7), e2977. doi:10.1371/journal.pntd.0002977

Porta, E. O., Carvalho, P. B., Avery, M. A., Tekwani, B. L., and Labadie, G. R. (2014). Click chemistry decoration of amino sterols as promising strategy to developed new leishmanicidal drugs. *Steroids* 79, 28–36. doi:10.1016/j.steroids.2013.10.010

Porta, E. O. J., Ballari, M. S., Carlucci, R., Wilkinson, S., Ma, G., Tekwani, B. L., et al. (2023). Systematic study of 1,2,3-triazolyl sterols for the development of new drugs against parasitic Neglected Tropical Diseases. *Eur. J. Med. Chem.* 254, 115378. doi:10.1016/j.ejmech.2023.115378

Porta, E. O. J., Bofill Verdaguer, I., Perez, C., Banchio, C., Ferreira de Azevedo, M., Katzin, A. M., et al. (2019). Repositioning Salirasib as a new antimalarial agent. *Medchemcomm* 10 (9), 1599–1605. doi:10.1039/c9md00298g

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., et al. (2019). Drug repurposing: Progress, challenges and recommendations. *Nat. Rev. Drug. Discov.* 18 (1), 41–58. doi:10.1038/nrd.2018.168

Ramsey, J. M., Elizondo-Cano, M., Sanchez-González, G., Peña-Nieves, A., and Figueroa-Lara, A. (2014). Opportunity cost for early treatment of Chagas disease in Mexico. *PLoS Negl. Trop. Dis.* 8 (4), e2776. doi:10.1371/journal.pntd.0002776

Reigada, C., Sayé, M., Phanstiel, O., Valera-Vera, E., Miranda, M. R., and Pereira, C. A. (2019a). Identification of trypanosoma cruzi polyamine transport inhibitors by computational drug repurposing. *Front. Med. (Lausanne)* 6, 256. doi:10.3389/fmed.2019.00256

Reigada, C., Sayé, M., Valera-Vera, E., Miranda, M. R., and Pereira, C. A. (2019b). Repurposing of terconazole as an anti Trypanosoma cruzi agent. *Heliyon* 25 (6), e01947. doi:10.1016/j.heliyon.2019.e01947

Reigada, C., Valera-Vera, E. A., Sayé, M., Errasti, A. E., Avila, C. C., Miranda, M. R., et al. (2017). Trypanocidal effect of isotretinoin through the inhibition of polyamine and amino acid transporters in trypanosoma cruzi. *PLoS Negl. Trop. Dis.* 11 (3), e0005472. doi:10.1371/journal.pntd.0005472

Rial, M. S., Reigada, C., Prado, N., Bua, J., Esteva, M., Pereira, C. A., et al. (2023). Effectiveness of the repurposed drug isotretinoin in an experimental murine model of Chagas disease. *Acta Trop.* 242, 106920. doi:10.1016/j.actatropica.2023.106920

Rivero, C. V., Martínez, S. J., Novick, P., Cueto, J. A., Salassa, B. N., Vanrell, M. C., et al. (2021). Repurposing carvedilol as a novel inhibitor of the trypanosoma cruzi autophagy flux that affects parasite replication and survival. *Front. Cell Infect. Microbiol.* 11, 657257. doi:10.3389/fcimb.2021.657257

Rocha-Hasler, M., de Oliveira, G. M., da Gama, A. N., Fiuza, L. F. A., Fesser, A. F., Cal, M., et al. (2021). Combination with tomatidine improves the potency of posaconazole against trypanosoma cruzi. *Front. Cell Infect. Microbiol.* 11, 617917. doi:10.3389/fcimb.2021.617917

Rodriguez, M. E., Tekiel, V., and Campo, V. A. (2022). *In vitro* evaluation of Resveratrol as a potential pre-exposure prophylactic drug against Trypanosoma cruzi infection. *Int. J. Parasitol. Drugs Drug Resist.* 20, 54–64. doi:10.1016/j.ijpddr.2022.08.003

Sánchez-Valdéz, F. J., Padilla, A., Wang, W., Orr, D., and Tarleton, R. L. (2018). Spontaneous dormancy protects Trypanosoma cruzi during extended drug exposure. *Elife* 7, e34039. doi:10.7554/eLife.34039

Saraiva, R. M., Portela, L. F., Silveira, G. P. E., Gomes, N. L. S., Pinto, D. P., Silva, A. C. d. A. d., et al. (2021). Disulfiram repurposing in the combined chemotherapy of Chagas disease: A protocol for phase I/II clinical trial. *Med. Case Rep. Study Protoc.* 2 (7), e0110. doi:10.1097/MD9.0000000000000110

Savage, D. G., and Antman, K. H. (2002). Imatinib mesylate--a new oral targeted therapy. *N. Engl. J. Med.* 346 (9), 683–693. doi:10.1056/NEJMra013339

Sayé, M., Gauna, L., Valera-Vera, E., Reigada, C., Miranda, M. R., and Pereira, C. A. (2020). Crystal violet structural analogues identified by *in silico* drug repositioning present anti-Trypanosoma cruzi activity through inhibition of proline transporter TcAAAP069. *PLoS Negl. Trop. Dis.* 14 (1), e0007481. doi:10.1371/journal.pntd.0007481

Sbaraglini, M. L., Bellera, C. L., Fraccaroli, L., Larocca, L., Carrillo, C., Talevi, A., et al. (2016). Novel cruzipain inhibitors for the chemotherapy of chronic Chagas disease. *Int. J. Antimicrob. Agents.* 48 (1), 91–95. doi:10.1016/j.ijantimicag.2016.02.018

Schenone, M., Dančík, V., Wagner, B. K., and Clemons, P. A. (2013). Target identification and mechanism of action in chemical biology and drug discovery. *Nat. Chem. Biol.* 9 (4), 232–240. doi:10.1038/nchembio.1199

Scotti, M. T., Scotti, L., Ishiki, H., Ribeiro, F. F., Cruz, R. M., Oliveira, M. P., et al. (2016). Natural products as a source for antileishmanial and antitrypanosomal agents. *Comb. Chem. High. Throughput Screen.* 19 (7), 537–553. doi:10.2174/1386207319666160506123921

Shyr, Z. A., Cheng, Y. S., Lo, D. C., and Zheng, W. (2021). Drug combination therapy for emerging viral diseases. *Drug. Discov. Today.* 26 (10), 2367–2376. doi:10.1016/j.drudis.2021.05.008

Simões-Silva, M. R., Brandão Peres, R., Britto, C., Machado Cascabulho, C., de Melo Oliveira, G., Nefertiti da Gama, A., et al. (2019b). Impact of levamisole in co-administration with benznidazole on experimental Chagas disease. *Parasitology* 146 (8), 1055–1062. doi:10.1017/S0031182019000374

Simões-Silva, M. R., De Araújo, J. S., Oliveira, G. M., Demarque, K. C., Peres, R. B., D'Almeida-Melo, I., et al. (2017). Drug repurposing strategy against trypanosoma cruzi infection: *In vitro* and *in vivo* assessment of the activity of metronidazole in mono- and combined therapy. *Biochem. Pharmacol.* 145, 46–53. doi:10.1016/j.bcp.2017.08.025

Simões-Silva, M. R., De Araújo, J. S., Peres, R. B., Da Silva, P. B., Batista, M. M., De Azevedo, L. D., et al. (2019a). Repurposing strategies for Chagas disease therapy: The effect of imatinib and derivatives against trypanosoma cruzi. *Parasitology* 146 (8), 1006–1012. doi:10.1017/S0031182019000234

Sun, W., Sanderson, P. E., and Zheng, W. (2016). Drug combination therapy increases successful drug repositioning. *Drug. Discov. Today.* 21 (7), 1189–1195. doi:10.1016/j.drudis.2016.05.015

Swinney, D. C., and Lee, J. A. (2020). Recent advances in phenotypic drug discovery. *F1000Res* 9, F1000 Faculty Rev-944. doi:10.12688/f1000research.25813.1

Swinney, D. C. (2013). Phenotypic vs. target-based drug discovery for first-in-class medicines. *Clin. Pharmacol. Ther.* 93 (4), 299–301. doi:10.1038/clpt.2012.236

Sykes, M. L., and Avery, V. M. (2015). Development and application of a sensitive, phenotypic, high-throughput image-based assay to identify compound activity against Trypanosoma cruzi amastigotes. *Int. J. Parasitol. Drugs Drug Resist.* 5 (3), 215–228. doi:10.1016/j.ijpddr.2015.10.001

Talevi, A., Carrillo, C., and Comini, M. (2019). The thiol-polyamine metabolism of trypanosoma cruzi: Molecular targets and drug repurposing strategies. *Curr. Med. Chem.* 26 (36), 6614–6635. doi:10.2174/0929867325666180926151059

Teixeira, D. E., Benchimol, M., Crepaldi, P. H., and de Souza, W. (2012). Interactive multimedia to teach the life cycle of Trypanosoma cruzi, the causative agent of Chagas disease. *PLoS Negl. Trop. Dis.* 6 (8), e1749. doi:10.1371/journal.pntd.0001749

Tempone, A. G., Pieper, P., Borborema, S. E. T., Thevenard, F., Lago, J. H. G., Croft, S. L., et al. (2021). Marine alkaloids as bioactive agents against protozoal neglected tropical diseases and malaria. *Nat. Prod. Rep.* 38 (12), 2214–2235. doi:10.1039/d0np00078g

Torreele, E., Bourdin Trunz, B., Tweats, D., Kaiser, M., Brun, R., Mazué, G., et al. (2010). Fexinidazole--a new oral nitroimidazole drug candidate entering clinical development for the treatment of sleeping sickness. *PLoS Negl. Trop. Dis.* 4 (12), e923. doi:10.1371/journal.pntd.0000923

Torrico, F., Gascón, J., Ortiz, L., Pinto, J., Rojas, G., Palacios, A., et al. (2023). A phase 2, randomized, multicenter, placebo-controlled, proof-of-concept trial of oral fexinidazole in adults with chronic indeterminate Chagas disease. *Clin. Infect. Dis.* 76 (3), e1186–e1194. doi:10.1093/cid/ciac579

Trevisan, R. O., Santos, M. M., Desidério, C. S., Alves, L. G., de Jesus Sousa, T., de Castro Oliveira, L., et al. (2020). *In silico* identification of new targets for diagnosis, vaccine, and drug candidates against *Trypanosoma cruzi*. *Dis. Markers* 2020, 9130719. doi:10.1155/2020/9130719

Trindade, J. D. S., Freire-de-Lima, C. G., Côrte-Real, S., Decote-Ricardo, D., and Freire de Lima, M. E. (2021). Drug repurposing for Chagas disease: *In vitro* assessment of nimesulide against trypanosoma cruzi and insights on its mechanisms of action. *PLoS One* 16 (10), e0258292. doi:10.1371/journal.pone.0258292

Urbina, J. A., Lazardi, K., Marchan, E., Visbal, G., Aguirre, T., Piras, M. M., et al. (1993). Mevinolin (lovastatin) potentiates the antiproliferative effects of ketoconazole and terbinafine against trypanosoma (schizotrypanum) cruzi: *In vitro* and *in vivo* studies. *Antimicrob. Agents Chemother.* 37 (3), 580–591. doi:10.1128/AAC.37.3.580

Valera-Vera, E. A., Sayé, M., Reigada, C., Miranda, M. R., and Pereira, C. A. (2020). *In silico* repositioning of etidronate as a potential inhibitor of the Trypanosoma cruzi enolase. *J. Mol. Graph. Model.* 95, 107506. doi:10.1016/j.jmgm.2019.107506

Villalta, F., and Rachakonda, G. (2019). Advances in preclinical approaches to Chagas disease drug discovery. *Expert Opin. Drug Discov.* 14 (11), 1161–1174. doi:10.1080/17460441.2019.1652593

WHO (2023). World health organization. Available at: https://www.who.int/health-topics/chagas-disease (Accessed May, 2023).

Wiggers, H. J., Rocha, J. R., Fernandes, W. B., Sesti-Costa, R., Carneiro, Z. A., Cheleski, J., et al. (2013). Non-peptidic cruzain inhibitors with trypanocidal activity discovered by virtual screening and *in vitro* assay. *PLoS Negl. Trop. Dis.* 7 (8), e2370. doi:10.1371/journal.pntd.0002370

Zaidel, E. J., and Sosa Liprandi, Á. (2021). Neglected of the neglected: Crude reality of Chagas disease. *Med. (B Aires)* 81 (4), 675–676.

Zhang, Q., Dong, J., and Yu, Z. (2020). Pleiotropic use of Statins as non-lipid-lowering drugs. *Int. J. Biol. Sci.* 16 (14), 2704–2711. doi:10.7150/ijbs.42965

Zhong, L., Li, Y., Xiong, L., Wang, W., Wu, M., Yuan, T., et al. (2021). Small molecules in targeted cancer therapy: Advances, challenges, and future perspectives. *Signal Transduct. Target Ther.* 6 (1), 201. doi:10.1038/s41392-021-00572-w

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Evaluation of the effect of direct-acting antiviral agents on melatonin level and lipid peroxidation in chronic hepatitis C patients

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Background: Oxidative stress and its end products, such as malondialdehyde (MDA) play a leading role in the pathogenesis of hepatitis C. Melatonin is a hormone that helps regulate circadian rhythms, which likely play a role in infectious diseases in terms of susceptibility, clinical expression, and outcome.

Objective: The present study was conducted to assess serum malondialdehyde and melatonin levels in patients with chronic hepatitis C infection before and after the intake of direct-acting antivirals.

Method: Forty hepatitis C patients were the subjects of this study. While ten healthy volunteers who matched in age and socioeconomic status served as the control subjects. Malondialdehyde and melatonin were assayed in the serum of the three groups, and the results were statistically analyzed.

Results: Hepatitis C patients had significantly higher malondialdehyde ($p < 0.001$) but significantly lower melatonin ($p < 0.001$) as compared to the healthy controls. After 12 weeks of treatment with direct-acting antivirals, the malondialdehyde level decreased significantly ($p < 0.001$) and the melatonin level increased significantly ($p < 0.001$). A significant negative correlation between malondialdehyde and melatonin was observed.

Conclusion: The present findings suggest that treatment of hepatitis C patients with Direct-acting antivirals improves liver function parameters and antioxidant profiles.

KEYWORDS

melatonin, malondialdehyde, direct-acting agents (DAAs), hepatitis C virus (HCV), oxidatie stress

Abbreviations: CHC, chronic hepatitis C; DAAs, direct-acting antivirals; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IFN, Interferon; MDA, malondialdehyde; ROS, reactive oxygen species.

Introduction

The hepatitis C virus (HCV) is a serious public health issue worldwide. Egypt has the highest global prevalence of HCV (Waked, 2022). 400,000 individuals worldwide are predicted to die from cirrhosis and hepatocellular carcinoma each year as a result of HCV, which affects an estimated 71 million people globally (HCC) (Moon et al., 2020). Despite the fact that HCV can now be accurately detected and treated with DAAs, which has cure rates of greater than 95% in 8–12 weeks, one of the most astonishing medical advancements in recent memory, viral hepatitis mortality increased by 22% between 2000 and 2015 (Schwander et al., 2022).

HCV replicates in the cytoplasm and causes chronic infections that may eventually result in cirrhosis, chronic hepatitis, and hepatocellular carcinoma (HCC) (Vrazas et al., 2022). It has been discovered that oxidative stress plays a significant role in HCV genome translation, which is mediated via PERK-mediated suppression of cap-dependent translation (Ivanov et al., 2013). Researchers previously discovered that the likely mechanism for viral escape from the immune system is reactive oxygen species (ROS)-generated viral genomic heterogeneity (Paracha et al., 2013).

Oxidative stress results in damage and the possibility of cell death due to the oxidation of many cellular components such as DNA, proteins, and lipids (lipid peroxidation) (apoptosis) (Avery, 2011). Once this process is started, cellular damage and the release of pro-inflammatory cytokines cycle continuously, causing hepatic inflammation, fibrosis, and cirrhosis (Vuppalanchi et al., 2011).

It has long been believed that one of the main effects of rogue free radicals is the peroxidation of membrane lipids. Polyunsaturated fatty acids undergo a chemical change as a result of lipid peroxidation, which also leads to the breakdown of the structural integrity of cellular and subcellular membranes. The dynamic properties of the lipid bilayer play a crucial role in controlling numerous of important physiological activities in the cell. Consequently, the disturbance of structural features brought on by oxidative stress has negative effects on cellular function (García et al., 2014).

Unsaturated reactive aldehydes and malondialdehyde (MDA) are two examples of lipid peroxidation end products that have been utilized as indicators of oxidative stress. As an indirect indicator of oxidative stress, MDA is relatively stable and simple to quantify in serum, plasma and urine (Kartavenka et al., 2022).

N-acetyl-5-methoxytryptamine, often known as melatonin, is a widely disseminated chemical in nature with a wide range of uses. To protect the morphological and functional components of the cell membrane from damage by free radicals, melatonin demonstrates exceptional functional properties (Radogna et al., 2010).

Melatonin, usually referred to as the "sleep hormone," has numerous important features, including being anti-inflammatory and anti-apoptotic (Tarocco et al., 2019).

The hypothalamic suprachiasmatic nucleus controls circadian rhythms and clock gene expression, and the pineal gland produces melatonin. Although the liver produces melatonin and has its own separate circadian rhythms and expressions, the brain senses light through the retinas and controls rhythms and melatonin secretion throughout the body. Circadian rhythm disturbance or clock gene expression may encourage the development of liver steatosis, inflammation, or cancer, according to earlier studies linking several liver disorders and circadian rhythms. The powerful antioxidant benefits of melatonin are well known. ROS and oxidative stress are produced in the liver by excessive fatty acid accumulation or alcohol consumption, which can harm the liver (Sato et al., 2020).

HCV infection, which results in continuous liver inflammation, can develop chronic hepatitis, cirrhosis, and HCC. Interferon (IFN)-based regimens, which have a low cure rate and the potential for serious side effects, were the only anti-HCV treatments available in the past. However, a number of oral anti-HCV medications (direct-acting antivirals, or DAAs) have become available recently (Endo et al., 2017). DAAs were first used with ribavirin and PEGIFN to boost response rates, but this increased toxicity (Pawlotsky, 2014). The development of interferon-free regimens with much improved therapeutic tolerance has been made possible by the very effective combination of DAAs that target several stages of the viral life cycle. The majority of patient populations' cure rates have now reached 90% thanks to well-tolerated oral regimens (Feld et al., 2014).

The National Committee for Control of Viral Hepatitis in Egypt states that patients fall into one of the two categories below: A) Easy-to-treat group meeting the following requirements: treatment-naive, total serum bilirubin of 1.2 mg/dL or lower, serum albumin of 3.5 g/dL or higher, INR of 1.2 or lower, and platelet count of 150,000/mm3 or higher. This group is eligible to receive treatment for a total of 12 weeks with any of the following regimens: sofosbuvir and daclatasvir or Qurevo® (Ombitasvir, Paritaprevir, and Ritonavir), as well as ribavirin. B) Group that is difficult to treat according to these standards: Having received Peg-INF treatment, having total serum bilirubin levels greater than 1.2 mg/dL, serum albumin levels lower than 3.5 g/dL, having an INR greater than 1.2, and having a platelet count below 150,000/mm3. This group is eligible to get a 12-week course of sofosbuvir, daclatasvir, and ribavirin treatment (Allam et al., 2022).

Sofosbuvir, daclatasvir with or without ribavirin, have been exclusively used for all patients in the Egyptian national program for managing HCV since early 2016 due to the high response rates with the locally produced generics, which support the use of low-cost generics in similar programs in limited resource settings (Waked, 2022).

Materials and methods

Study design

This cohort study was performed on 10 healthy individuals (6 men, 4 women) who served as controls, or Group I, and 40 Egyptian patients who had chronic infection with hepatitis C treated with a combination of IFN-free DAAs at the Hepatology and Virology outpatient clinic at Tanta University Hospital during the period from May 2022 to July 2022 after the study was approved by the Institutional Review Board of Tanta University (IRB study protocol code: TP/RE/9/4/22 ph-001). The study was registered as a clinical trial (ClinicalTrials.gov identifier: NCT05372874). Written informed consent was obtained from all participants. HCV Patients were divided into two groups (Group II and Group III). Group II included 20 patients with CHC infection who were treated with DAAs (one sofosbuvir 400 mg tablet and

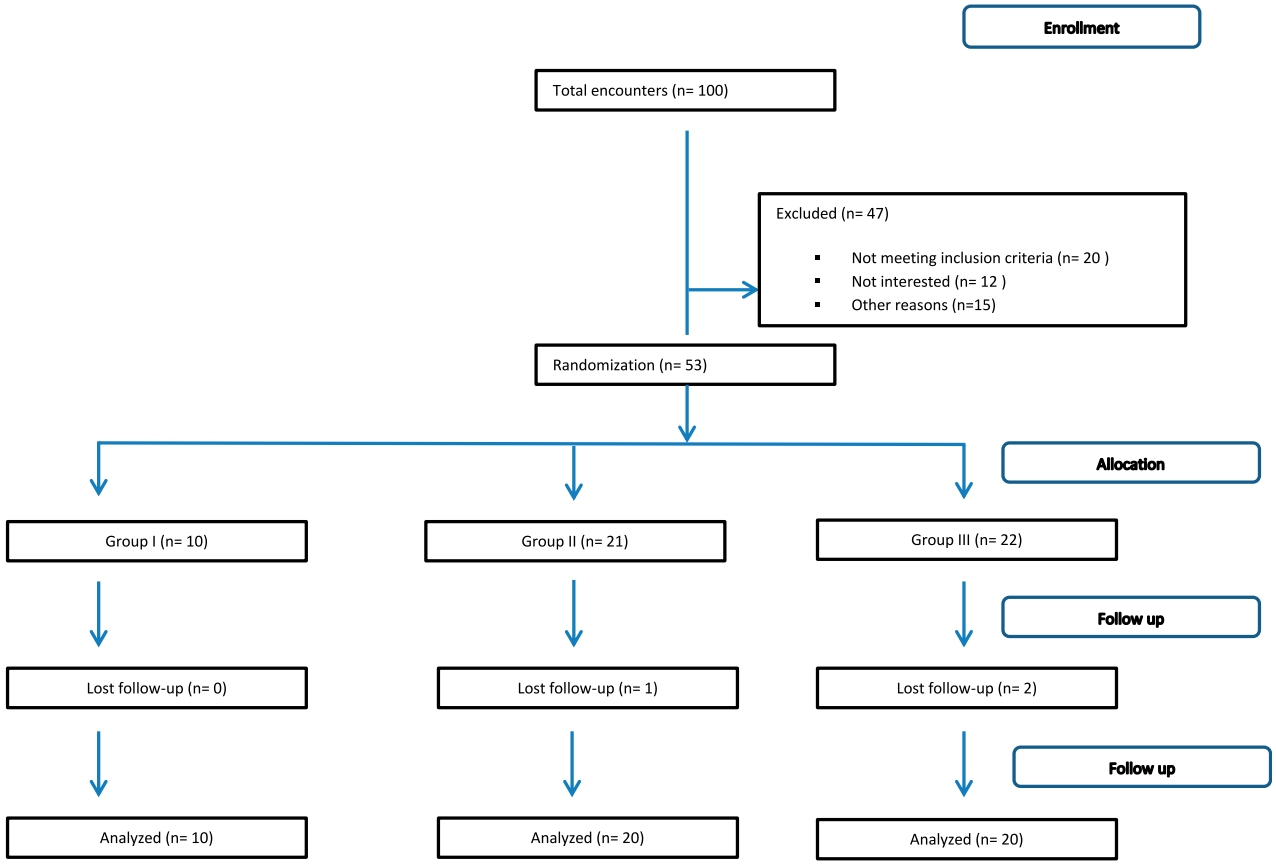

FIGURE 1
Flowchart illustrating the participants' screening, enrollment, and randomization.

TABLE 1 Demographic data of the study participants.

Parameter	Group I: (n = 10)	Group II: (n = 20)	Group III: (n = 20)	p-value
Age[a], y	48.300 ± 4.322	48.900 ± 8.416	55.250 ± 15.986	0.160
Mean ± SD				
Sex[a]				0.353
Male	6 (60.00 %)	7 (35.00 %)	7 (35.00 %)	
Female	4 (40.00 %)	13 (65.00 %)	13 (65.00 %)	

$p < 0.05$ is considered significant.
Values are presented as mean (SD).
[a]Values are presented as n (%).

one daclatasvir 60 mg capsule once a day) for 12 weeks. Patients in this group had one or more of the following conditions: treatment naïve, total serum bilirubin of 1.2 mg/dL or lower, serum albumin of 3.5 g/dL or higher, INR of 1.2 or lower, and a platelet count of 150,000/mm^3 or higher.

Group III included 20 patients with CHC infection who were treated with DAAs (one sofosbuvir 400 mg tablet, one daclatasvir 60 mg capsule once a day, and 600–1000 ribavirin) according to tolerance for 12 weeks (this group belongs to the difficult-to-treat group according to the National Committee for Control of Viral Hepatitis in Egypt, 2016). Patients in this group had one or more of the following conditions: total serum bilirubin is higher than 1.2 mg/dL, serum albumin is lower than 3.5 g/dL, INR is higher than 1.2, and platelet count is less than 150,000/mm^3. Figure 1.

The enrolled patients, besides being HCV positive, were recruited based on specific eligibility criteria, including the absence of HBV infection, renal insufficiency, Hepatocellular carcinoma (HCC) or other types of malignancy, current use of melatonin or any medications that have interactions with melatonin, consuming a lot of caffeine, being heavy smokers, or working night shifts. Also, patients were excluded for another reasons, such as pregnancy or lactation, alcohol consumption, liver transplantation,

TABLE 2 Baseline selected laboratory data of all patients in the 3 study group.

Parameter	Group I: (*n* = 10)	Group II: (*n* = 20)	Group III: (*n* = 20)	*p*-value (among all groups)
ALT, IU/L	23.100 ± 2.470	38.700 ± 4.067$	91.685 ± 44.368$#	<0.001*
AST, IU/L	25.900 ± 1.729	40.450 ± 3.649$	53.235 ± 34.641$	0.009*
BIL-T, mg/dL	0.853 ± 0.049	0.902 ± 0.099	1.024 ± 0.204$#	0.006*
BIL-D, mg/dL	0.120 ± 0.027	0.143 ± 0.017	0.202 ± 0.111$#	0.008*
Albumin, g/dL	4.450 ± 0.158	3.940 ± 0.185$	3.790 ± 0.484$	<0.001*
INR, IU	1.079 ± 0.152	1.094 ± 0.067	1.200 ± 0.138$#	0.008*
PT, sec	13.000 ± 1.826	13.500 ± 1.680	14.612 ± 1.626$	0.031*
Hb, g/dL	14.250 ± 1.461	11.818 ± 1.489$	12.268 ± 1.562$	0.001*
Platelets, 10^9/L	238.800 ± 60.487	207.650 ± 59.063	129.050 ± 43.686$#	<0.001*
WBCs, 10^9/L	5.038 ± 0.698	5.536 ± 1.412	8.549 ± 2.140$#	<0.001*
Creatinine, mg/dL	0.838 ± 0.116	0.875 ± 0.096	0.958 ± 0.196	0.077
GGT, U/L	24.500 ± 1.581	39.000 ± 2.714$	42.100 ± 6.512$	<0.001*
Cholesterol, mg/dL	179.300 ± 26.277	165.600 ± 7.074$	161.950 ± 5.356$	0.004*
LDL, mg/dL	129.340 ± 8.935	115.500 ± 18.392$	114.835 ± 7.251$	0.014*
HDL, mg/dL	58.200 ± 2.201	39.600 ± 2.186$	41.000 ± 2.884$	<0.001*
MDA, nmol/mL	1.400 ± 0.302	4.270 ± 0.254$	4.435 ± 0.292$	<0.001*
Melatonin, pg/mL	45.167 ± 3.410	17.970 ± 2.116$	17.895 ± 1.927$	<0.001*
HCV RNA (IU/mL)	Not detected	1114033.650 ± 1451732.743	955572.300 ± 4176787.175	0.874

$p < 0.05$ indicates a significant difference compared to the healthy normal control group, and # $p < 0.05$ indicates a significant difference compared to group II. ALT, alanine aminotranferase; AST, aspartate transaminase; BIL-T, total bilirubin; BIL-D, direct bilirubin; INR, international normalized ratio; PT, prothrombin time; Hb, hemoglobin; WBCs, white blood cells; GGT, gamma-glutamyl transferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein and MDA, malondialdehyde. *Values are presented as mean (SD). * Significant at $p < 0.05$.

the presence of neurological disorders or major psychiatric diseases, patients with cerebral disease or trauma that may affect the pineal gland, and difficulty of follow-up. The control group comprised 10 healthy volunteers who met the same eligibility criteria, i.e., they were HCV-negative and were age- and gender-matched.

Patients were followed-up by weekly phone calls and directed to meet at monthly intervals to assess their average sleep time, adherence to the study medications, and any medication adverse effects.

Clinical and laboratory assessment

All participants were subjected to a full history and a thorough clinical examination, with a focus on sex, age, and route of HCV transmission. Two fasting blood samples, 10 mL each, were withdrawn from every hepatitis C patient at 9 a.m.: one before the recommended antiviral therapy intake and the second after 12 weeks of treatment. Complete blood cell counts were done immediately. Then, the remaining blood was allowed to clot at room temperature and centrifuged at 3,000 rpm for 10 min. Sera were separated. 2 mL of serum from each sample was kept frozen at −70°C until the quantitative determination of MDA and melatonin levels. The remaining serum of each sample was used immediately for the determination of liver function parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, Gamma-glutamyl transferase (GGT), albumin and international normalized ratio (INR). Fasting lipid profile [total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL)] and serum creatinine were also determined.

Analysis of MDA

Lipid peroxidation was estimated by determination of thiobarbituric acid-reactive substance (TBARS) content, reflective of the MDA end-product of membrane oxidation, using a commercial kit (Biodiagnostic, Giza, Egypt, Catalog No. MD 25029).

Analysis of melatonin

Colorimetric melatonin levels were measured by a double antibody sandwich ELISA using commercially available ELISA kits and in accordance with the manufacturer's instructions (SunRed; SunRed Biological Technology Co. Ltd., Shanghai, China, Catalog No. 201-12-1014). At the same time, 10 healthy subjects were similarly investigated.

Assessment of HCV-RNA

Assessment of HCV-RNA load at baseline and at the end of treatment (12 weeks). Undetectable HCV-RNA at the end of treatment response was defined as undetectable HCV-RNA at the completion of treatment.

TABLE 3 Comparison among the 3 study groups 12 weeks after treatment.

Parameter	Group I: (n = 10)	Group II: (n = 20)	Group III: (n = 20)	p-value) among all groups)
ALT, IU/L	23.100 ± 2.470	24.778 ± 7.395	25.050 ± 3.137	0.612
AST, IU/L	25.900 ± 1.729	26.316 ± 6.371	26.800 ± 3.302	0.873
BIL-T, mg/dL	0.853 ± 0.049	0.862 ± 0.129	0.894 ± 0.161	0.651
BIL-D, mg/dL	0.120 ± 0.027	0.138 ± 0.026	0.139 ± 0.011	0.069
Albumin, g/dL	4.450 ± 0.158	4.215 ± 0.201$^{\$}$	4.115 ± 0.225$^{\$}$	<0.001*
INR, IU	1.079 ± 0.152	1.074 ± 0.053	1.105 ± 0.081	0.536
PT, sec	13.000 ± 1.826	13.125 ± 0.599	13.480 ± 0.574	0.346
Hb, g/dL	14.250 ± 1.461	12.671 ± 1.533$^{\$}$	12.499 ± 1.098$^{\$}$	0.004*
Platelets, 10^9/L	238.800 ± 60.487	232.600 ± 50.543	174.950 ± 38.465$^{\$\#}$	<0.001*
WBCs, 10^9/L	5.038 ± 0.698	5.124 ± 0.856	7.203 ± 1.436$^{\$\#}$	<0.001*
Creatinine, mg/dL	0.838 ± 0.116	0.850 ± 0.170	0.938 ± 0.145	2.251
GGT, U/L	24.500 ± 1.581	29.000 ± 7.980	32.750 ± 2.511$^{\$}$	0.001*
Cholesterol, mg/dL	179.300 ± 26.277	207.300 ± 14.012$^{\$}$	210.450 ± 10.782$^{\$}$	<0.001*
LDL, mg/dL	129.340 ± 8.935	141.600 ± 11.686$^{\$}$	140.920 ± 13.539$^{\$}$	0.026*
HDL, mg/dL	58.200 ± 2.201	51.150 ± 4.648$^{\$}$	44.650 ± 3.117$^{\$\#}$	<0.001*
MDA, nmol/mL	1.400 ± 0.302	3.295 ± 0.437$^{\$}$	2.555 ± 0.307$^{\$\#}$	<0.001*
Melatonin, pg/mL	45.167 ± 3.410	28.273 ± 5.436$^{\$}$	43.890 ± 4.106$^{\#}$	<0.001*
HCV RNA (IU/mL)	Not detected	Not detected	Not detected	—

$p < 0.05$ indicates a significant difference compared to the healthy normal control group, and # $p < 0.05$ indicates a significant difference compared to group II. ALT, alanine aminotranferase; AST, aspartate transaminase; BIL-T, total bilirubin; BIL-D, direct bilirubin; INR, international normalized ratio; PT, prothrombin time; Hb, hemoglobin; WBCs, white blood cells; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein and MDA, malondialdehyde. *Values are presented as mean (SD). * Significant at $p < 0.05$.

Data analysis

The data were analyzed using a statistical package for the social science (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, United States). Quantitative data were expressed as mean ± Standard deviation. Qualitative data are expressed as numbers and percentages and analyzed by Chi–square test (X2). Student's t-test was used to compare means between the two groups. Multiple comparisons were performed by one-way analysis of variance (ANOVA) followed by the Chi-square test for multiple comparisons. Correlations were analyzed using the Pearson test. p-values of 0.05 were considered statistically significant.

Results

Our cohort was conducted on 50 individuals divided into 3 groups: group I: 10 patients as a control group, group II: 20 patients with CHC infection who were treated with DAAs (sofosbuvir/daclatasvir) for 12 weeks; and group III: 20 patients with CHC infection who were treated with DAAs (sofosbuvir/daclatasvir/ribavirin) for 12 weeks. Table 1 shows that no significant differences were observed in age or gender between groups ($p > 0.05$).

Table 2 shows that CHC patients (pre-treatment, group II and group III) had significantly higher ALT, AST, GGT, and MDA than the control group (group I) ($p < 0.001$, $p = 0.009$, $p < 0.001$, $p < 0.001$, respectively), but significantly lower albumin, hemoglobin, cholesterol, LDL, HDL, and melatonin ($p < 0.001$, $p = 0.001$, $p < 0.001$, $p = 0.004$, $p = 0.014$, $p < 0.001$, $p < 0.001$ respectively). However, there were insignificant differences between CHC patients (pre-treatment, group II and group III) and the control group (group I) regarding serum creatinine level ($p = 0.077$). Also, group II had significantly higher total bilirubin, direct bilirubin, INR, prothrombin time, and WBCs than the control group (group I) ($p = 0006$, $p = 0.008$, $p = 0.008$, $p = 0.031$, $p < 0.001$ respectively), but significantly lower Platelets ($p < 0.001$) than the control group (group I).

Table 3 shows that after treatment of CHC patients (group II and group III) there were no significant differences compared to the healthy control group (group I) regarding ALT, AST, total bilirubin, direct bilirubin, INR, prothrombin time, serum creatinine level ($p = 0.612$, $p = 0.873$, $p = 0.651$, $p = 0.069$, $p = 0.536$, $p = 0.346$, respectively), while there were significant differences regarding albumin, hemoglobin, cholesterol, LDL, HDL, MDA, and melatonin ($p < 0.001$, p =0.004, $p < 0.001$, $p = 0.026$, $p < 0.001$, $p < 0.001$, $p < 0.001$, respectively). However, group III had significant differences regarding platelets, WBCs, and GGT (p=<0.001, $p < 0.001$, $p = 0.001$, respectively).

Table 4 shows that there was a significant decrease in ALT, AST, GGT, and MDA ($p < 0.001$, $p < 0.001$, $p < 0.001$, and $p < 0.001$ respectively). However, there was a significant increase in Albumin, hemoglobin, platelets, cholesterol, LDL, HDL, and

TABLE 4 Laboratories characterization of HCV-patients treated with dual therapy (Group II).

Parameter	Baseline	After 12 weeks of treatment	*p*-value
ALT, IU/L	38.700 ± 4.067	24.778 ± 7.395	<0.001*
AST, IU/L	40.450 ± 3.649	26.316 ± 6.371	<0.001*
BIL-T, mg/dL	0.902 ± 0.099	0.862 ± 0.129	0.138
BIL-D, mg/dL	0.143 ± 0.017	0.138 ± 0.026	0.459
Albumin, g/dL	3.940 ± 0.185	4.215 ± 0.201	<0.001*
INR, IU	1.094 ± 0.067	1.074 ± 0.053	0.202
PT, sec	13.500 ± 1.680	13.125 ± 0.599	0.223
Hb, g/dL	11.818 ± 1.489	12.671 ± 1.533	0.016*
Platelets, 10^9/L	207.650 ± 59.063	232.600 ± 50.543	<0.001*
WBCs, 10^9/L	5.536 ± 1.412	5.124 ± 0.856	0.064
Creatinine, mg/dL	0.875 ± 0.096	0.850 ± 0.170	0.543
GGT, U/L	39.000 ± 2.714	29.000 ± 7.980	<0.001*
Cholesterol, mg/dL	165.600 ± 7.074	207.300 ± 14.012	<0.001*
LDL, mg/dL	115.500 ± 18.392	141.600 ± 11.686	<0.001*
HDL, mg/dL	39.600 ± 2.186	51.150 ± 4.648	<0.001*
MDA, nmol/mL	4.270 ± 0.254	3.295 ± 0.437	<0.001*
Melatonin, pg/mL	17.970 ± 2.116	28.273 ± 5.436	<0.001*
HCV RNA (IU/mL)	1114033.650 ± 1451732.743	Not detected	

ALT, alanine aminotranferase; AST, aspartate transaminase; BIL-T, total bilirubin; BIL-D, direct bilirubin; INR, international normalized ratio; PT, prothrombin time; Hb, hemoglobin; WBCs, white blood cells; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein and MDA, malondialdehyde.
*Values are presented as mean (SD). *Significant at $p < 0.05$.

melatonin ($p < 0.001$, $p = 0.016$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$) In addition, there were insignificant decreases in total bilirubin, direct bilirubin, INR, prothrombin time, WBCs, and serum creatinine level ($p = 0.138$, $p = 0.459$, $p = 0.202$, $p = 0.223$, $p = 0.064$, $p = 0.543$ respectively).

Table 5 shows that there was a significant decrease in ALT, AST, total bilirubin, direct bilirubin, INR, prothrombin time, WBCs, GGT, and MDA ($p < 0.001$, $p = 0.003$, $p = 0.004$, $p = 0.020$, $p = 0.006$, $p < 0.001$, $p = 0.014$, $p < 0.001$, $p < 0.001$ respectively). However, there was a significant increase in albumin, platelets, cholesterol, LDL, HDL, and melatonin ($p = 0.006$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively). In addition, there was no significant change in either hemoglobin or serum creatinine levels ($p = 0.507$ and 0.724 respectively).

Figures 2, 3 illustrate the changes in melatonin and MDA in the 3 study groups before and 3 months after treatment.

The percentage of changes after 12 weeks of treatment on MDA in group II and group III is (22.47% ($p < 0.001$), 42.15($p < 0.001$), respectively).

Also, the percentage of changes after 12 weeks of treatment on melatonin in group II and group III is (60.02% ($p < 0.001$), 146.11 ($p < 0.001$), respectively).

Figures 4, 5 illustrate the Pearson correlation between melatonin and MDA in the 3 study groups before and 3 months after treatment

There was a negative non-significant correlation between melatonin and both total cholesterol and LDL before treatment (r= −0.049 [$p = 0.766$], r= −0.164 [$p = 0.312$], respectively). Also, there was a positive non-significant correlation between melatonin and HDL (r= 0.075 [$p = 0.646$]. In addition, MDA had a negative non-significant correlation with total cholesterol, LDL and HDL (r= −0.239 [$p = 0.138$], r= −0.091 [$p = 0.577$], r= −0.053 [$p = 0.745$], respectively).

Table 6 shows that Fatigue and headache were the most commonly reported adverse effects of DAAs.

Discussion

This study was dedicated to investigating the efficacy of using sofosbuvir/daclatasvir ± ribavirin on lipid peroxidation and melatonin levels in HCV patients. The results of this study suggest that sofosbuvir and daclatasvir, with or without ribavirin, were successful in decreasing lipid peroxidation and increasing melatonin levels. Infection with HCV affects millions of people worldwide. In Egypt, this infection's prevalence rate is as high as 19% (Abdalla et al., 2005).

With the introduction of numerous novel antivirals, notably DAAs, the area of HCV treatment has undergone a significant transformation. Combinations of these medications in IFN-free

TABLE 5 Laboratories characterization of HCV-patients treated with triple therapy (Group III).

Parameter	Baseline	After 12 weeks of treatment	*p*-value
ALT, IU/L	91.685 ± 44.368	25.050 ± 3.137	<0.001*
AST, IU/L	53.235 ± 34.641	26.800 ± 3.302	0.003*
BIL-T, mg/dL	1.024 ± 0.204	0.894 ± 0.161	0.004*
BIL-D, mg/dL	0.202 ± 0.111	0.139 ± 0.011	0.020*
Albumin, g/dL	3.790 ± 0.484	4.115 ± 0.225	0.006*
INR, IU	1.200 ± 0.138	1.105 ± 0.081	<0.001*
PT, sec	14.612 ± 1.626	13.480 ± 0.574	<0.001*
Hb, g/dL	12.268 ± 1.562	12.499 ± 1.098	0.507
Platelets, 10^9/L	129.050 ± 43.686	174.950 ± 38.465	<0.001*
WBCs, 10^9/L	8.549 ± 2.140	7.203 ± 1.436	0.014*
Creatinine, mg/dL	0.958 ±0.196	0.938 ± 0.145	0.724
GGT, U/L	42.100 ± 6.512	32.750 ± 2.511	<0.001*
Cholesterol, mg/dL	161.950 ± 5.356	210.450 ± 10.782	<0.001*
LDL, mg/dL	114.835 ± 7.251	140.920 ± 13.539	<0.001*
HDL, mg/dL	41.000 ± 2.884	44.650 ± 3.117	<0.001*
MDA, nmol/mL	4.435 ± 0.292	2.555 ± 0.307	<0.001*
Melatonin, pg/mL	17.895 ± 1.927	43.890 ± 4.106	<0.001*
HCV RNA, IU/mL	955572.300 ± 4176787.175	Not detected	—

ALT, alanine aminotranferase; AST, aspartate transaminase; BIL-T, total bilirubin; BIL-D, direct bilirubin; INR, international normalized ratio; PT, prothrombin time; Hb, hemoglobin; WBCs, white blood cells; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein and MDA, malondialdehyde.
*Values are presented as mean (SD). * Significant at $p < 0.05$.

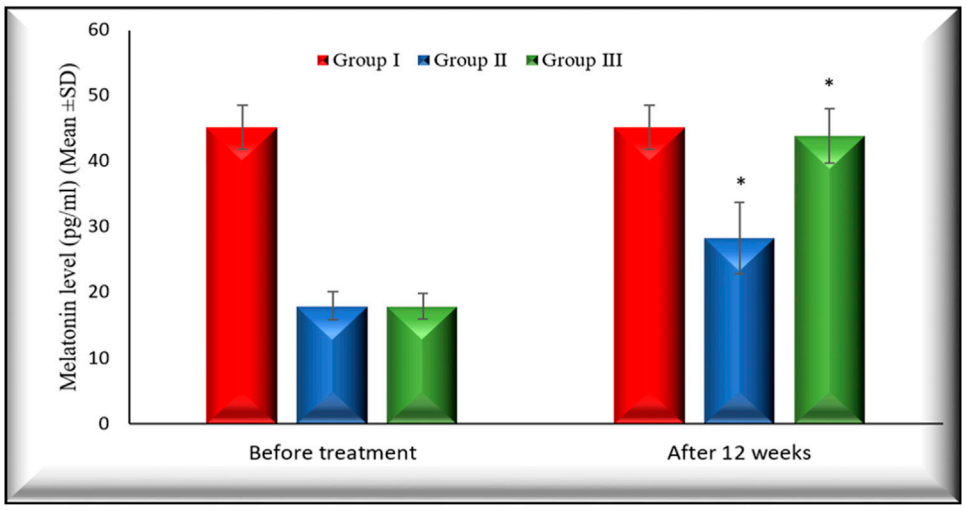

FIGURE 2
Changes in melatonin levels in the 3 studied groups before treatment and 3 months after treatment. Melatonin levels in both group II and group III increased significantly ($p < 0.001$) 3 months after treatment in comparison with their baseline. Group I: healthy control. Group II: CHC patients treated with sofosbuvir and daclatasvir. Group III: CHC patients treated with sofosbuvir, daclatasvir and ribavirin. Values are presented as mean (SD). *Significant difference.

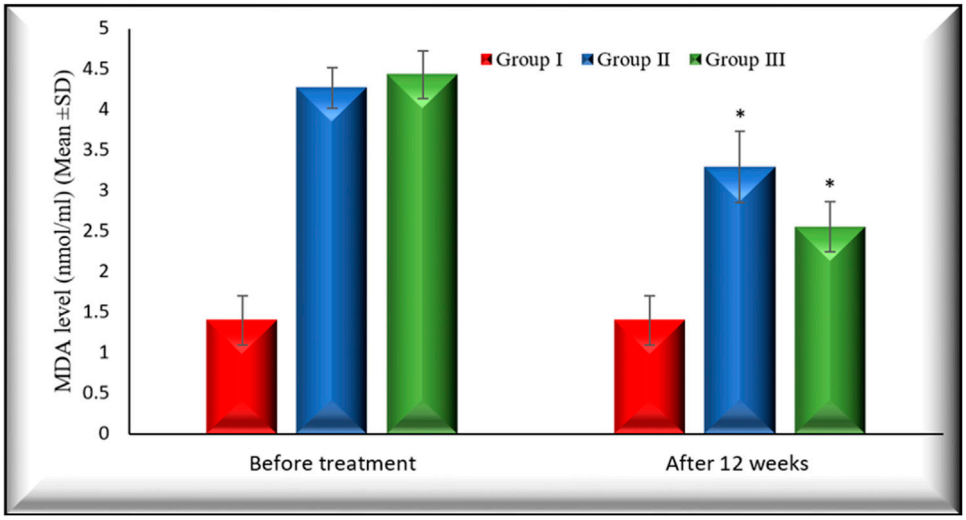

FIGURE 3
Changes in MDA level in the 3 studied groups before treatment and 3 months after treatment. MDA levels in both group II and group III decreased significantly ($p < 0.001$) 3 months after treatment in comparison with their baseline. Group I: healthy control. Group II: CHC patients treated with sofosbuvir and daclatasvir. Group III: CHC patients treated with sofosbuvir, daclatasvir, and ribavirin Values are presented as mean (SD). *Significant difference.

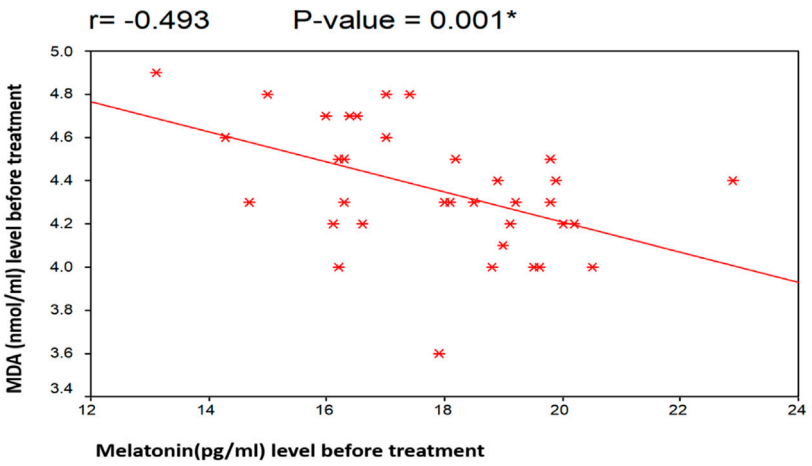

FIGURE 4
Illustrate the Pearson correlation analysis between the measured variables, which revealed the presence of a significant negative correlation between MDA and melatonin before treatment ($r = -0.493$ [$p = 0.001$]). Group II: CHC patients treated with sofosbuvir and daclatasvir. Group III: CHC patients treated with sofosbuvir, daclatasvir, and ribavirin.

regimens are now the norm for treating HCV infection due to their superior safety profiles and antiviral effectiveness.

Clinical study findings and preliminary results from real-world applications show that the combination of sofosbuvir, an NS5B inhibitor, and an NS5A inhibitor, such as the first-in-class drug daclatasvir, is one of the most effective antiviral medicines available. Sofosbuvir and an NS5A inhibitor given together for 12 weeks in combination with ribavirin seem to be a very good option for treating cirrhotic and treatment-experienced patients with any stage of fibrosis, regardless of the severity of the underlying liver disease and the baseline characteristics of the patients (Pol et al., 2016).

In the current study, basal serum MDA levels were shown to be significantly higher for CHC disease than for the comparable normal value. Other studies have reported similar outcomes (Romero et al., 1998; Seren et al., 2011). CHC is typically linked to an excess of oxidants and a deficiency of antioxidants. Free radicals produce lipid peroxidation, which results in the oxidative breakdown of polyunsaturated fatty acids that are essential to cellular membranes. When they are destroyed, harmful and reactive aldehyde metabolites like MDA are created (Levent et al., 2006).

Before treatment, the levels of serum MDA were significantly higher in patients in group II and group III than in the controls ($p < 0.001$). At the end of treatment, there was a significant decrease in

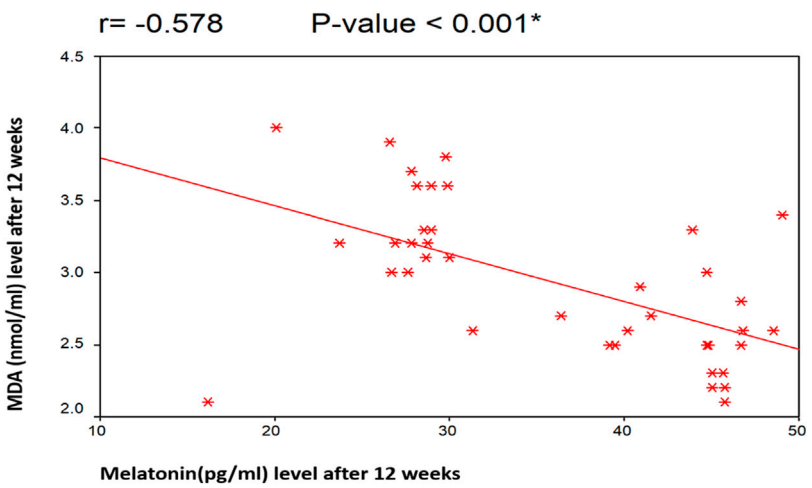

FIGURE 5
Illustrate the Pearson correlation analysis between the measured variables, which revealed the presence of a significant negative correlation between MDA and melatonin after treatment (r = −0.578 [p < 0.001]). Group II: CHC patients treated with sofosbuvir and daclatasvir. Group III: CHC patients treated with sofosbuvir, daclatasvir, and ribavirin.

TABLE 6 Side effects among studied groups.

Parameters	Group II (n= 20)	Group III (n= 20)
Anorexia	2 (10%)	3 (15%)
Decreased appetite	3 (15%)	4 (20%)
Itching	1 (5%)	0 (0%)
Headache	6 (30%)	7 (35%)
Fatigue	8 (40%)	10 (50%)
Fever	4 (20%)	5 (25%)
Serious adverse events or death	0 (0%)	0 (0%)

serum MDA (p < 0.001). This agrees with Villani Rosanna et al. (Villani et al., 2020).

This data supports the idea that DAAs therapy partially reverses this negative effect by lowering the formation of reactive species caused by HCV. This may help partially explain the observed decrease in circulating MDA levels, compared to prior therapy in our patients.

A substantial association between the HCV virus and intracellular lipids has been observed in studies on the HCV life cycle, indicating that host lipids are crucial for viral replication (Vogt et al., 2013). Host blood lipid levels have an impact on the movement of hepatitis C virion and entry into hepatocytes. A fraction of host triacylglycerol-rich lipoproteins, also referred to as lipoviroparticles, complexes with circulating HCV particles (Schaefer and Chung, 2013).

Before treatment, the levels of serum LDL-C, high-density lipoprotein-cholesterol, and cholesterol were significantly lower in patients in group II and group III than the controls (p = 0.014, p < 0.001, p =0.004 respectively). At the end of treatment, there was a significant increase in serum LDL-C, high-density lipoprotein-cholesterol, cholesterol, and cholesterol (p = 0.026, p < 0.001, p < 0.001, respectively), This is in agreement with Ayman M. El-Lehleh et al. (El-Lehleh et al., 2019).

This effect is attributed to a reversal of the impact of HCV replication on hepatic lipid metabolism.

In the current study, we observed that HCV patients produced less endogenous melatonin. This is in agreement with Vikram Mehraj et al. (Mehraj and Routy, 2015). The accumulation of toxic chemicals caused by hepatic insufficiency or pineal dysfunction may be the mechanism for decreased melatonin production in people with liver illness. Another theory is that persistent viral infections manipulate the host immune system to develop illness tolerance through kynurenine catabolites. HCV infection indoleamine-2, 3-dioxygenase (IDO) expression was reported by (Lepiller et al., 2015). These study findings showed that HCV infection directly induced IDO, which could lead to decreased melatonin levels.

Group III has a higher level of melatonin after treatment compared with group II. This enhanced efficacy of ribavirin combination therapy in this group may be the result of ribavirin's ability to induce a cellular immune reaction against HCV. Ribavirin acts on Human peripheral blood mononuclear cells (PBMCs) and induces T helper (Th) differentiation. T cells have the four enzymes required for the synthesis of melatonin (aromatic L-amino acid decarboxylase, arylalkylamine N-acetyltransferase, N- acetylserotonin methyltransferase, and tryptophan hydroxylase) and produce high levels of melatonin (Shiina et al., 2004; Ren et al., 2017).

Despite not being an antioxidant, ribavirin's antiviral properties might reduce viral load and inflammation. This process could additionally reduce oxidative stress caused on by viruses and MDA in group III compared to group II (Levent et al., 2006).

In the current study, we found that DAAs have a safe profile, as no patient in our series stopped treatment due to severe adverse events, which is in line with (Abd El Rhman. et al., 2019).

Based on our study results, HCV decreases melatonin level, which enables it to evade the immune system and develop illness tolerance. We therefore urge the conduct of further clinical studies to examine the advantages of melatonin supplements as an adjunctive therapy to antiviral medication in hepatitis C patients. Also, our result shows that after using DAA, we found a significant increase in melatonin levels compared to before treatment. Consequently, other studies are needed to explain the mechanism by which DAAs increase melatonin production. In addition, our data showed that there was a significant negative correlation between serum MDA and serum melatonin concentrations in CHC patients.

From the present study, it is established that CHC is associated with oxidative stress, as evidenced by an increased MDA level and a decrease in melatonin level. Further study is needed with a large sample size.

Conclusion

In conclusion, these preliminary findings suggest that DAAs increase endogenous melatonin and decrease MDA levels, which are associated with improved antiviral treatment outcomes in patients with HCV.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Research Ethics Committee—University of Tanta—Faculty of Pharmacy. The patients/participants provided their written informed consent to participate in this study.

Author contributions

NE-M, MA, and HH reviewed the literature and constructed the study design. The eligibility assessment, enrollment of participants, randomization and collection of clinical data were performed by SA-S and HH. SA-R and MH performed laboratory and statistical analyses. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abd El Rhman, M. M., Galal, G. M., Abd El Hamid, R. M., and Abd Allah, S. K. (2019). Efficacy, safety, and biochemical response of sofosbuvir-based combinations in treatment of chronic hepatitis C patients in sohag governorate. *Sohag Med. J.* 24, 104–111. doi:10.21608/SMJ.2019.18730.1067

Abdalla, M., Im, A., Dr, S., Wn, S., and Be, B. (2005). Hepatitis C virus-core and non structural proteins lead to different effects on cellular antioxidant defenses. *J. Med. Virol.* 76, 489–497. doi:10.1002/jmv.20388

Allam, A. S., Elmeged, M. L. A., Ghaly, S. M., Ahmed, O. A., Naguib, G. G., and Abohalima, A. S. (2022). Impact of direct-acting antiviral therapy on metabolic profiles and adiponectin serum level in different categories of patients with chronic hepatitis C infection. *Egypt. Liver J.* 12, 32. doi:10.1186/s43066-022-00194-z

Avery, S. V. (2011). Molecular targets of oxidative stress. *Biochem. J.* 434, 201–210. doi:10.1042/BJ20101695

El-Lehleh, A. M., El-Shiekh, M. M., and El-Hamouly, M. S. (2019). Study of serum lipid profile before and after hepatitis C virus treatment. *Menoufia Med. J.* 32, 1126. doi:10.4103/mmj.mmj_141_19

Endo, D., Satoh, K., Shimada, N., Hokari, A., and Aizawa, Y. (2017). Impact of interferon-free antivirus therapy on lipid profiles in patients with chronic hepatitis C genotype 1b. *World J. gastroenterology* 23, 2355–2364. doi:10.3748/wjg.v23.i13.2355

Feld, J. J., Kowdley, K. V., Coakley, E., Sigal, S., Nelson, D. R., Crawford, D., et al. (2014). Treatment of HCV with ABT-450/r–ombitasvir and dasabuvir with ribavirin. *N. Engl. J. Med.* 370, 1594–1603. doi:10.1056/NEJMoa1315722

García, J. J., López-Pingarrón, L., Almeida-Souza, P., Tres, A., Escudero, P., García-Gil, F. A., et al. (2014). Protective effects of melatonin in reducing oxidative stress and in preserving the fluidity of biological membranes: A review. *J. pineal Res.* 56, 225–237. doi:10.1111/jpi.12128

Ivanov, A. V., Bartosch, B., Smirnova, O. A., Isaguliants, M. G., and Kochetkov, S. N. (2013). HCV and oxidative stress in the liver. *Viruses* 5, 439–469. doi:10.3390/v5020439

Kartavenka, K., Panuwet, P., Yakimavets, V., Jaikang, C., Thipubon, K., D'Souza, P. E., et al. (2022). LC-MS quantification of malondialdehyde-dansylhydrazine derivatives in urine and serum samples. *J. Anal. Toxicol.* 44, 470–481. doi:10.1093/jat/bkz112

Lepiller, Q., Soulier, E., Li, Q., Lambotin, M., Barths, J., Fuchs, D., et al. (2015). Antiviral and immunoregulatory effects of indoleamine-2, 3-dioxygenase in hepatitis C virus infection. *J. innate Immun.* 7, 530–544. doi:10.1159/000375161

Levent, G., Ali, A., Ahmet, A., Polat, E. C., Aytaç, Ç., Ayşe, E., et al. (2006). Oxidative stress and antioxidant defense in patients with chronic hepatitis C patients before and after pegylated interferon alfa-2b plus ribavirin therapy. *J. Transl. Med.* 4, 25–26. doi:10.1186/1479-5876-4-25

Mehraj, V., and Routy, J. P. (2015). Tryptophan catabolism in chronic viral infections: Handling uninvited guests. *Int. J. Tryptophan Res.* 8, 41–48. doi:10.4137/IJTR.S26862

Moon, A. M., Singal, A. G., and Tapper, E. B. (2020). Contemporary epidemiology of chronic liver disease and cirrhosis. *Clin. Gastroenterology Hepatology* 18, 2650–2666. doi:10.1016/j.cgh.2019.07.060

Paracha, U. Z., Fatima, K., Alqahtani, M., Chaudhary, A., Abuzenadah, A., Damanhouri, G., et al. (2013). Oxidative stress and hepatitis C virus. *Virology J.* 10, 251–259. doi:10.1186/1743-422X-10-251

Pawlotsky, J. M. (2014). New hepatitis C therapies: The toolbox, strategies, and challenges. *Gastroenterology* 146, 1176–1192. doi:10.1053/j.gastro.2014.03.003

Pol, S., Corouge, M., and Vallet-Pichard, A. (2016). Daclatasvir–sofosbuvir combination therapy with or without ribavirin for hepatitis C virus infection: From the clinical trials to real life. *Hepatic Med. Evid. Res.* 4, 21–26. doi:10.2147/HMER.S62014

Radogna, F., Diederich, M., and Ghibelli, L. (2010). Melatonin: A pleiotropic molecule regulating inflammation. *Biochem. Pharmacol.* 80, 1844–1852. doi:10.1016/j.bcp.2010.07.041

Ren, W., Liu, G., Chen, S., Yin, J., Wang, J., Tan, B., et al. (2017). Melatonin signaling in T cells: Functions and applications. *J. pineal Res.* 62 (3), e12394. doi:10.1111/jpi.12394

Romero, M. J., Bosch-Morell, F., Romero, B., Rodrigo, J. M., Serra, M. A., and Romero, F. J. (1998). Serum malondialdehyde: Possible use for the clinical management of chronic hepatitis C patients. *Free Radic. Biol. Med.* 25, 993–997. doi:10.1016/s0891-5849(98)00118-x

Sato, K., Meng, F., Francis, H., Wu, N., Chen, L., Kennedy, L., et al. (2020). Melatonin and circadian rhythms in liver diseases: Functional roles and potential therapies. *J. pineal Res.* 68 (3), e12639. doi:10.1111/jpi.12639

Schaefer, E. A., and Chung, R. T. (2013). HCV and host lipids: An intimate connection. *Seminars liver Dis.* 33 (04), 358–368. doi:10.1055/s-0033-1358524

Schwander, B., Feldstein, J., Sulo, S., Gonzalez, L., Elshishiney, G., and Hassany, M. (2022). Pursuing elimination of hepatitis C in Egypt: Cost-effectiveness and economic evaluation of a country-wide program. *Infect. Dis. Ther.* 11, 1193–1203. doi:10.1007/s40121-022-00631-x

Seren, Ö., Rler, G. E., Özlem Gülbahar, Ö. B., Bostankolu, O., Ozbaş Demırel, O., Bılgıhan, A., et al. (2011). Serum levels of advanced oxidation protein products, malonyldialdehyde, and total radical trapping antioxidant parameter in patients with chronic hepatitis C. *Turk J. Gastroenterol.* 22, 47–53. doi:10.4318/tjg.2011.0156

Shiina, M., Kobayashi, K., Satoh, H., Niitsuma, H., Ueno, Y., and Shimosegawa, T. (2004). Ribavirin upregulates interleukin-12 receptor and induces T cell differentiation towards type 1 in chronic hepatitis C. *J. gastroenterology hepatology* 19, 558–564. doi:10.1111/j.1440-1746.2003.03329.x

Tarocco, A., Caroccia, N., Morciano, G., Wieckowski, M. R., Ancora, G., Garani, G., et al. (2019). Melatonin as a master regulator of cell death and inflammation: Molecular mechanisms and clinical implications for newborn care. *Cell. death Dis.* 10, 1–12. doi:10.1038/s41419-019-1556-7

Villani, R., Bellanti, F., Cavallone, F., Di Bello, G., Tamborra, R., Vidyasagar, N. B., et al. (2020). Direct-acting antivirals restore systemic redox homeostasis in chronic HCV patients. *Free Radic. Biol. Med.* 156, 200–206. doi:10.1016/j.freeradbiomed.2020.06.007

Vogt, D. A., Camus, G., Herker, E., Webster, B. R., Tsou, C. L., Greene, W. C., et al. (2013). Lipid droplet-binding protein TIP47 regulates hepatitis C Virus RNA replication through interaction with the viral NS5A protein. *PLoS Pathog.* 9, e1003302. doi:10.1371/journal.ppat.1003302

Vrazas, V., Moustafa, S., Makridakis, M., Karakasiliotis, I., Vlahou, A., Mavromara, P., et al. (2022). A proteomic approach to study the biological role of hepatitis C virus protein Core+ 1/ARFP. *Viruses.* 14, 1694. doi:10.3390/v14081694

Vuppalanchi, R., Ghabril, M., Chalasani, N., Juluri, R., Bell, L. N., Kamendulis, L., et al. (2011). Oxidative stress in chronic liver disease: Relationship between peripheral and hepatic measurements. *Am. J. Med. Sci.* 342, 314–317. doi:10.1097/MAJ.0b013e31821d9905

Waked, I. (2022). Case study of hepatitis C virus control in Egypt: Impact of access program. *Antivir. Ther.* 27, 13596535211067592. doi:10.1177/13596535211067592

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Efficacy and safety evaluation of Azvudine in the prospective treatment of COVID-19 based on four phase III clinical trials

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Azvudine (FNC) is a synthetic nucleoside analog used to treat adult patients living with human immunodeficiency virus-1 (HIV-1) infection with high viral load. After phosphorylation, Azvudine inhibits RNA-dependent RNA polymerase, leading to the discontinuation of RNA chain synthesis in viruses. In addition, Azvudine is the first dual-target nucleoside oral drug worldwide to simultaneously target reverse transcriptase and viral infectivity factors in the treatment of HIV infection. On 9 August 2022, Azvudine was incorporated into the Guidelines for the Diagnosis and Treatment of Coronavirus Disease 2019 (version ninth) issued by the National Health Commission and the National Administration of Traditional Chinese Medicine. The recommended oral dose of Azvudine for the treatment of moderate coronavirus disease 2019 (COVID-19) is 5 mg once daily, and the duration of Azvudine treatment should not exceed 14 days. Four phase III clinical trials were performed during 2020–2022 to evaluate the efficacy and safety of Azvudine in the treatment of COVID-19. The results revealed that Azvudine could reduce nucleic acid-negative conversion time, viral load, and time to improvement in clinical conditions in patients with moderate COVID-19. In addition, Azvudine exhibited good safety and tolerance. Thereafter, Azvudine was incorporated into the Chinese guidelines and expert consensus for the treatment of COVID-19 and was highly approbated. Furthermore, Azvudine was also included in the Chinese guidelines for HIV infection.

KEYWORDS

Azvudine (FNC), viral load, severe acute respiratory syndrome coronavirus 2, RNA-dependent RNA polymerase, nucleic acid-negative conversion time

1 Introduction

Azvudine (FNC) is a synthetic nucleoside analog used to treat adult patients with plasma human immunodeficiency virus-1 (HIV-1) RNA of more than 100,000 copies/mL through the combination of another nucleoside reverse transcriptase inhibitor (NRTI) or non-nucleoside reverse transcriptase inhibitor (NNRTI). Azvudine inhibits RNA-dependent RNA polymerase (RdRp) after phosphorylation, leading to the discontinuation of RNA chain synthesis in viruses, thereby playing an antiviral role (Henan Genuine Biotech, 2022). On 21 July 2021, Azvudine (Shuangxinaike®, Henan Genuine Biotech Co., Ltd., Pingdingshan, Henan Province, China) was approved by the National Medical Products Administration (NMPA, Beijing, China) for the treatment of adult HIV-1-infected patients

(National Medical Products Administration, 2021). In addition, Azvudine is the first dual-target nucleoside oral agent worldwide to simultaneously target both reverse transcriptase and viral infectivity factors for the treatment of HIV infection. As a Chinese homegrown innovative drug for the treatment of acquired immunodeficiency syndrome (AIDS), Azvudine was developed with funding from the National Science and Technology Major Projects for Major New Drug Innovation (Genuine Biotech, 2021). Thereafter, Azvudine was incorporated into the Chinese guidelines for the diagnosis and treatment of AIDS (Acquired Immunodeficiency Syndrome and Hepatitis C Professional Group, Society of Infectious Diseases, Chinese Medical Association and Chinese Center for Disease Control and Prevention, 2021).

The ChemSrc database (https://www.chemsrc.com/en) was used to inquire about the chemical structure of Azvudine. The molecular formula of Azvudine is $C_9H_{11}FN_6O_4$ with a molecular weight of 286.22, and the chemical name is 4-amino-1-[(2R,3S,4R,5R)-5-azido-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydro-2-furanyl]-2(1H)-pyrimidinone (Supplementary Figure S1).

RdRp is highly conserved in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is an excellent target for the development of anti-SARS-CoV-2 agents (Rabie, 2021a; Rabie, 2021b; Rabie, 2021c; Vicenti et al., 2021; Rabie et al., 2023; Zhu, 2023a). Nucleoside analogs were considered candidate drugs for the treatment of coronavirus disease 2019 (COVID-19), and several nucleoside analogs were identified as promising anti-SARS-CoV-2 drugs based on satisfactory results of preclinical trials (Rabie and Abdalla, 2022; Eltayb et al., 2023; Rabie and Abdalla, 2023). Nucleoside analogs targeting RdRp are ideal anti-SARS-CoV-2 candidate drugs in consideration of the broad conservation of RdRp in all SARS-CoV-2 variants (Rabie, 2022a; Rabie, 2022b; Rabie and Abdalla, 2023; Rabie and Eltayb, 2023). Azvudine, as a nucleoside analog targeting highly conserved RdRp, had potential anti-SARS-CoV-2 effects and was selected as a possible effective candidate drug for the treatment of COVID-19 (Yu and Chang, 2020). A preclinical trial revealed that Azvudine was transformed into active Azvudine triphosphate concentrated in the thymus after phosphorylation, inhibited SARS-CoV-2 replication, and improved immunity. The subsequent pilot clinical study of Azvudine exhibited its excellent efficacy and satisfactory safety in the treatment of COVID-19 (Zhang et al., 2021). In addition, Ren et al. (2020) performed a preliminary clinical trial of Azvudine with a randomized, open-label, controlled design. A total of 20 patients with mild-to-moderate COVID-19 were randomly assigned to receive Azvudine or standard antiviral treatment with a ratio of 1:1. The results revealed that Azvudine shortens the time of nucleic acid-negative conversion (NANC) compared to standard antiviral treatment. Moreover, Azvudine displayed satisfactory safety without adverse events occurring during the treatment.

However, the two pilot clinical studies of Azvudine are short of a large sample size. Thereafter, four phase III clinical trials were performed during 2020–2022 to evaluate the efficacy and safety of Azvudine in the treatment of COVID-19. Here, the therapeutic effects of Azvudine on COVID-19 were comprehensively analyzed using the clinical data from four phase III clinical trials.

2 Four phase III clinical trials

2.1 A phase III clinical trial in China

The four phase III clinical trials were carried out in China, Russia, and Brazil. In China, the phase III clinical trial was registered in the Chinese Clinical Trial Registry (ChiCTR2000032769; registration date: 9 May 2020; website: https://www.chictr.org.cn/showproj.aspx?proj=53368), and the clinical trial was simultaneously registered in the International Clinical Trials Registry Platform (NCT04425772; registration date: 9 May 2020; website: https://trialsearch.who.int/Trial2.aspx?TrialID=NCT04425772). The randomized, double-blinded, parallel-controlled clinical trial was carried out in Beijing Ditan Hospital affiliated to Capital Medical University (Beijing, China) from June 2020 to March 2022. The sponsor was Henan Genuine Biotech Co., Ltd. A total of 348 patients with COVID-19 were enrolled in the study, and the patients were randomly assigned to the Azvudine group or control group with a 1:1 ratio. The patients in the Azvudine group received oral Azvudine tablets (5 mg once daily) plus standard treatment, and the patients in the control group received Azvudine dummy tablets (placebo) and standard treatment. The duration of treatment in both groups was less than 14 days. The primary efficacy outcome was the change (reduction) from baseline in viral load on days 7 and 14. The secondary primary efficacy outcomes included the time of NANC, nucleic acid conversion rate, the proportion of patients changing from mild-or-moderate COVID-19 to severe COVID-19 in the severity of the disease, the proportion of patients changing from severe COVID-19 to critical COVID-19 in severity, time and proportion of improvement in pulmonary imaging, time and rate of improvement in respiratory symptoms and other symptoms, frequency of requirement for supplemental oxygen or non-invasive ventilation, changes in blood oxygen detection index, and time and proportion of temperature return to normal levels. As a result, among the patients with viral load ≥ 3 $\log_{10}$ copies/mL, the reduction from baseline in viral load on days 3, 5, and 7 in the Azvudine group was higher than that in the placebo group. However, only the difference in the reduction from baseline in viral load on day 5 between the Azvudine group and placebo group was statistically significant. This result was similar to that in the patients with viral load ≥ 4 $\log_{10}$ copies/mL. Unfortunately, there were no significant differences in secondary primary efficacy outcomes between the Azvudine and placebo groups. In addition, 341 subjects were involved in the safety analysis. A total of 62 subjects experienced 119 adverse events (AEs) in the Azvudine group, while 76 subjects experienced 175 AEs in the placebo group. Most of the AEs were evaluated as grade 1 or grade 2 according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 of the National Cancer Institute. One patient experienced an AE that was evaluated as grade 3 in the Azvudine group, while three patients experienced three AEs that were evaluated as grade 3 in the placebo group. No AEs were evaluated as grade 4, or no serious AEs occurred. There was no significant difference in the incidence or severity of AEs between the Azvudine and placebo groups (ClinicalTrials, 2022; Zhang, 2022).

TABLE 1 Median time to improvement in clinical conditions and the proportion of improvement in clinical conditions on day 7 after the administration of Azvudine and placebo.

Parameters	Full analysis set		Per protocol set	
	Azvudine	Placebo	Azvudine	Placebo
N (missing)	157 (0)	157 (0)	141 (0)	138 (0)
Endpoint (%)	138 (87.90%)	136 (86.62%)	137 (97.16%)	136 (98.55%)
Censoring (%)	19 (12.10%)	21 (13.38%)	4 (2.84%)	2 (1.45%)
Improvement	57 (36.31%)	15 (9.55%)	57 (40.43%)	15 (10.87%)
No improvement	100 (63.69%)	142 (90.45%)	84 (59.57%)	123 (89.13%)
p-value	<0.001		<0.001	
Median time	10.00	13.00	10.00	13.00
p-value	<0.001		<0.001	

[a]The clinical data were obtained from the package insert of Azvudine tablets manufactured by Henan Sincere Biotechnology Co., Ltd.
[b]The missing values in the primary efficacy outcome were imputed using the worst value method.
[c]Clinical conditions were determined according to the WHO Ordinary Clinical Progression Scale (Jun/2020), score of 4 to 10. The data were acquired according to the WHO score between day 1 and day 31.
[d]The time to improvement in clinical conditions was calculated in terms of the first time improvement in clinical conditions emerged, which was determined by a decrease of ≥ 2 in the WHO score. Patients without any improvement in clinical condition by the end of the study were denoted as censoring patients. The last time that the WHO score was determined was used to calculate the censoring time.

2.2 A phase III clinical trial in Russia

In Russia, the phase III clinical trial was approved by the Russian Ministry of Health (http://www.minzdravsoc.ru) in January 2021 and was initiated in June 2021. The results of the two phase III clinical trials performed in China and Russia were disclosed in detail by Fujie Zhang at the 17th National Infectious Diseases Conference, which was hosted by the Chinese Medical Association in Hefei (Anhui, China) during 11-13 August 2022 (Chinese Society of Infectious Diseases and Chinese Medical Association, 2022). Fujie Zhang, a chief physician at Beijing Ditan Hospital affiliated with Capital Medical University (Beijing, China), was also the principal investigator (PI) in the Chinese phase III clinical trial. In addition, the results of the phase III clinical trial performed in Russia were recorded in the package insert of Azvudine tablets, manufactured by Henan Genuine Biotech Co., Ltd., and presented in Chinese language (Henan Genuine Biotech, 2022). A total of 314 patients with moderate COVID-19 were enrolled as the full analysis set (FAS) in the study, and the patients were randomly assigned to the Azvudine group or control group with a 1:1 ratio. The primary efficacy outcome was the median time to improvement in clinical conditions and the proportion of improvement in clinical conditions on day 7. A total of 279 patients were eligible according to inclusion and exclusion criteria and were included in the per-protocol set (PPS), which consisted of 141 subjects in the Azvudine group and 138 subjects in the control group. The mean baseline age was 48 years, with 39% and 61% of subjects falling under age groups 18–45 years (inclusive of 18 years) and 45–65 years (inclusive of 45 years), respectively. Male and female subjects accounted for 43% and 57%, respectively. There was no significant difference in the demographic or clinical characteristics between the Azvudine and control groups. As shown in Table 1, Azvudine significantly elevated the proportion of improvement in clinical conditions on day 7 and reduced the median time to improvement in clinical conditions compared to placebo in both FAS and PPS (Henan Genuine Biotech, 2022). With regard to safety, 34 patients underwent 47 AEs in the Azvudine group, and 35 patients underwent 50 AEs in the placebo group. Most of the AEs were determined as grade 1 or grade 2 according to CTCAE version 4.03. Only one serious AE occurred in the placebo group, and no serious AE occurred in the Azvudine group. There was no statistically significant difference in the frequency or severity of AEs between the Azvudine and placebo groups (ClinicalTrials, 2022; Zhang, 2022).

2.3 Two phase III clinical trials in Brazil

In Brazil, two phase III clinical trials of Azvudine were registered on the International Clinical Trials Registry Platform (NCT05033145 and NCT04668235). One trial was performed on patients with mild COVID-19, and the other one was performed on patients with moderate COVID-19. In the phase III clinical trial performed on patients with mild COVID-19, the primary outcome was the proportion of patients hospitalized within 4 weeks after randomization. The secondary outcomes involved the negative conversion time of the SARS-CoV-2 viral load, the proportion of patients cured during treatment, the severity and duration of COVID-19 symptoms, and the duration of Azvudine treatment until the second negative conversion. Patients with mild COVID-19 were randomly assigned to receive Azvudine 10 mg once a night or a placebo. Additionally, all the patients received standard treatment. A total of 281 patients with mild COVID-19 were included in the analysis (da Silva et al., 2023). For the phase III clinical trial performed in patients with moderate COVID-19, the primary outcome was the proportion of patients with improved clinical status. The secondary outcomes included the proportion of

patients with a clinical outcome of cure after treatment, time to improvement in COVID-19 symptoms, hospital length of stay (LOS), and negative conversion time of the SARS-CoV-2 viral load. Patients with moderate COVID-19 were randomly assigned with a ratio of 1:1 to receive Azvudine 10 mg once a night plus standard treatment or placebo plus standard treatment. A total of 180 eligible patients with moderate COVID-19 were enrolled, of whom 172 completed the study (Cabral et al., 2022). The results of both phase III clinical trials revealed that Azvudine significantly reduced the NANC time and viral load of the patients compared to the placebo group (da Silva et al., 2023; Cabral et al., 2022).

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2.4 Significant contributions to the fight against the COVID-19 pandemic by the end of 2022

The State Council of the People's Republic of China (http://english.www.gov.cn), also known as the Chinese central government, announced 10 new measures for the COVID-19 response on 7 December 2022. Henceforth, the dynamic zero-COVID-19 policy was abrogated (Xinhua, 2022a; Zhu, 2023). Without the restrictions of quarantine measures, SARS-CoV-2 Omicron variants rapidly spread across the Chinese mainland and caused large-scale infections. To date, only two anti-SARS-CoV-2 agents were approved by the NMPA for the treatment of COVID-19, and another one was nirmatrelvir–ritonavir (Paxlovid®, Pfizer, New York, State of New York, USA). NMPA granted conditional approval for the imports of Paxlovid on 11 February 2022 (Xinhua, 2022b). However, the price of Paxlovid was too high relative to Azvudine in the Chinese market. The prices of Paxlovid and Azvudine in medical insurance were 2,300 Yuan per box and 270 Yuan per bottle, respectively. Therefore, the preferred antiviral drug was Azvudine instead of Paxlovid for Chinese people during the COVID-19 pandemic. In this situation, numerous domestic public hospitals introduced Azvudine to cope with the current SARS-CoV-2 Omicron wave. A total of 113 community hospitals admitted Azvudine as a prescription drug in the treatment of COVID-19 on 3 January 2023. Genuine Biotech, together with Shanghai Fosun Pharmaceutical (Group) Co., Ltd., donated 100 million Yuan's worth of Azvudine to the rural areas of Midwestern China, covering 180 counties, in several phases through the Shanghai Fosun Foundation on 9 January 2023 (Genuine Biotech, 2023a). In fact, patients with COVID-19 in rural and remote areas should be given more attention due to poor medical conditions and health outcomes (Zhu, 2023). Within 48 h of the announcement, the first batch of 6,000 bottles of Azvudine tablets was conveyed to 10 county-/village-level clinics in four provinces through human-powered transportation. As of 28 February 2023, the two pharmaceutical companies had donated nearly 300,000 bottles of Azvudine tablets to Chinese rural areas, covering 250 cities/counties in nine provinces and the Shanghai municipality (Genuine Biotech, 2023b). Thereafter, in the award ceremony for "Henan Socially Responsible Enterprise of 2022 and Entrepreneur with Outstanding Social Contribution," Genuine Biotech became the winner after several rounds of selection and was honored with the "2022 Annual Award of Henan Socially Responsible Enterprise" (Genuine Biotech, 2023c).

3 Discussion

In summary, Azvudine could decrease the time of NANC, viral load, and the median time for improvement in clinical conditions and increase the proportion of improvement in clinical conditions on day 7 in patients with moderate COVID-19. Additionally, Azvudine could reduce body temperature normalization time, fever duration, and hospital LOS. In safety analyses, Azvudine exhibited good safety and tolerance. In summary, Azvudine was effective and well tolerated in the treatment of moderate COVID-19.

However, there were some limitations in the four phase III trials. In the trial performed in China, there were no significant differences between the Azvudine and control groups for most of the clinical outcomes; only the difference in the reduction in viral load from baseline on day 5 between the Azvudine and placebo groups was statistically significant. Azvudine did not display effectiveness in the alleviation of respiratory symptoms and prevention of progression to severe or critical COVID-19. For the phase III clinical trial performed in Brazilian patients with mild COVID-19, there also were no significant differences between Azvudine and placebo for most of the clinical outcomes, especially the primary outcome, and the researchers attributed the failure to the self-limiting nature of mild COVID-19 (da Silva et al., 2023). In the phase III clinical trial performed on Brazilian patients with moderate COVID-19, Azvudine significantly improved the clinical status at the time of discharge and reduced body temperature normalization time, fever duration, and hospital LOS compared to placebo (Cabral et al., 2022). The two phase III clinical trials in Brazil suggested Azvudine exhibited good efficacy in patients with moderate COVID-19 instead of in patients with mild COVID-19. In the four phase III clinical trials, whether Azvudine could reduce COVID-19 mortality or prevent progression to severe/critical COVID-19 was unclear and remained a major concern. Moreover, the sample sizes in the four phase III clinical trials were relatively small as compared to other high-quality phase III clinical trials of anti-SARS-CoV-2 drugs (Hammond et al., 2022; Jayk Bernal et al., 2022; Cao et al., 2023). Nevertheless, the four phase III clinical trials have demonstrated that Azvudine effectively reduced the time of NANC and viral load in patients with COVID-19.

After entering a host cell, the genomic RNA of SARS-CoV-2 acts as a template and is translated to yield two polyproteins by utilizing the host cell protein synthesis system, followed by proteolytic cleavage with two cysteine proteases to form a replication–transcription complex mediating SARS-CoV-2 RNA synthesis, capping, and proofreading. New SARS-CoV-2 genomic RNA is translated to produce additional non-structural proteins, which are used for further RNA synthesis or new virion assembly. Then, SARS-CoV-2 genomic RNA is coated with nucleocapsid proteins to generate nucleocapsid structures. Lipid

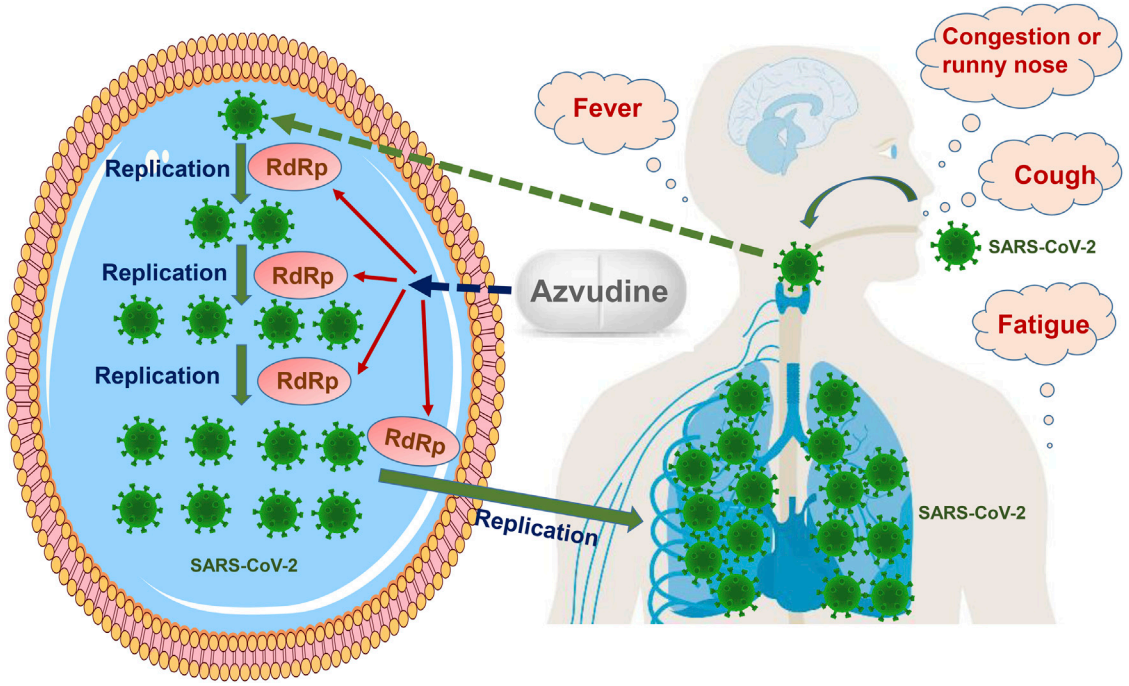

FIGURE 1
Therapeutic effect of Azvudine on COVID-19. Azvudine inhibits RNA-dependent RNA polymerase (RdRp), which is a vital enzyme mediating SARS-CoV-2 replication and transcription. Azvudine plays an anti-SARS-CoV-2 role by blocking virus replication.

bilayers consisting of viral spikes, membranes, and envelope proteins are synthesized in the endoplasmic reticulum–Golgi intermediate compartment. A mature progeny SARS-CoV-2 virion emerges after assembly and is released through exocytosis. Eventually, numerous SARS-CoV-2 virions are produced after several cycles of replication, leading to another round of infection (Malone et al., 2022; Yang and Rao, 2021; V'Kovski et al., 2021). The degree of infection is related to the innate antiviral response, and disease severity can promote the enhancement of the antibody response, which is associated with clinical outcomes. The entry of SARS-CoV-2 through the respiratory tract tends to cause the involvement of the upper and lower respiratory tracts, leading to a series of respiratory or systemic symptoms, such as cough, fever, and fatigue (Attaway et al., 2021; Gattinoni et al., 2021) (Figure 1).

RdRp is a vital enzyme that controls SARS-CoV-2 replication and transcription by catalyzing the phosphodiester binding process for RNA synthesis and is identified as a promising molecular target in COVID-19 (Tian et al., 2021; Ng et al., 2022). Azvudine, as an RdRp inhibitor, plays an anti-SARS-CoV-2 role by blocking virus replication (Figure 1). It should be noted that Azvudine has no role in the clearance of SARS-CoV-2, and it might be the fundamental reason that Azvudine did not exhibit effectiveness for several clinical outcomes in the four phase III clinical trials. On the other hand, in order to control viral load and prevent progression to severe COVID-19, Azvudine should be administered as soon as possible after the diagnosis of COVID-19 in accordance with the package insert of Azvudine tablets (Henan Genuine Biotech, 2022).

COVID-19 was added to the Azvudine list of indications in the package insert to resist the COVID-19 Omicron wave, and this application was conditionally approved by the NMPA on 25 July 2022 (National Medical Products Administration, 2022). On 9 August 2022, the National Health Commission and the National Administration of Traditional Chinese Medicine announced the inclusion of Azvudine in the Guidelines for the Diagnosis and Treatment of Coronavirus Disease 2019 (version ninth). The recommended oral dose of Azvudine for the treatment of moderate COVID-19 is 5 mg once daily, and the duration of Azvudine treatment should not exceed 14 days (National Health Commission of the People's Republic of China and National Administration of Traditional Chinese Medicine, 2022; None, 2022). Azvudine was incorporated into the Guidelines for the Diagnosis and Treatment of Coronavirus Disease 2019 (version 10th) issued by the National Health Commission and National Administration of Traditional Chinese Medicine on 6 January 2023 (General Office of National Health Commission of the People's Republic of China and General comprehensive affairs department of national administration of traditional Chinese medicine of the People's Republic of China, 2023). Thereafter, Azvudine was included in the Expert Consensus on Antiviral Therapy for COVID-19, which was compiled by 34 Chinese experts, and was highly approbated (Zhang and Li, 2023). On 18 January 2023, Azvudine was officially included in the National Reimbursement Drug List and was priced at 11.58 Yuan/tablet/3 mg in medical insurance (Genuine Biotech, 2023d).

4 Conclusion and therapeutic applications

In patients with moderate COVID-19, Azvudine could reduce NANC time, viral load, and time to improvement in clinical

conditions and exhibited good safety and tolerance. Azvudine is a promising anti-SARS-CoV-2 drug. However, randomized controlled trials with large sample sizes or large-scale real-world clinical studies are needed to evaluate the efficacy of Azvudine in preventing disease progression and reducing mortality in patients with COVID-19.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material; further inquiries can be directed to the corresponding author.

Author contributions

K-WZ is the sole author of this paper.

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This work is a pooled analysis of the results of four phase III clinical trials, and only introduces the most important results of the four phase III clinical trials. The author thanks all the researchers for conducting the four phase III clinical trials and for disclosing and sharing the results with the public.

Conflict of interest

Author K-WZ was employed by Guangzhou Baiyunshan Pharmaceutical Holding Co., Ltd. Baiyunshan Pharmaceutical General Factory.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1228548/full#supplementary-material

References

Acquired Immunodeficiency Syndrome and Hepatitis C Professional Group, Society of Infectious Diseases, Chinese Medical Association, Chinese Center for Disease Control and Prevention (2021). Chinese guidelines for diagnosis and treatment of human immunodeficiency virus infection/acquired immunodeficiency syndrome (2021 edition). *Med. J. Peking Union Med. Coll. Hosp. [CNKI]* 13 (2), 203–226. doi:10.12290/xhyxzz.2022-0097

Attaway, A. H., Scheraga, R. G., Bhimraj, A., Biehl, M., and Hatipoglu, U. (2021). Severe covid-19 pneumonia: pathogenesis and clinical management. *BMJ* 372, n436. doi:10.1136/bmj.n436

Cabral, P., de Souza, S., Silva, Rd, Arruda, R., Cabral, S., de Assis, A., et al. *Serial viral load analysis by ddpcr to evaluate fnc efficacy and safety in the treatment of moderate cases of covid-19, 21 november 2022, PREPRINT*. (Version 1) available at Research Square. doi:10.21203/rs.3.rs-2273657/v1

Cao, Z., Gao, W., Bao, H., Feng, H., Mei, S., Chen, P., et al. (2023). VV116 versus nirmatrelvir-ritonavir for oral treatment of covid-19. *N. Engl. J. Med.* 388 (5), 406–417. doi:10.1056/NEJMoa2208822

Chinese Society of Infectious Diseases, Chinese Medical Association (2022). *The 17th national congress of Chinese society of infectious diseases and the 3rd international forum on infectious diseases and clinical microbiology*. Available at: https://csi2022.sciconf.cn/cn/web/index/13482 (accessed June 9, 2022).

ClinicalTrials (2022). *Clinical data and adverse reactions of the oral drug Azvudine for COVID-19*. Available at: https://baijiahao.baidu.com/s?id=1753640204148539705&wfr=spider&for=pc (accessed December 30, 2022).

da Silva, R. M., Gebe Abreu Cabral, P., de Souza, S. B., Arruda, R. F., Cabral, S. P. F., de Assis, A., et al. (2023). Serial viral load analysis by DDPCR to evaluate FNC efficacy and safety in the treatment of mild cases of COVID-19. *Front. Med. (Lausanne)* 10, 1143485. doi:10.3389/fmed.2023.1143485

Eltayb, W. A., Abdalla, M., and Rabie, A. M. (2023). Novel investigational anti-SARS-CoV-2 agent ensitrelvir "S-217622": a very promising potential universal broad-spectrum antiviral at the therapeutic frontline of coronavirus species. *ACS Omega* 8 (6), 5234–5246. doi:10.1021/acsomega.2c03881

Gattinoni, L., Gattarello, S., Steinberg, I., Busana, M., Palermo, P., Lazzari, S., et al. (2021). COVID-19 pneumonia: pathophysiology and management. *Eur. Respir. Rev.* 30 (162), 210138. doi:10.1183/16000617.0138-2021

General Office of National Health Commission of the People's Republic of China, General comprehensive affairs department of national administration of traditional Chinese medicine of the People's Republic of China (2023). Guidelines for the Diagnosis and Treatment of Coronavirus Disease 2019 (trial version tenth). *China Med. [CNKI]* 18 (2), 161–166. doi:10.3760/j.issn.1673-4777.2023.02.001

Genuine Biotech (2021). *Genuine Biotech raises $100 million in series B financing, accelerating commercialization of world's first dual-target anti-HIV drug*. Available at: http://en.genuine-bio.com/news2021/275.html (accessed August 27, 202).

Genuine Biotech (2023a). *Genuine Biotech and Fosun pharma donated 100 million yuan's worth of oral drug for COVID-19, azvudine, to rural areas*. Available at: http://en.genuine-bio.com/2023a/359.html (accessed January 9, 2023).

Genuine Biotech (2023b). *Genuine Biotech and Fosun pharma donated nearly 300,000 bottles of azvudine tablets to China's rural areas*. Available at: http://en.genuine-bio.com/2023a/368.html (accessed March 10, 2023).

Genuine Biotech (2023c). *Good news! Genuine Biotech won the 2022 annual award of henan socially responsible Enterprise*. Available at: http://en.genuine-bio.com/2023a/371.html (accessed June 2, 2023).

Genuine Biotech (2023d). *Good news, azvudine tablets is officially included in the national reimbursement drug list*. Available at: http://en.genuine-bio.com/2023a/362.html (accessed January 18, 2023).

Hammond, J., Leister-Tebbe, H., Gardner, A., Abreu, P., Bao, W., Wisemandle, W., et al. (2022). Oral nirmatrelvir for high-risk, nonhospitalized adults with covid-19. *N. Engl. J. Med.* 386 (15), 1397–1408. doi:10.1056/NEJMoa2118542

Henan Genuine Biotech (2022). *Package insert of Azifudine tablets*. Pingdingshan, China: Henan Genuine Biotech Co.Ltd.

Jayk Bernal, A., Gomes da Silva, M. M., Musungaie, D. B., Kovalchuk, E., Gonzalez, A., Delos Reyes, V., et al. (2022). Molnupiravir for oral treatment of covid-19 in nonhospitalized patients. *N. Engl. J. Med.* 386 (6), 509–520. doi:10.1056/NEJMoa2116044

Malone, B., Urakova, N., Snijder, E. J., and Campbell, E. A. (2022). Structures and functions of coronavirus replication-transcription complexes and their relevance for SARS-CoV-2 drug design. *Nat. Rev. Mol. Cell. Biol.* 23 (1), 21–39. doi:10.1038/s41580-021-00432-z

National Health Commission of the People's Republic of China, National Administration of Traditional Chinese Medicine (2022). Guidelines for the diagnosis and treatment of coronavirus disease 2019 (trial version ninth). *Chin. J. Viral Dis. [CNKI]* 12 (3), 161–169. doi:10.16505/j.2095-0136.2022.0023

National Medical Products Administration (2021). *Azvudine was conditionally approved by the national medical products administration*. Available at: https://www.nmpa.gov.cn/yaowen/ypjgyw/20210721142223181.html (accessed July 21, 2021).

National Medical Products Administration (2022). *The application that coronavirus disease 2019 (COVID-19) was added into the indications of Azvudine was conditionally*

approved by the National Medical Products Administration. Available at: https://www.nmpa.gov.cn/yaowen/ypjgyw/20220725165620176.html (accessed July 25, 2022).

Ng, T. I., Correia, I., Seagal, J., DeGoey, D. A., Schrimpf, M. R., Hardee, D. J., et al. (2022). Antiviral drug discovery for the treatment of COVID-19 infections. *Viruses* 14 (5), 961. doi:10.3390/v14050961

None (2022). The national health commission includes azvudine into the guidelines for the diagnosis and treatment of coronavirus disease 2019 (version ninth). *Chin. J. Viral Dis. [CNKI]* 12 (4), 283. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=ZRYX202204016&DbName=DKFX2022.

Rabie, A. M., and Abdalla, M. (2023). Evaluation of a series of nucleoside analogs as effective anticoronaviral-2 drugs against the Omicron-B.1.1.529/BA.2 subvariant: a repurposing research study. *Med. Chem. Res.* 32 (2), 326–341. doi:10.1007/s00044-022-02970-3

Rabie, A. M., and Abdalla, M. (2022). Forodesine and riboprine exhibit strong anti-SARS-CoV-2 repurposing potential: *in silico* and *in vitro* studies. *ACS Bio Med Chem Au* 2 (6), 565–585. doi:10.1021/acsbiomedchemau.2c00039

Rabie, A. M., Abdel-Dayem, M. A., and Abdalla, M. (2023). Promising experimental anti-SARS-CoV-2 agent "SLL-0197800": the prospective universal inhibitory properties against the coming versions of the coronavirus. *ACS Omega* 8. doi:10.1021/acsomega.2c08073

Rabie, A. M. (2022a). Efficacious preclinical repurposing of the nucleoside analogue didanosine against COVID-19 polymerase and exonuclease. *ACS Omega* 7 (25), 21385–21396. doi:10.1021/acsomega.1c07095

Rabie, A. M., and Eltayb, W. A. (2023). Potent dual polymerase/exonuclease inhibitory activities of antioxidant aminothiadiazoles against the COVID-19 Omicron virus: a promising in silico/*in vitro* repositioning research study. *Mol. Biotechnol.*, 1–20. doi:10.1007/s12033-022-00551-8

Rabie, A. M. (2022b). Potent inhibitory activities of the adenosine analogue cordycepin on SARS-CoV-2 replication. *ACS Omega* 7 (3), 2960–2969. doi:10.1021/acsomega.1c05998

Rabie, A. M. (2021b). Potent toxic effects of Taroxaz-104 on the replication of SARS-CoV-2 particles. *Chem. Biol. Interact.* 343, 109480. doi:10.1016/j.cbi.2021.109480

Rabie, A. M. (2021c). Teriflunomide: a possible effective drug for the comprehensive treatment of COVID-19. *Curr. Res. Pharmacol. Drug Discov.* 2, 100055. doi:10.1016/j.crphar.2021.100055

Rabie, A. M. (2021a). Two antioxidant 2,5-disubstituted-1,3,4-oxadiazoles (CoViTris2020 and ChloViD2020): successful repurposing against COVID-19 as the first potent multitarget anti-SARS-CoV-2 drugs. *New J. Chem.* 45 (2), 761–771. doi:10.1039/d0nj03708g

Ren, Z., Luo, H., Yu, Z., Song, J., Liang, L., Wang, L., et al. (2020). A randomized, open-label, controlled clinical trial of azvudine tablets in the treatment of mild and Common COVID-19, a pilot study. *Adv. Sci. (Weinh)* 7 (19), e2001435. doi:10.1002/advs.202001435

Tian, L., Qiang, T., Liang, C., Ren, X., Jia, M., Zhang, J., et al. (2021). RNA-dependent RNA polymerase (RdRp) inhibitors: the current landscape and repurposing for the COVID-19 pandemic. *Eur. J. Med. Chem.* 213, 113201. doi:10.1016/j.ejmech.2021.113201

V'Kovski, P., Kratzel, A., Steiner, S., Stalder, H., and Thiel, V. (2021). Coronavirus biology and replication: implications for SARS-CoV-2. *Nat. Rev. Microbiol.* 19 (3), 155–170. doi:10.1038/s41579-020-00468-6

Vicenti, I., Zazzi, M., and Saladini, F. (2021). SARS-CoV-2 RNA-dependent RNA polymerase as a therapeutic target for COVID-19. *Expert Opin. Ther. Pat.* 31 (4), 325–337. doi:10.1080/13543776.2021.1880568

Xinhua (2022a). *China focus: COVID-19 response further optimized with 10 new measures*. Available at: https://english.news.cn/20221207/ca014c043bf24728b8dcbc0198565fdf/c.html (accessed December 7, 2022).

Xinhua (2022b). *China grants conditional approval for Pfizer's oral COVID-19 drug*. Available at: http://english.nmpa.gov.cn/2022-02/14/c_707085.htm (accessed February 14, 2022).

Yang, H., and Rao, Z. (2021). Structural biology of SARS-CoV-2 and implications for therapeutic development. *Nat. Rev. Microbiol.* 19 (11), 685–700. doi:10.1038/s41579-021-00630-8

Yu, B., and Chang, J. (2020). Azvudine (FNC): a promising clinical candidate for COVID-19 treatment. *Signal Transduct. Target Ther.* 5 (1), 236. doi:10.1038/s41392-020-00351-z

F. Zhang (Editor) (2022). "Clinical study of Azfudine against COVID-19," *The 17th national congress of Chinese society of infectious diseases and the 3rd international forum on infectious diseases and clinical microbiology* (Anhui, China: Hefei).

Zhang, F., and Li, X. (2023). Interpretation of Expert Consensus on Antiviral Therapy of COVID-19. *Chin. J. Clin. Infect. Dis.* 16 (1), 21–25. doi:10.3760/cma.j.issn.1674-2397.2023.01.003

Zhang, J. L., Li, Y. H., Wang, L. L., Liu, H. Q., Lu, S. Y., Liu, Y., et al. (2021). Azvudine is a thymus-homing anti-SARS-CoV-2 drug effective in treating COVID-19 patients. *Signal Transduct. Target Ther.* 6 (1), 414. doi:10.1038/s41392-021-00835-6

Zhu, K. W. (2023). Why is it necessary to improve COVID-19 vaccination coverage in older people? How to improve the vaccination coverage? *Hum. Vaccin Immunother.* 19 (2), 2229704. doi:10.1080/21645515.2023.2229704

Zhu, K-.W. (2023a). Deuremidevir and Simnotrelvir–Ritonavir for the Treatment of COVID-19 *ACS Pharmacol. Transl. Sci*. doi:10.1021/acsptsci.3c00134

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Treatment of non-tuberculosis mycobacteria skin infections

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Non-tuberculosis mycobacteria (NTM) skin infections have become increasingly prevalent in recent years, presenting a unique challenge in clinical management. This review explored the complexities of NTM infections localized to the superficial tissues and provided valuable insights into the optimal therapeutic strategies. The antibiotic selection should base on NTM species and their susceptibility profiles. It is recommended to adopt a comprehensive approach that considers the unique characteristics of superficial tissues to improve treatment effectiveness and reduce the incidence of adverse reactions, infection recurrence, and treatment failure. Infection control measures, patient education, and close monitoring should complement the treatment strategies to achieve favorable outcomes in managing NTM skin infections. Further efforts are warranted to elucidate factors and mechanisms contributing to treatment resistance and relapse. Future research should focus on exploring novel treatment options, innovative drug development/delivery platforms, and precise methodologies for determining therapeutic duration. Longitudinal studies are also needed to assess the long-term safety profiles of the integrated approaches.

KEYWORDS

non-tuberculosis mycobacteria (NTMs), skin diseases, infectious, anti-bacterial, mycobacterium infections, nontuberculous, atypical mycobacteria

1 Introduction

Non-tuberculosis mycobacteria (NTM) (Sharma and Upadhyay, 2020; Pavlik et al., 2022), mycobacteria other than *Mycobacterium tuberculosis* and *Mycobacterium leprae*, have emerged as a significant source of infections (Kumar et al., 2021; Nogueira et al., 2021). Among the diverse manifestations of NTM infections, skin and soft tissue involvements are prevalent clinical presentations. Although not posing an immediate life-threatening risk, these infections can result in significant morbidity and adversely affect the quality of life for affected individuals. Notably, there has been a global increase in reported cases of superficial NTM infections (Mei et al., 2019; Philips et al., 2019; Gopalaswamy et al., 2020) with contributing factors including the expanding population of immunocompromised individuals (Blakney et al., 2022; Toth et al., 2022) who face heightened susceptibility during injury or cosmetic procedures (Ahmed et al., 2020; Wang C. J. et al., 2022; Ni et al., 2023). Furthermore, the rise in NTM infections can be attributed, in part, to the ongoing adaptation of NTM to the human host. NTM's remarkable capacity to thrive within the diverse skin environment, while effectively evading immune responses, also plays a role in the escalating incidence of these infections (Luo et al., 2021).

In light of these observations, understanding and effectively managing NTM skin infections are crucial for public health and patient wellbeing. Regarding treatment choices for NTM infections, various factors come into play due to the unique nature of

these infections. Generally, the choice of treatment for NTM infections depends on the specific species and susceptibility pattern of the isolated organisms, as well as the severity and extent of the infection (Pennington et al., 2021). Current treatment strategies often reference the guidelines (Daley et al., 2020) of the Infectious Disease Society of America (IDSA) and American Thoracic Society (ATS) on pulmonary NTM-infected diseases. However, it should be noted that the ATS/IDSA guidelines for pulmonary diseases may not be directly applied to all skin-involved cases due to the unique characteristics of cutaneous NTM infections. The invasive or disseminated NTM infections may require a greater variety of drugs and a more extended treatment duration (Liu et al., 2023). When it comes to superficial involved cases, consideration must be given to the potential variations in pathogen types resulting from diverse infection pathways. The unique physiological structures and functions of the skin (Gravitz, 2018), compared to other anatomical sites, may also influence drug absorption and distribution, warranting tailored treatment approaches. In addition, the influence of local microbiota (Grice and Segre, 2011) and differences in host immune responses (Nguyen and Soulika, 2019) should not be underestimated, as they significantly impact treatment outcomes. Surgical interventions, phototherapy (Yang et al., 2022), and heat application (Lee and Lee, 2017) should also be considered as viable alternative treatment options due to anatomical variations in the infected sites. Furthermore, the emergence of newly invented antimycobacterial agents, such as MmpL3 inhibitors and Efflux Pump inhibitors (Rindi, 2020; North et al., 2023), are believed to have potent against slow-growing mycobacteria (SGM) and rapid-growing mycobacteria (RGM), also highlights the need for timely revision of treatment approaches. Finally, although NTM and *M. tuberculosis* shares similar physiological characteristics, virulence factors, and genetic drug targets (Rifat et al., 2021; Mei et al., 2023), it is still not advisable to fully copy the treatment regimens of TB. Many drugs being developed for treating TB do not exhibit any antimicrobial activity against NTM (Saxena et al., 2021). In summary, those factors highlight the need for updating of targeted treatment approaches to enhance skin-involved patient outcomes.

The management of cutaneous infections caused by various NTM subtypes poses significant challenges, necessitating a careful balance between therapeutic benefits and potential risks. The optimization of diverse treatment approaches, as well as the mitigation of adverse effects and infection recurrence, remain critical objectives. Through this comprehensive review, our aim is to provide an in-depth analysis of the treatment strategies for NTM skin infections and shed light on the complexities involved in addressing these clinical aspects.

2 Main text

2.1 Slow-growing mycobacteria (SGM)

SGM (>7 days for mature colony formation in solid media) mainly includes *Mycobacterium* marinum (*M. marinum*), *M. kansasii*, *Mycobacterium avium complex* (MAC), and many others (Yan et al., 2023). Common risk factors for SGM skin infections include exposure to contaminated water sources, such as swimming in contaminated water bodies or handling fish tanks, and skin injuries like cuts or scrapes that serve as entry points for the bacteria. Clinical presentations of SGM infections typically involve the development of nodules or raised skin lesions at the site of entry, which gradually enlarge and may lead to non-healing wounds or abscess formation (Riccardi et al., 2022). Infected areas may become painful and swollen, and the infection can spread along lymphatic vessels in a linear fashion. The final goal of the targeted treatment of SGM infection is to facilitate rehabilitation, shorten the treatment course, and prevent the pathogen from further progressing to deeper tissues to avoid multiple distributions.

2.1.1 *Mycobacterium* marinum

M. marinum is the predominant pathogen responsible for SGM, often leading to skin and soft tissue infections (Hashish et al., 2018). Early diagnosis and prompt treatment of *M. marinum* infections pose significant challenges, especially during the atypical stage, potentially complicating the subsequent course of medication (Trčko et al., 2021). Presently, there is a lack of standardized norms concerning the selection, dosages, and treatment duration of drugs, as well as the consideration of surgery as an adjunct to treatment options (Seidel et al., 2022). According to the recommendations of IDSA/ATS guidelines, treatment typically involves using a combination of two active drugs, such as ethambutol-macrolide combinations, and continuing therapy until 1–2 months after symptom resolution. However, it is essential to acknowledge that no randomized controlled trials have been conducted in this domain, and the available data are insufficient to establish statistically significant evidence on drug efficacy and tolerability. The scarcity of verified data necessitates further research to comprehensively evaluate the effectiveness and safety of different treatment regimens for *M. marinum* infections.

2.1.1.1 Susceptibility test and drug resistance characteristics of *M. marinum*

The *in vitro* drug sensitivity test (Hendrikx et al., 2022; Seidel et al., 2022) shows that *M. marinum* is moderately sensitive to streptomycin and resistant to azithromycin, isoniazid, and pyrazinamide. The minimum inhibitory concentrations (MICs) of levofloxacin, ciprofloxacin, and quinolones about *M. marinum* are high while keeping lower for rifampin, moxifloxacin, ethambutol, clarithromycin, linezolid, and tetracyclines. The results (Koushk-Jalali et al., 2019; Yeo et al., 2019; Castillo et al., 2020; Strobel et al., 2022) show that rifampicin, clarithromycin, sulfonamides, doxycycline, minocycline, and ethambutol are more suitable choices. Hence, given *M. marinum*'s susceptibility to numerous antibiotics, empirical treatment can be initiated at first, especially when susceptibility tests are unavailable (Oh et al., 2018). However, a test is needed when the condition is not improved after adequate treatment, or the mycobacterial culture is still positive after several months.

2.1.1.2 Alone or combined use of antibiotics?

Several studies (Rallis et al., 2012; Chung et al., 2018; Franco-Paredes et al., 2018) indicated that oral monotherapy (single antibiotics such as clarithromycin, trimethoprim, and ciprofloxacin) is effective in immunocompetent patients only

with superficial cutaneous *M. marinum* infections in the early stage, which also recommends that a suitable course of treatment need to last up 3–6 months, or the focus is limited and then proceed for 1–2 months (Aubry et al., 2017). Combined use of various active antimycobacterial agents is recommended under the involvement of deeper tissues, disseminated extracutaneous infection, and immunosuppressive status of hosts (Holden et al., 2018). Combinations, such as clarithromycin combined with rifampicin, clarithromycin combined with ethambutol, ethambutol combined with rifampicin, or three, are preferred (Strobel et al., 2022). The duration of therapy depends on the infection's severity and treatment effects and could be extended moderately according to the host-drug interactions.

2.1.1.3 Other treatments?

Surgeries (incision and drainage) are needed when the *M. marinum* goes deeper or poor curative effect for a long time. This may involve drainage of abscesses, removal of infected tissue, or excision of nodules or lesions that are unresponsive to antimicrobial therapy. Keeping the affected area clean and dry, avoiding activities that may traumatize the skin, and using appropriate dressings or bandages to protect the affected skin from further irritation or contamination are necessary. It is also recommended that amputation (Hendrikx et al., 2022) might be considered in case of severe cutaneous infections caused by multidrug-resistant isolates. Other than that, hot compress therapy (Strobel et al., 2022) might be a choice due to its high-temperature intolerance. (The optimal temperature is 30 Degrees Celsius).

2.1.2 *Mycobacterium* kansasii

Cutaneous infections caused by *M. kansasii* predominantly affect immunocompromised hosts, including individuals with conditions such as diabetes or those who have undergone renal transplantation (Zhang et al., 2017; Okuno et al., 2020). Often, these cutaneous infections are concomitant with pulmonary involvement. As a result, when managing *M. kansasii* infections in superficial tissues, it is reasonable to refer to the guidelines established for the treatment of pulmonary NTM infections. According to the official ATS/ERS/ESCMID/IDSA clinical practice guidelines, for patients with rifampin-susceptible *M. kansasii*, a treatment course lasting over 12 months, comprising rifampicin, ethambutol, and either isoniazid or a macrolide, is advised.

There are a few differences between the guidelines of ATS/IDSA and the consensus of the British Thoracic Society (BTS) (Haworth et al., 2017). Rifampin and ethambutol are the same, while the part combined with isoniazid or clarithromycin differs. Both regimes mentioned above need years of treatment duration. A recent study (Chapagain et al., 2020) indicated that the regime of BTS and a novel one (rifapentine + tedizolid + minocycline) show better efficacy on *M. kansasii* of pulmonary diseases. Another study (Moon et al., 2019) found that the macrolide-containing regimen is as effective as the isoniazid-containing regimen, which might reduce the cumulative side effects of long-term use of anti-tuberculosis drugs. Clofazimine only shows a modest to poor impact on *M. kansasii* in a clinic in an observational study (Srivastava and Gumbo, 2018) on the antimicrobial effect of clofazimine monotherapy in the intracellular-infection hollow fiber model of *M. kansasii*.

Although the result of *in vitro* susceptibility testing of *M. kansasii* correlates little with clinical outcomes generally, it is still something to be learned from this. According to the effects of susceptibility tests (Zhang et al., 2017; Bakuła et al., 2018; Wang et al., 2019), *M. kansasii* is susceptible to ethambutol, rifampin, ethambutol, clarithromycin, aminoglycosides, and fluoroquinolones, which might guide clinical work of complicated cases. *M. kansasii* is resistant to pyrazinamide, and its potential resistance mechanism is not necessarily related to gene mutation but to great genetic diversity globally (Guo et al., 2022).

2.1.3 *Mycobacterium avium* complex (MAC)

MAC (Daley, 2017), such as *M. avium*, *M. Intracellulare*, *M. Chimaera*, *M. indicuspranii*, is a group of SGM commonly identified in the respiratory system of patients with severely immunocompromised statuses (To et al., 2020). Given the circumstances, MAC infections often exhibit a propensity to disseminate from the initial infected site to involve other organs and tissues. Thus, the management of MAC infections affecting the skin and soft tissues warrants a comprehensive approach akin to treating invasive or disseminated cases (Chen et al., 2020; Crilly et al., 2020). A 3 year cross-sectional study (Akrami et al., 2023) found that MAC was susceptible to amikacin, moxifloxacin, and clarithromycin, while resistant to linezolid, rifampin, isoniazid, and clofazimine. In a cases report (Fukushi et al., 2022) of pulmonary and disseminated MAC patients confirmed by tissue-direct polymerase chain reaction-based nucleic acid lateral flow immunoassay, clinicians treated two patients with clarithromycin (CAM, 800 mg/day), rifampicin (RIP, 600 mg/day), and ethambutol (EB, 700 mg/day) for a year. No adverse side effects or recurrence were founded during the treatment. Omadacycline was tested as a potential treatment option for pulmonary MAC in hollow fibre system model, possibly as an alternative treatment for a new MAC regimen. The results of susceptibility testing in a retrospective study (Mok et al., 2019) of 88 isolates showed that *M. chimaera* is susceptible to clarithromycin, amikacin, rifabutin, and streptomycin while resistant to moxifloxacin and linezolid with a high probability, which might influence the overall therapeutic strategy. Several studies (Maurer et al., 2019; Li et al., 2022; Schulthess et al., 2023) on susceptibility testing found that *M. chimaera* and other members of the MAC generally have similar susceptibility (clarithromycin, amikacin, and rifabutin). In summary, for MAC infections that are susceptible to macrolides, a regimen of at least three drugs, including a macrolide and ethambutol, is preferred over a monotherapy of just a macrolide or ethambutol.

2.1.4 Other less common SGM

Less common organisms include *M. xenopi, M. malmoense, M. simiae, and M. szulgai*. Despite the close phylogenetic relationship among these organisms, they exhibit discrete epidemiological characteristics and pathogenic behaviors. Therefore, the management of these SGM demand a nuanced approach that meticulously considers the distinctive attributes of each individual species. According to the established consensus, the recommended treatment approach involves a combination of two to three types of antibiotics, administered for a duration of at least 12 months beyond the point of culture conversion (Yan et al., 2023).

For example, in the case of *M. xenopi* infections, a daily regimen that consists of at least three drugs: rifampicin, ethambutol, and either a macrolide or a fluoroquinolone (e.g., moxifloxacin) is recommended according to the official ATS/ERS/ESCMID/IDSA clinical practice guidelines. Nevertheless, the consensus regarding treatment recommendations (such as azithromycin, clarithromycin, rifampicin, ethambutol, amikacin, and moxifloxacin) for less common NTM species is largely based on low-quality evidence derived from published scientific literature.

2.2 Rapid-growing bacteria (RGM)

Similar to SGM infections, direct inoculation of RGM can occur through various routines, including trauma, surgical procedures, injections, tattoos, and other operations that involve the disruption of the skin barrier. In such instances, RGM can potentially spread to deeper tissues and cause infections beyond the initial site of entry. Clinical presentations of RGM skin infections often involve the development of nodules, abscesses, or ulcers at the site of entry. These skin lesions can be painful, red, and may contain pus. In immunocompromised individuals or those with underlying medical conditions, RGM skin infections can be more severe.

Treatment of RGM infections includes various regimens with different response rates (Kasperbauer and De Groote, 2015). The selection of antibiotics is mainly based on the results of drug susceptibility tests. According to the results (Chang and Whipps, 2015; Dumic and Lutwick, 2021), RGM is more sensitive to tigecycline, tobramycin, clarithromycin, and amikacin. However, the susceptibility profile varies from species to species. Drug resistance (Forbes et al., 2018; Shrivastava et al., 2020) still poses a significant challenge to a successful outcome due to the presence of the erm41 gene, which could lead to inducible resistance to macrolide, prolong the therapy, and increase the incidence of drug-induced toxicity. Below are details of the most common RGM species.

2.2.1 *Mycobacterium* fortuitum complex

Mycobacterium fortuitum complex consists of *M. peregrinum*, *M. porcinum*, *M. fortuitum* and many others. Combined antibiotics treatment is often required, and surgical therapy may be needed optionally (Philips et al., 2019). After reviewing several *in vitro* antimicrobial susceptibility research (Forbes et al., 2018; Yeo et al., 2019; Da et al., 2020; Kumar et al., 2021; Das et al., 2022), we found that *M. fortuitum* strains were susceptible to many antibiotics. The isolates are susceptible to amikacin (intermediate to highly sensitive), ciprofloxacin (highly susceptible), doxycycline (intermediate susceptible), clofazimine, trimethoprim-sulfamethoxazole (TMP-SMX) and linezolid, resistance to all the antituberculosis agents, while different to macrolides (decreased sensitivity due to inducible susceptibility) and imipenem. A study (Chew et al., 2021) of 86 isolates showed that *M. fortuitum* is resistant to clarithromycin and tobramycin but susceptible to tetracyclines and quinolones. Similarly, a retrospective case series (Wang J. et al., 2022) of 18 patients with cutaneous *M. fortuitum* complex infections found that five uncomplicated infection cases showed an excellent response to the treatments. One patient received monotherapy of doxycycline for 8 weeks with no recurrence; the other four patients were treated with combined antibiotics, clarithromycin-minocycline, clarithromycin-ciprofloxacin, clarithromycin-TMP-SMX, and ciprofloxacin-TMP/SMX. Treatment courses range from 10 weeks to 40 weeks. While only three complicated infection patients with a prolonged period of the same therapy showed satisfactory clinical consequences, which meant immunosuppressed hosts were at higher risk of having persistent SGM infection than the immunocompetent population. Moreover, the findings from an *in vitro* and *in vivo* experiments (Ahmad et al., 2022) have demonstrated that gepotidacin, a first-in-class triazaacenapthylene topoisomerase inhibitor, exhibits a promising and potentially novel mechanism of action, allowing it to evade prevailing resistance mechanisms. These results underscore the potential of gepotidacin as a valuable therapeutic candidate with the ability to overcome resistance challenges commonly encountered with existing antimicrobial agents.

2.2.2 Mycobacterium chelonae

The results of susceptibility testing (Franco-Paredes et al., 2018; Uslu et al., 2019; Watanabe et al., 2022) indicated that *M. chelonae* is often susceptible to macrolides, cefoxitin, fluoroquinolones, and tobramycin. The monotherapy (clarithromycin) can be sufficient for localized or superficial infections but not enough for patients who develop potential resistance. At least two antibiotic agents (oral macrolide combined with cefoxitin, amikacin, or imipenem) and 4–6 months of systemic treatment are recommended for these complicated cases. A biologics side-effects induced case (Frizzell et al., 2020) showed that omadacycline monotherapy at a dose of 300 mg orally daily for 4 months was efficient against *M. chelonae* skin and skin structure infections without recurrence in a 1-year follow-up. Surgical debridement, incising, draining, and source control are recommended in treatment if there is extensive involvement of extra-pulmonary *M. chelonae* infection (Dumic and Lutwick, 2021). Like *M. marinum*, thermal therapy was efficacious due to its thermal sensitivity. In addition, routine treatment (antimicrobial and surgical therapies) added a single bacteriophage (Little et al., 2022) showed stable disease improvement with no evidence of bacterial resistance to the phage. Bacteriophage therapy involves using viruses to infect and target specific bacteria, leading to the destruction and elimination of the bacterial population. Given the current challenges posed by antimicrobial resistance, bacteriophage therapy has emerged as a promising and attractive therapeutic option.

2.2.3 *Mycobacterium* abscessus group

The *M. abscessus* group (*M. abscessus*, *M. massiliense*, and *M. bolletii*) is the primary source of cutaneous involvement of RGM (Jeong et al., 2017; Franco-Paredes et al., 2018). M. abscessus has an irregular resistance pattern to numerous anti-NTM agents (Lee et al., 2015; To et al., 2020; Kumar et al., 2021). Compared to *M. massiliense*, some *M. abscessus* and *M. bolletii* isolates (not all) have inducible macrolide resistance due to the functional erm41 gene, which could lead to inadequate response to a macrolides-dominant therapeutic schedule. Hence, antimicrobial susceptibility testing on all clinically significant isolates is strongly recommended before starting the therapy. The susceptibility list should include at least amikacin, cefoxitin, imipenem, clarithromycin, linezolid, doxycycline, tigecycline, ciprofloxacin, and moxifloxacin. Per the

official ATS/ERS/ESCMID/IDSA clinical practice guidelines, for *M. abscessus* infections, whether the strains possess inducible or mutational macrolide resistance or not, it is recommended to initiate with a macrolide-inclusive multidrug regimen, which should encompass at least three drugs proven effective *in vitro*. An observational study (Da et al., 2020) showed that all strains of the *M. abscessus* group were susceptible to amikacin, linezolid, clofazimine, and tigecycline and suggested a prolonged drug resistance testing of 14 days to determine the presence of inducible resistance to macrolides is necessary. Monotherapy (clarithromycin) has shown promising efficacy in uncomplicated non-pulmonary disease, probably because its hand and foot lesions may represent a self-limited characteristic (Lee et al., 2015). However, invasive or disseminated *M. abscessus* and *M. bolletii* infections are complicated to treat; a combination of medication and a more comprehensive treatment course are necessary (Comba et al., 2021). Surgical resection of the infected tissues following chemotherapy to lessen the extensive progress might be a possible curative treatment for complex cases.

In the context of treating *M. abscessus* infection, the preclinical and clinical data derived from a study (Singh et al., 2023) suggest that the inclusion of omadacycline at a dosage of 300 mg per day in combination regimens holds promise for potential evaluation in Phase III trials involving patients with pulmonary involvement of *M. abscessus*. Such investigations could potentially bear significant guiding implications for addressing skin-related issues as well. Moreover, bacteriophages have also been explored as a potential therapeutic option. A study (Gorzynski et al., 2023) revealed that the lytic efficiency of phages is influenced by environmental factors, particularly when dealing with biofilm and intracellular states of *M. abscessus*. This observation has important implications, as it aids in the identification of therapeutic phages capable of reducing bacterial fitness by hindering antibiotic efflux function and attenuating the intrinsic resistance mechanisms of *M. abscessus* through targeted therapeutic interventions. Thiostrepton, a promising novel therapeutic drug candidate, has demonstrated substantial inhibition of *M. abscessus* growth in various contexts, including wild-type strains, subspecies, clinical isolates, and drug-resistant mutants, as evidenced by *in vitro* experiments and macrophage models. Additionally, it exhibited a dose-dependent reduction in proinflammatory cytokine production, suggesting its potential as an anti-inflammatory agent in the context of *M. abscessus* infection (Kim et al., 2019).

2.2.4 Other RGM

According to the *in vitro* antimicrobial drug susceptibility testing, A study (Cantillon et al., 2022) using an open drug discovery approach found that oxazolidinones such as linezolid and doxycycline have excellent tissue penetration properties and are actively potent against *M. chimaera*. Two case reports (Shimizu et al., 2012; Wang C. J. et al., 2022) recommend combined therapy with adequate debridement and sensitive antibiotic administration for soft tissues in patients infected with *M. smegmatis*.

3 Discussion

Since more and more extrapulmonary NTM-infected cases have been reported recently and no unified treatment proposal could be referred to, a safer, more effective, higher adherent, a broader spectrum of anti-NTM activities, and more cutaneous-specific treatment strategy is needed. Thus, we reviewed the treatment of NTM infections involving skin or soft tissues in recent years to give some suggestions on this topic.

NTM skin involvements exhibit distinctive therapeutic disparities compared to other NTM-infected manifestations, owing to the unique structural characteristics of the skin, variations in drug distribution patterns, diverse modes of infection, relatively confined lesion distribution, milder disease severity, and a greater array of treatment modalities available. The management of NTM infections frequently entails the administration of multiple antimicrobial agents over extended durations, requiring vigilant clinical and laboratory monitoring. Nevertheless, the dearth of well-structured controlled trials investigating first-line treatment regimens, including optimal drug selection, dosage, and duration, poses challenges in formulating evidence-based guidelines for effectively managing a wide array of NTM species and associated diseases. Consequently, regimen selection should generally be guided by drug susceptibility testing. This testing involves assessing the susceptibility of the NTM isolate to various antimicrobial drugs, allowing for informed decisions on the most appropriate therapeutic approach. According to the results of drug sensitivity tests, our recommended treatment choices were summarized in Table 1. The establishment of rigorous clinical trials will be instrumental in addressing these knowledge gaps and facilitating the development of more effective and targeted treatment strategies for NTM skin infections. Waiting for species identification and susceptibility before treatment is reasonable without any delay for most superficial cases. However, the correlation between clinical outcomes and *in vitro* susceptibility thresholds remains undefined for the majority of NTM species (van Ingen et al., 2012a; Timmins, 2020). Different NTM subspecies have other susceptibility profiles to antimicrobial agents. The susceptible antibiotics against SGM differ from that of RGM (Alffenaar et al., 2021; Sharma et al., 2021). Therefore, subspecies level identification (no higher than the species level) and sensitivity testing of NTM, especially RGM, is recommended. For example, the Clinical and Laboratory Standards Institute (CLSI) recommends (Schoutrop et al., 2018) clarithromycin and amikacin susceptibility testing only for MAC, clarithromycin, and rifampicin for *M. kansasii*, and clarithromycin for *M. abscessus* complex. In addition, susceptibility testing should be prolonged as long as 6 weeks for SGM and 2 weeks for macrolides (Dartois and Dick, 2022). Recent advancements in molecular diagnostic techniques have improved the accuracy and speed of identifying NTM species and their drug susceptibilities, allowing for more precise and targeted treatment. However, over time, the stability of some antimycobacterial drugs is gradually affected by the pathogen, leading to a variable minimum inhibitory concentration (MIC), which then affects the final interpretation of the DST result. This partly explains why drug susceptibility testing results do not necessarily translate to a positive clinical response. The local microenvironments (Dey et al., 2010; Dartois and Dick, 2022), which can decrease therapeutic concentrations of drugs at the anatomical sites, might be another reason.

In addition, there is no well-defined treatment endpoint for superficial NTM infections. In contrast to TB or NTM pulmonary

TABLE 1 The summary of preferred options for treating NTM skin infection.

Species		Recommended choices[a]	Unrecommended choices	Supplementary choices
Slow-growing mycobacteria (SGM)	*M. marinum*	Ethambutol, Azithromycin, Isoniazid, Pyrazinamide, Rifampicin, Clarithromycin, Sulfonamides, Doxycycline, Minocycline	Azithromycin, Isoniazid, Pyrazinamide, Levofloxacin, Ciprofloxacin, Quinolones	Surgery[b] Hot Compress Therapy
	M. kansasii	Rifampin, Ethambutol, Clarithromycin, Clarithromycin, Aminoglycosides, Fluoroquinolones, Moxifloxacin	Clofazimine, Pyrazinamide, Linezolid, Isoniazid	Surgery
	MAC	Clarithromycin, Ethambutol, Amikacin, Rifabutin, Streptomycin	Linezolid, Isoniazid. Clofazimine, Rifampicin Moxifloxacin Linezolid	Surgery
	Other SGM	Azithromycin, Ethambutol, Ethambutol, Rifabutin	Unavailable	Surgery
Rapid-growing mycobacteria (RGM)	*M. fortuitum* complex	Amikacin, Ciprofloxacin, Doxycycline, Clofazimine, Trimethoprim-Sulfamethoxazole, Linezolid, Tetracyclines, Quinolones, Gepotidacin, Minocycline	Clarithromycin, Tobramycin Macrolides, Imipenem	Surgery
	M. chelonae	Clarithromycin, Cefoxitin, Fluoroquinolones, Tobramycin, Omadacycline	Macrolide (Inducible Resistant)	Surgery Bacteriophage Therapy
	M. abscessus complex	Amikacin, Linezolid, Clofazimine, Tigecycline, Clarithromycin, Omadacycline, Thiostrepton	Macrolide (Inducible Resistant)	Surgery Bacteriophage Therapy
	Other RGM	Amikacin, Linezolid, Doxycycline, Moxifloxacin, Moxifloxacin, Ciprofloxacin	Unavailable	Surgery

[a]Monotherapy or combined therapy depends on the specific situation (NTM, species, infection sites, and disease severities).
[b]The surgery operations include excision, debridement, drainage, and amputation, etc.

diseases, where the treatment endpoint can be determined by sputum specimen culture conversion and imaging results, defining the endpoint of treatment for superficial NTM infections remains uncertain. Typically, a treatment duration of 2–4 months is recommended for skin and soft-tissue NTM infections, while NTM pulmonary diseases often require at least 12 months of therapy after sputum culture reversion. To improve treatment efficacy while minimizing the risk of drug resistance, long-term and multidrug therapy is often necessary for NTM infections. However, this approach may lead to challenges such as drug interactions, drug-related adverse reactions (AEs), and high medication costs, potentially compromising treatment efficacy and patient compliance. Another method for determining the endpoint of treatment involves obtaining post-treatment specimens for culture to assess treatment efficacy, but this invasive procedure carries a heightened risk of reinfection, particularly in individuals with compromised immune systems. Finding a consensus on a specific and effective treatment endpoint for superficial NTM infections is imperative and demands further research and clinical investigation to ensure optimal patient outcomes and successful management of these challenging infections (Haworth et al., 2017; Wi, 2019). Currently, the determination of the treatment endpoint for skin NTM infection primarily relies on the assessment of changes in the patient's clinical manifestations. These assessments typically involve evaluating the complete or substantial disappearance of preexisting skin lesions, the absence of new skin lesions, and the persistence of unchanged skin lesions after a specific duration of treatment. However, it is important to note that this criterion is subjective and lacks well-defined objective measures. To establish a more standardized and evidence-based approach for defining the treatment endpoint of skin NTM infection, further research and clinical investigations are necessary.

The low susceptibility of NTM to a wide range of antibiotics is attributed to their several characteristics. 1. Intrinsic resistance mechanisms: the first barrier is the unique metabolic condition [hydrophobicity of cell wall, and thereby low permeability (van Ingen et al., 2012a)] and the absence of porin or ABC transporter superfamily of the cell wall, which weakens the uptake and biotransformation of drugs and decreases the affinity with the drug target. 2. Inducible resistance mechanisms (Alffenaar et al., 2021): the second barrier is the genomic mutations of NTM, which could confer high-level resistance. Resistant strains are due to mutations at nucleotides. For instance, the changes of the 23S rRNA (functional erm genes) in *M. abscessus* isolates and of the RNA polymerase binding protein A (RbpA) in *M. smegmatis* are linked to the resistance to the macrolides and rifampicin (Dey et al., 2010). Comparative genomics and population genetics studies can provide insights into the genetic variability, evolution, and adaptation of NTM species. 3. Adaptative resistance mechanisms: he adaptability of NTM is the third barrier. NTM has extraordinary abilities in generation-upgrade time, and metabolic capabilities, which means they can adapt to stress before the cells are killed. They can form biofilms on the skin, which are complex microbial communities encased in an extracellular matrix. For example, one of the persistence strategies of NTM is hidden in biofilms (Slany et al., 2016), which generally leads to ten times less susceptibility to antibiotics than their counterparts. Understanding the mechanisms and dynamics of NTM biofilm formation on the

TABLE 2 Recommendations on treatments of NTM skin infections.

	Recommendations
1. The characteristics required for novel anti-NTM drugs	1). Are ideally active against a broader spectrum of NTM; 2). Are bactericidal ideally against growing, and various drug-tolerant persist pathogens; 3). Could penetrate the multilayered structure of granulomas; 4). Drug interactions should be as minimal as possible
2. Choose the right treatment choices for each patient	After careful interpretation of the drug sensitivity results and the characteristics of the different cases, the choice of using a single therapy, a combination of antibiotics, physical therapy, or multiple parallel approaches is made
3. Drug monitoring and sensitivity tests are necessary	Therapeutic drug monitoring and prolonged drug sensitivity tests are always necessary during treatment. Clinical and laboratory monitoring of patients is essential. Treatment should be also tailored to the NTM species and susceptibility profile
4. Develop/screen drugs with the help of new platforms and new ideas	Referring to formal pharmacokinetics/pharmacodynamics research (such as CRISPR/Cas9 system and nanotechnology) might lead to safer and shorter-duration regimens. Novel molecular diagnostic technology can offer more effective, targeted multidrug treatments at the species level
5. Prevention and skin care are essential	Prevention is equally essential during percutaneous invasive operations or trauma. Immunosuppressed hosts need to pay more attention to infection during antimicrobial therapy. Proper wound care is also an important aspect of NTM skin disease management
6. Patient education and condition explanation	Counseling patients about the characteristics of NTM infections, such as choices of treatment, length of treatment, and possible side effects, to moderate their expectations for an unrealistic solution. Patients should also be advised to promptly report any new symptoms or changes in the affected skin to their healthcare provider
7. Multidisciplinary Cooperation	Multidisciplinary approach involving dermatologists, infectious disease specialists, and surgeons are recommended. Collaboration among healthcare professionals is important in determining the appropriate treatment plan, monitoring treatment response, and addressing potential complications

skin is an active area of research, aiming to develop strategies to disrupt biofilms for improved treatment outcomes, including the use of biofilm-targeting agents and biofilm-disrupting techniques (such as enzymes, peptides, nanoparticles, and ultrasound). In addition, some studies (Huh et al., 2019) believe that NTM can enter a nonreplicating state and exhibit phenotypic drug resistance. However, up to now, the survey of resistance mechanisms associated with NTM still needs to be completed. Except for macrolides, the resistance mechanisms of many drugs still need to be clarified. It is essential to understand the basis for resistance and, more importantly, how to revise treatment choices to prevent the development of resistance.

For uncomplicated skin-involved cases, primary empirical treatments and antibiotic monotherapy could respond well in most patients. Single-drug or combined (clarithromycin, rifampin, and ethambutol) treatments depend on the specific characteristics of the hosts, location, and identification of species. When a poor response to treatment or rapid progression is found, *in vitro* susceptibility testing should be addressed throughout the treatment. Compared to antimicrobial agents' therapy alone, additional surgical operation of the localized infection with medication has proven to have better outcomes for extracutaneous involvement. Regular monitoring of the patient's clinical response to treatment, as well as laboratory testing to assess the effectiveness of antimicrobial therapy, is important in the management of NTM skin infections. Follow-up appointments with the treating physician should be scheduled as recommended to monitor progress and make any necessary adjustments to the treatment plan. Our recommendations for treating NTM skin infections and recommended procedures are summarized in Table 2 and Figure 1.

Emerging strategies are being explored to overcome drug resistance and improve treatment efficacy of complicated cases. 1. Screen existing drugs and new drugs: Novel antimicrobial drugs have shown promising activity against NTM species and may be considered in the treatment of NTM skin infections, particularly in cases where standard treatment regimens have failed or in the presence of drug-resistant strains. A study (Kaushik et al., 2019) showed that the new β-lactamase inhibitors relebactam and vaborbactam in combination with β-lactams have potent against *M. abscessus* complex clinical isolates *in vitro*. Clofazimine (Meir and Barkan, 2020), used for treating leprosy, is repurposed against *M. abscessus*. Besides, delamanid, pretomanid, and PIPD1 were also tested against *M. abscessus*. Telacebec is a promising novel drug with the potency of shorter duration and better tolerability (Lee and Pethe, 2022). However, the use of newer drugs may be limited by their potential side effects, higher costs, and availability. 2. Recombination of existing drugs: this is a very economical and efficient option. Previous studies (van Ingen et al., 2012b; Lee and Pethe, 2022) found that some antibiotics could increase cell wall permeability for the uptake of the second drug and accelerate durable cure, which indicates that the synergistic drug interactions could provide additional support in treating NTM infections. 3. Find new drugs according to new targets (Dartois and Dick, 2022): RNA polymerase, DNA gyrase, the ribosome, F-ATP synthase, and several enzymes. For instance, antibiotics that target oxidative phosphorylation energy-generate pathways could be a new choice. Alternatively, MmpL3 (Sethiya et al., 2020), a transporter crucial for exporting trehalose monomycolates to the periplasmic space and outer membrane,

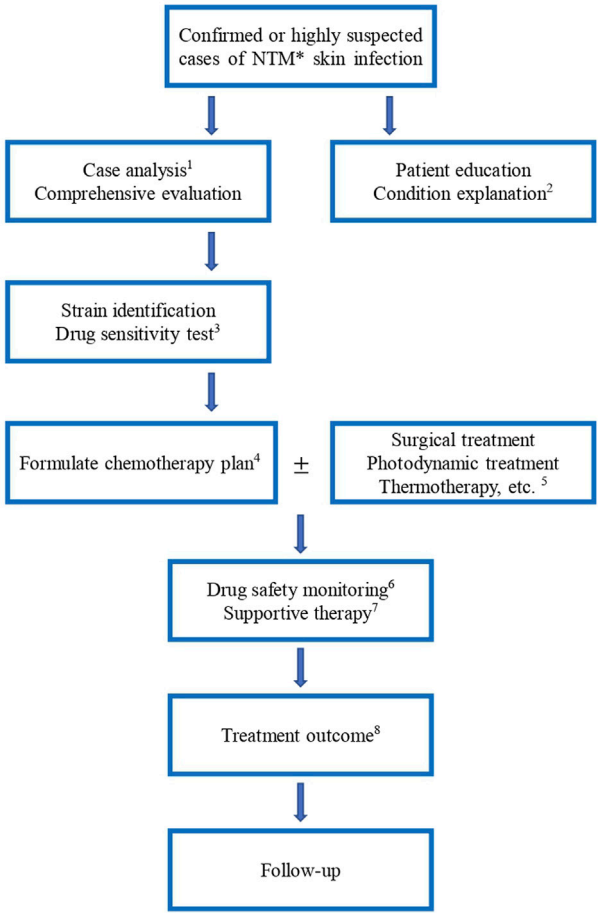

FIGURE 1
Procedure of NTM skin infection treatment. *: NTM, non-tuberculosis mycobacteria 1: The treatment should distinguish between mild and severe, drug resistance and non-resistance, initial and continuous stages, drug composition and dosage, children and adults, and HIV and non-HIV, etc. 3: Anti-NTM drugs include clarithromycin, azithromycin, ethambutol, amikacin, ciprofloxacin, moxifloxacin, rifampicin, rifampicin, isoniazid, cefoxitin, linezolid, chlorfazimine, tegacycline, imipenem/cilastatin, doxycycline, minocycline and compound sulfamethoxazole, etc. 4: Formulate the chemotherapy plan for NTM skin infections, the drugs should be selected according to the above-mentioned results. The type of medication and course of treatment are different for different NTM species. Experimental treatment of suspected NTM infections is not recommended. 5. Other treatment modalities are added depending on the patient's condition. For patients with extensive lesions, abscess formation and poor drug efficacy, surgical debridement or foreign body removal can be actively used. 6. Monitor blood routine, liver and kidney function, blood electrolyte, urine routine, body mass, *mycobacterium* culture, hearing, visual field and color vision, electrocardiogram, etc. 7. Provide good patient education and explanation of the condition. For example, reduce contact with patients with NTM disease, and protect against human-to-human transmission. 8. Treatment outcome includes bacteriological negative conversion, bacteriological cure, clinical cure, cure, treatment failure, bacteriological recurrence, and death.

could also be a novel target in treating NTM. A study (Swain et al., 2021) found 15 new targets through screening 537 core proteins that researchers could further utilize to design inhibitors for discovering antimicrobial agents. In addition, some new drug research approaches are equally exciting. Macrophage infection assays, persister-specific assays, nonreplicating assays, biofilm assays, animal models, and lesion- or infection-site-specific pharmacokinetic assays, which can help us focus on skin and soft tissue, are instrumental methods to measure and evaluate effects when developing new anti-NTM drugs (Wu et al., 2018). For example, interferon-gamma, a cytokine that plays a role in the immune response against mycobacterial infections, has been used as an adjunct to antimicrobial therapy in some cases of NTM skin infections, particularly in patients with underlying immunosuppressive conditions. Other immune-enhancing agents, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), have also been studied in the management of NTM infections. Fragment-based drug discovery (Togre et al., 2022) (FBDD) can concentrate on designing optimal inhibitors against potential therapeutic targets of NTM. A rabbit model could provide an acceptable surrogate model to study antibiotic penetration and simulate pharmacokinetic-pharmacodynamic tracks *in vivo* (Kaya et al., 2022).

Our article also has many limitations. First, randomized studies need to be added, and data regarding optimal treatment are limited. Clinical data on the efficacy of different treatment of NTM skin diseases in humans is limited, and further research is needed to determine its safety and effectiveness in clinical practice. Second, the resistance mechanism of NTM (genetic and pathogenic variations among species) infections needs to be understood more.

In summary, a comprehensive understanding of the various aspects discussed in this study is crucial for the effective management of cutaneous involvement caused by NTM. The ideal therapeutic approach should encompass a broader spectrum of anti-NTM activities while simultaneously considering the specific characteristics of cutaneous infections. The optimal treatment approach for NTM infections is still evolving, continuous research, clinical trials, and innovative therapeutic strategies are essential in the quest for safer, more effective, and tailored treatment options to combat NTM cutaneous involvement effectively. By addressing these aspects, clinicians can enhance patient outcomes and reduce the burden of NTM infections in affected populations.

Author contributions

Conceptualization and design: JL, literature search and writing (original draft): X-YW, methodology and writing (review and editing): Q-NJ. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Ahmad, M. N., Garg, T., Singh, S., Shukla, R., Malik, P., Krishnamurthy, R. V., et al. (2022). *In vitro* and *in vivo* activity of gepotidacin against drug-resistant mycobacterial infections. *Antimicrob. Agents Ch.* 66, e0056422. doi:10.1128/aac.00564-22

Ahmed, I., Tiberi, S., Farooqi, J., Jabeen, K., Yeboah-Manu, D., Migliori, G. B., et al. (2020). Non-tuberculous mycobacterial infections-A neglected and emerging problem. *Int. J. Infect. Dis.* 92S, S46–S50. doi:10.1016/j.ijid.2020.02.022

Akrami, S., Dokht, K. A., and Hashemzadeh, M. (2023). Drug resistance profiles and related gene mutations in slow-growing non-tuberculous mycobacteria isolated in regional tuberculosis reference laboratories of Iran: a three year cross-sectional study. *Pathog. Glob. Health* 117, 52–62. doi:10.1080/20477724.2022.2049029

Alffenaar, J. W., Märtson, A. G., Heysell, S. K., Cho, J. G., Patanwala, A., Burch, G., et al. (2021). Therapeutic drug monitoring in non-tuberculosis mycobacteria infections. *Clin. Pharmacokinet.* 60, 711–725. doi:10.1007/s40262-021-01000-6

Aubry, A., Mougari, F., Reibel, F., and Cambau, E. (2017). Mycobacterium marinum. *Mycobacterium Mar.* Microbiol. Spectr. 5. doi:10.1128/microbiolspec.TNMI7-0038-2016

Bakuła, Z., Modrzejewska, M., Pennings, L., Proboszcz, M., Safianowska, A., Bielecki, J., et al. (2018). Drug susceptibility profiling and genetic determinants of drug resistance in Mycobacterium kansasii. *Antimicrob. Agents Ch.* 62, e01788-17. doi:10.1128/AAC.01788-17

Blakney, R. A., Ricotta, E. E., Frankland, T. B., Honda, S., Zelazny, A., Mayer-Barber, K. D., et al. (2022). Incidence of nontuberculous mycobacterial pulmonary infection, by ethnic group, Hawaii, USA, 2005-2019. *Emerg. Infect. Dis.* 28, 1543–1550. doi:10.3201/eid2808.212375

Cantillon, D., Goff, A., Taylor, S., Salehi, E., Fidler, K., Stoneham, S., et al. (2022). Searching for new therapeutic options for the uncommon pathogen Mycobacterium chimaera: an open drug discovery approach. *Lancet Microbe* 3, e382–e391. doi:10.1016/S2666-5247(21)00326-8

Castillo, N. E., Gurram, P., Sohail, M. R., Fida, M., and Abu, S. O. (2020). Fishing for a diagnosis, the impact of delayed diagnosis on the course of Mycobacterium marinum infection: 21 Years of experience at a tertiary care hospital. *Open Forum Infect. Di* 7, ofz550. doi:10.1093/ofid/ofz550

Chang, C. T., and Whipps, C. M. (2015). Activity of antibiotics against Mycobacterium species commonly found in laboratory zebrafish. *J. Aquat. Anim. Health* 27, 88–95. doi:10.1080/08997659.2015.1007176

Chapagain, M., Gumbo, T., Heysell, S. K., and Srivastava, S. (2020). Comparison of a novel regimen of rifapentine, tedizolid, and minocycline with standard regimens for treatment of pulmonary Mycobacterium kansasii. *Antimicrob. Agents Ch.* 64, e00810-20. doi:10.1128/AAC.00810-20

Chen, L. C., Huang, H. N., Yu, C. J., Chien, J. Y., and Hsueh, P. R. (2020). Clinical features and treatment outcomes of Mycobacterium chimaera lung disease and antimicrobial susceptibility of the mycobacterial isolates. *J. Infect.* 80, 437–443. doi:10.1016/j.jinf.2020.01.005

Chew, K. L., Octavia, S., Go, J., Yeoh, S. F., and Teo, J. (2021). MIC distributions of routinely tested antimicrobials and of rifabutin, eravacycline, delafloxacin, clofazimine, and bedaquiline for Mycobacterium fortuitum. *Antimicrob. Agents Ch.* 65, e0059321. doi:10.1128/AAC.00593-21

Chung, J., Ince, D., Ford, B. A., and Wanat, K. A. (2018). Cutaneous infections due to nontuberculosis Mycobacterium: recognition and management. *Am. J. Clin. Dermatol* 19, 867–878. doi:10.1007/s40257-018-0382-5

Comba, I. Y., Tabaja, H., Almeida, N., Fida, M., and Saleh, O. A. (2021). Bloodstream infections with rapidly growing nontuberculous mycobacteria. *J. Clin. Tuber. Oth Myc* 25, 100288. doi:10.1016/j.jctube.2021.100288

Crilly, N. P., Ayeh, S. K., and Karakousis, P. C. (2020). The new frontier of host-directed therapies for *Mycobacterium avium* complex. *Front. Immunol.* 11, 623119. doi:10.3389/fimmu.2020.623119

Da, M. O., Angulo, A., Rodríguez, M., Fernández-Figueiras, S., and de Waard, J. H. (2020). Drug susceptibility patterns of rapidly growing mycobacteria isolated from skin and soft tissue infections in Venezuela. *Eur. J. Clin. Microbiol.* 39, 433–441. doi:10.1007/s10096-019-03740-7

Daley, C. L., Iaccarino, J. M., Lange, C., Cambau, E., Wallace, R. J., Andrejak, C., et al. (2020). Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline: executive summary. *Clin. Infect. Dis.* 71, e1–e36. doi:10.1093/cid/ciaa241

Daley, C. L. (2017). *Mycobacterium avium* complex disease. *Microbiol. Spectr.* 5. doi:10.1128/microbiolspec.TNMI7-0045-2017

Dartois, V., and Dick, T. (2022). Drug development challenges in nontuberculous mycobacterial lung disease: tb to the rescue. *J. Exp. Med.* 219, e20220445. doi:10.1084/jem.20220445

Das, S., Mishra, B., Mohapatra, P. R., Preetam, C., and Rath, S. (2022). Clinical presentations of nontuberculous mycobacteria as suspected and drug-resistant tuberculosis: experience from a tertiary care center in Eastern India. *Int. J. Mycobact.* 11, 167–174. doi:10.4103/ijmy.ijmy_68_22

Dey, A., Verma, A. K., and Chatterji, D. (2010). Role of an RNA polymerase interacting protein, MsRbpA, from Mycobacterium smegmatis in phenotypic tolerance to rifampicin. *Microbiol-Sgm* 156, 873–883. doi:10.1099/mic.0.033670-0

Dumic, I., and Lutwick, L. (2021). Successful treatment of rapid growing mycobacterial infections with source control alone: case series. *Idcases* 26, e01332. doi:10.1016/j.idcr.2021.e01332

Forbes, B. A., Hall, G. S., Miller, M. B., Novak, S. M., Rowlinson, M. C., Salfinger, M., et al. (2018). Practical guidance for clinical microbiology laboratories: mycobacteria. *Clin. Microbiol. Rev.* 31, e00038-17. doi:10.1128/CMR.00038-17

Franco-Paredes, C., Marcos, L. A., Henao-Martínez, A. F., Rodríguez-Morales, A. J., Villamil-Gómez, W. E., Gotuzzo, E., et al. (2018). Cutaneous mycobacterial infections. *Clin. Microbiol. Rev.* 32, e00069-18. doi:10.1128/CMR.00069-18

Frizzell, M., Carr, E., and Brust, K. (2020). Omadacycline for treatment of *Mycobacterium chelonae* skin infection. *Proc. (Bayl Univ. Med. Cent.* 33, 610–611. doi:10.1080/08998280.2020.1792748

Fukushi, D., Murakami, K., Watanabe, Y., Sugimoto, N., Uehara, H., and Seki, M. (2022). Pulmonary and disseminated *Mycobacterium avium* complex cases confirmed by tissue-direct polymerase chain reaction-based nucleic acid lateral flow immunoassay of formalin-fixed paraffin-embedded tissues. *Infect. Drug Resist* 15, 1049–1054. doi:10.2147/IDR.S358112

Gopalaswamy, R., Shanmugam, S., Mondal, R., and Subbian, S. (2020). Of tuberculosis and non-tuberculous mycobacterial infections - a comparative analysis of epidemiology, diagnosis and treatment. *J. Biomed. Sci.* 27, 74. doi:10.1186/s12929-020-00667-6

Gorzynski, M., De Ville, K., Week, T., Jaramillo, T., and Danelishvili, L. (2023). Understanding the phage-host interaction mechanism toward improving the efficacy of current antibiotics in Mycobacterium abscessus. *Biomedicines* 11, 1379. doi:10.3390/biomedicines11051379

Gravitz, L. (2018). Skin. *Nature* 563, S83. doi:10.1038/d41586-018-07428-4

Grice, E. A., and Segre, J. A. (2011). The skin microbiome. *Nat. Rev. Microbiol.* 9, 244–253. doi:10.1038/nrmicro2537

Guo, Y., Cao, Y., Liu, H., Yang, J., Wang, W., Wang, B., et al. (2022). Clinical and microbiological characteristics of Mycobacterium kansasii pulmonary infections in China. *Microbiol. Spectr.* 10, e0147521. doi:10.1128/spectrum.01475-21

Hashish, E., Merwad, A., Elgaml, S., Amer, A., Kamal, H., Elsadek, A., et al. (2018). Mycobacterium marinum infection in fish and man: epidemiology, pathophysiology and management; a review. *Vet. Quart.* 38, 35–46. doi:10.1080/01652176.2018.1447171

Haworth, C. S., Banks, J., Capstick, T., Fisher, A. J., Gorsuch, T., Laurenson, I. F., et al. (2017). British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). *Thorax* 72, ii1–ii64. doi:10.1136/thoraxjnl-2017-210927

Hendrikx, L., van Hees, C., de Steenwinkel, J., Bax, H. I., Sprong, T., Mulder, B., et al. (2022). Treatment and outcome of culture-confirmed Mycobacterium marinum disease. *Open Forum Infect. Di* 9, ofac077. doi:10.1093/ofid/ofac077

Holden, I. K., Kehrer, M., Andersen, A. B., Wejse, C., Svensson, E., and Johansen, I. S. (2018). Mycobacterium marinum infections in Denmark from 2004 to 2017: a retrospective study of incidence, patient characteristics, treatment regimens and outcome. *Sci. Rep-Uk* 8, 6738. doi:10.1038/s41598-018-24702-7

Huh, H. J., Kim, S. Y., Jhun, B. W., Shin, S. J., and Koh, W. J. (2019). Recent advances in molecular diagnostics and understanding mechanisms of drug resistance in nontuberculous mycobacterial diseases. *Infect. Genet. Evol.* 72, 169–182. doi:10.1016/j.meegid.2018.10.003

Jeong, S. H., Kim, S. Y., Huh, H. J., Ki, C. S., Lee, N. Y., Kang, C. I., et al. (2017). Mycobacteriological characteristics and treatment outcomes in extrapulmonary Mycobacterium abscessus complex infections. *Int. J. Infect. Dis.* 60, 49–56. doi:10.1016/j.ijid.2017.05.007

Kasperbauer, S. H., and De Groote, M. A. (2015). The treatment of rapidly growing mycobacterial infections. *Clin. Chest Med.* 36, 67–78. doi:10.1016/j.ccm.2014.10.004

Kaushik, A., Ammerman, N. C., Lee, J., Martins, O., Kreiswirth, B. N., Lamichhane, G., et al. (2019). *In vitro* activity of the new β-lactamase inhibitors relebactam and vaborbactam in combination with β-lactams against Mycobacterium abscessus complex clinical isolates. *Antimicrob. Agents Ch.* 63, e02623-18. doi:10.1128/AAC.02623-18

Kaya, F., Ernest, J. P., LoMauro, K., Gengenbacher, M., Madani, A., Aragaw, W. W., et al. (2022). A rabbit model to study antibiotic penetration at the site of infection for nontuberculous mycobacterial lung disease: macrolide case study. *Antimicrob. Agents Ch.* 66, e0221221. doi:10.1128/aac.02212-21

Kim, T. H., Hanh, B., Kim, G., Lee, D. G., Park, J. W., Lee, S. E., et al. (2019). Thiostrepton: a novel therapeutic drug candidate for Mycobacterium abscessus infection. *Molecules* 24, 4511. doi:10.3390/molecules24244511

Koushk-Jalali, B., Freitag, A. P., Tigges, C., Oellig, F., Hillemann, D., and Kreuter, A. (2019). Sporotrichoid fish tank granuloma. *Qjm-Int J. Med.* 112, 147. doi:10.1093/qjmed/hcy203

Kumar, C., Shrivastava, K., Singh, A., Chauhan, V., and Varma-Basil, M. (2021). Skin and soft-tissue infections due to rapidly growing mycobacteria: an overview. *Int. J. Mycobact.* 10, 293–300. doi:10.4103/ijmy.ijmy_110_21

Lee, B. S., and Pethe, K. (2022). Telacebec: an investigational antibacterial for the treatment of tuberculosis (TB). *Expert Opin. Inv Drug* 31, 139–144. doi:10.1080/13543784.2022.2030309

Lee, C. N., and Lee, J. Y. (2017). Local hyperthermia therapy for refractory cutaneous Mycobacterium marinum infection. *Dermatol Ther.* 30, e12561. doi:10.1111/dth.12561

Lee, M. R., Sheng, W. H., Hung, C. C., Yu, C. J., Lee, L. N., and Hsueh, P. R. (2015). Mycobacterium abscessus complex infections in humans. *Emerg. Infect. Dis.* 21, 1638–1646. doi:10.3201/2109.141634

Li, Y., Liu, C., Ma, A., He, W., Qiu, Q., Zhao, Y., et al. (2022). Identification and drug susceptibility testing of the subspecies of *Mycobacterium avium* complex clinical isolates in mainland China. *J. Glob. Antimicrob. Re* 31, 90–97. doi:10.1016/j.jgar.2022.05.027

Little, J. S., Dedrick, R. M., Freeman, K. G., Cristinziano, M., Smith, B. E., Benson, C. A., et al. (2022). Bacteriophage treatment of disseminated cutaneous *Mycobacterium chelonae* infection. *Nat. Commun.* 13, 2313. doi:10.1038/s41467-022-29689-4

Liu, Q., Du, J., An, H., Li, X., Guo, D., Li, J., et al. (2023). Clinical characteristics of patients with non-tuberculous mycobacterial pulmonary disease: a seven-year follow-up study conducted in a certain tertiary hospital in beijing. *Front. Cell Infect. Mi* 13, 1205225. doi:10.3389/fcimb.2023.1205225

Luo, T., Xu, P., Zhang, Y., Porter, J. L., Ghanem, M., Liu, Q., et al. (2021). Population genomics provides insights into the evolution and adaptation to humans of the waterborne pathogen Mycobacterium kansasii. *Nat. Commun.* 12, 2491. doi:10.1038/s41467-021-22760-6

Maurer, F. P., Pohle, P., Kernbach, M., Sievert, D., Hillemann, D., Rupp, J., et al. (2019). Differential drug susceptibility patterns of Mycobacterium chimaera and other members of the Mycobacterium avium-intracellulare complex. *Clin. Microbiol. Infec* 25, 371–379. doi:10.1016/j.cmi.2018.06.010

Mei, Y., Zhang, W., Shi, Y., Jiang, H., Chen, Z., Chokkakula, S., et al. (2019). Cutaneous tuberculosis and nontuberculous mycobacterial infections at a national specialized hospital in China. *Acta Derm-Venereol* 99, 997–1003. doi:10.2340/00015555-3283

Mei, Y. M., Zhang, W. Y., Sun, J. Y., Jiang, H. Q., Shi, Y., Xiong, J. S., et al. (2023). Genomic characteristics of *Mycobacterium tuberculosis* isolates of cutaneous tuberculosis. *Front. Microbiol.* 14, 1165916. doi:10.3389/fmicb.2023.1165916

Meir, M., and Barkan, D. (2020). Alternative and experimental therapies of Mycobacterium abscessus infections. *Int. J. Mol. Sci.* 21, 6793. doi:10.3390/ijms21186793

Mok, S., Hannan, M. M., Nölke, L., Stapleton, P., O'Sullivan, N., Murphy, P., et al. (2019). Antimicrobial susceptibility of clinical and environmental Mycobacterium chimaera isolates. *Antimicrob. Agents Ch.* 63, e00755-19. doi:10.1128/AAC.00755-19

Moon, S. M., Choe, J., Jhun, B. W., Jeon, K., Kwon, O. J., Huh, H. J., et al. (2019). Treatment with a macrolide-containing regimen for Mycobacterium kansasii pulmonary disease. *Resp. Med.* 148, 37–42. doi:10.1016/j.rmed.2019.01.012

Nguyen, A. V., and Soulika, A. M. (2019). The dynamics of the skin's immune system. *Int. J. Mol. Sci.* 20, 1811. doi:10.3390/ijms20081811

Ni, L., Zou, D., Yang, H., Gao, Z., Yu, Q., and Yang, L. (2023). Case report: subcutaneous Mycobacterium haemophilum infection in an immunocompetent patient after lipolysis injections. *Front. Med-Lausanne* 10, 1098047. doi:10.3389/fmed.2023.1098047

Nogueira, L. B., Garcia, C. N., Costa, M., Moraes, M. B., Kurizky, P. S., and Gomes, C. M. (2021). Non-tuberculous cutaneous mycobacterioses. *Bras Dermatol* 96, 527–538. doi:10.1016/j.abd.2021.04.005

North, E. J., Schwartz, C. P., Zgurskaya, H. I., and Jackson, M. (2023). Recent advances in mycobacterial membrane protein large 3 inhibitor drug design for mycobacterial infections. *Expert Opin. Drug Dis.* 18, 707–724. doi:10.1080/17460441.2023.2218082

Oh, T. H., Kim, U. J., Kang, S. J., Jang, H. C., Park, K. H., Jung, S. I., et al. (2018). Disseminated invasive Mycobacterium marinum infection involving the lung of a patient with diabetes mellitus. *Infect. Chemother.* 50, 59–64. doi:10.3947/ic.2018.50.1.59

Okuno, H., Tanaka, H., Hagiya, H., Yoshida, H., Hamaguchi, S., Hori, Y., et al. (2020). Mycobacterium kansasii arthritis of the elbow in an immunocompetent patient with a suspected soft-tissue tumor. *J. Infect. Chemother.* 26, 261–264. doi:10.1016/j.jiac.2019.07.015

Pavlik, I., Ulmann, V., and Falkinham, J. R. (2022). Nontuberculous mycobacteria: ecology and impact on animal and human health. *Microorganisms* 10, 1516. doi:10.3390/microorganisms10081516

Pennington, K. M., Vu, A., Challener, D., Rivera, C. G., Shweta, F., Zeuli, J. D., et al. (2021). Approach to the diagnosis and treatment of non-tuberculous mycobacterial disease. *J. Clin. Tuber. Oth Myc* 24, 100244. doi:10.1016/j.jctube.2021.100244

Philips, R. C., Hoyer, P. E., White, S. M., Tinkey, K. T., Loeffelholz, M., Andersen, C. R., et al. (2019). Cutaneous nontuberculous mycobacteria infections: a retrospective case series of 78 patients from the Texas gulf coast region. *J. Am. Acad. Dermatol* 81, 730–739. doi:10.1016/j.jaad.2019.04.022

Rallis, E., Falidas, E., and Stavropoulos, P. (2012). Mycobacterium marinum cutaneous infection with sporotrichoid distribution treated with azithromycin monotherapy. *Skinmed* 10, 54–55.

Riccardi, N., Antonello, R. M., Canetti, D., and Polidori, M. (2022). An octopus gift: mycobacterium marinum multiple skin lesions. *Infection* 50, 1631–1632. doi:10.1007/s15010-022-01851-6

Rifat, D., Chen, L., Kreiswirth, B. N., and Nuermberger, E. L. (2021). Genome-wide essentiality analysis of Mycobacterium abscessus by saturated transposon mutagenesis and deep sequencing. *Mbio* 12, e0104921. doi:10.1128/mBio.01049-21

Rindi, L. (2020). Efflux Pump inhibitors against nontuberculous mycobacteria. *Int. J. Mol. Sci.* 21, 4191. doi:10.3390/ijms21124191

Saxena, S., Spaink, H. P., and Forn-Cuní, G. (2021). Drug resistance in nontuberculous mycobacteria: mechanisms and models. *Biology-Basel* 10, 96. doi:10.3390/biology10020096

Schoutrop, E., Brouwer, M., Jenniskens, J., Ferro, B. E., Mouton, J. W., Aarnoutse, R. E., et al. (2018). The stability of antimycobacterial drugs in media used for drug susceptibility testing. *Diagn Micr Infec Dis.* 92, 305–308. doi:10.1016/j.diagmicrobio.2018.06.015

Schulthess, B., Schäfle, D., Kälin, N., Widmer, T., and Sander, P. (2023). Drug susceptibility distributions of Mycobacterium chimaera and other non-tuberculous mycobacteria. *Antimicrob. Agents Ch.* 65, e02131-20. doi:10.1128/AAC.02131-20

Seidel, A., Nunes, D. H., Fernandes, C., and Funchal, G. (2022). Skin infection by Mycobacterium marinum - diagnostic and therapeutic challenge. *Bras Dermatol* 97, 366–368. doi:10.1016/j.abd.2021.03.013

Sethiya, J. P., Sowards, M. A., Jackson, M., and North, E. J. (2020). MmpL3 inhibition: a new approach to treat nontuberculous mycobacterial infections. *Int. J. Mol. Sci.* 21, 6202. doi:10.3390/ijms21176202

Sharma, M., Malhotra, B., and Khandelwal, S. (2021). Drug susceptibiity testing of nontuberculous mycobacteria by broth microdilution method. *Indian J. Med. Microbi* 39, 306–310. doi:10.1016/j.ijmmb.2021.03.015

Sharma, S. K., and Upadhyay, V. (2020). Epidemiology, diagnosis and treatment of non-tuberculous mycobacterial diseases. *Indian J. Med. Res.* 152, 185–226. doi:10.4103/ijmr.IJMR_902_20

Shimizu, F., Hatano, Y., Okamoto, O., Katagiri, K., Fujiwara, S., Sato, S., et al. (2012). Mycobacterium smegmatis soft tissue infection. *Int. J. Dermatol* 51, 1518–1520. doi:10.1111/j.1365-4632.2010.04835.x

Shrivastava, K., Kumar, C., Singh, A., Narang, A., Giri, A., Sharma, N. K., et al. (2020). An overview of pulmonary infections due to rapidly growing mycobacteria in South Asia and impressions from a subtropical region. *Int. J. Mycobact.* 9, 62–70. doi:10.4103/ijmy.ijmy_179_19

Singh, S., Wang, J. Y., Heysell, S. K., McShane, P. J., Wadle, C., Shankar, P., et al. (2023). Omadacycline pharmacokinetics/pharmacodynamics in the hollow fiber model and clinical validation of efficacy to treat pulmonary Mycobacterium abscessus disease. *Int. J. Antimicrob. Ag.* 62, 106847. doi:10.1016/j.ijantimicag.2023.106847

Slany, M., Ulmann, V., and Slana, I. (2016). Avian mycobacteriosis: still existing threat to humans. *Biomed. Res. Int.* 2016, 4387461. doi:10.1155/2016/4387461

Srivastava, S., and Gumbo, T. (2018). Clofazimine for the treatment of Mycobacterium kansasii. *Antimicrob. Agents Ch.* 62, e00248-18. doi:10.1128/AAC.00248-18

Strobel, K., Sickenberger, C., Schoen, C., Kneitz, H., Kolb-Mäurer, A., and Goebeler, M. (2022). Diagnosis and therapy of Mycobacterium marinum: a single-center 21-year retrospective analysis. *J. Dtsch. Dermatol Ges.* 20, 1211–1218. doi:10.1111/ddg.14847

Swain, A., Gnanasekar, P., Prava, J., Rajeev, A. C., Kesarwani, P., Lahiri, C., et al. (2021). A comparative genomics approach for shortlisting broad-spectrum drug targets in nontuberculous mycobacteria. *Microb. Drug Resist* 27, 212–226. doi:10.1089/mdr.2020.0161

Timmins, G. S. (2020). What are the challenges in commercial non-tuberculous mycobacteria (NTM) drug discovery and how should we move forward? *Expert Opin. Drug Dis.* 15, 7–9. doi:10.1080/17460441.2020.1673362

To, K., Cao, R., Yegiazaryan, A., Owens, J., and Venketaraman, V. (2020). General overview of nontuberculous mycobacteria opportunistic pathogens: *mycobacterium avium* and Mycobacterium abscessus. *J. Clin. Med.* 9, 2541. doi:10.3390/jcm9082541

Togre, N. S., Vargas, A. M., Bhargavi, G., Mallakuntla, M. K., and Tiwari, S. (2022). Fragment-based drug discovery against mycobacteria: the success and challenges. *Int. J. Mol. Sci.* 23, 10669. doi:10.3390/ijms231810669

Toth, E., Waller, J. L., Bollag, W. B., Siddiqui, B., Mohammed, A., Kheda, M., et al. (2022). Non-tuberculous mycobacterial infections in patients with end-stage renal disease: prevalence, risk factors, and mortality. *J. Invest. Med.* 2022, 002462–12022. doi:10.1136/jim-2022-002462

Trčko, K., Plaznik, J., and Miljković, J. (2021). Mycobacterium marinum hand infection masquerading as tinea manuum: a case report and literature review. *Acta Dermatoven Alp.* 30, 91–93. doi:10.15570/actaapa.2021.23

Uslu, U., Böhm, O., Heppt, F., and Sticherling, M. (2019). Skin and soft tissue infections caused by *Mycobacterium chelonae*: more common than expected? *Acta Derm-Venereol* 99, 889–893. doi:10.2340/00015555-3230

van Ingen, J., Boeree, M. J., van Soolingen, D., and Mouton, J. W. (2012a). Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. *Drug Resist Update* 15, 149–161. doi:10.1016/j.drup.2012.04.001

van Ingen, J., Totten, S. E., Helstrom, N. K., Heifets, L. B., Boeree, M. J., and Daley, C. L. (2012b). *In vitro* synergy between clofazimine and amikacin in treatment of nontuberculous mycobacterial disease. *Antimicrob. Agents Ch.* 56, 6324–6327. doi:10.1128/AAC.01505-12

Wang, C. J., Song, Y., Li, T., Hu, J., Chen, X., and Li, H. (2022a). Mycobacterium smegmatis skin infection following cosmetic procedures: report of two cases. *Clin. Cosmet. Inv Derm.* 15, 535–540. doi:10.2147/CCID.S359010

Wang, D. M., Liao, Y., Li, Q. F., Zhu, M., Wu, G. H., Xu, Y. H., et al. (2019). Drug resistance and pathogenic spectrum of patients coinfected with nontuberculous mycobacteria and human-immunodeficiency virus in Chengdu, China. *Chin. Med. J-Peking* 132, 1293–1297. doi:10.1097/CM9.0000000000000235

Wang, J., Huang, J., Peng, S., Li, L., Zhong, K., and Chen, T. (2022b). A clinical case and a review of Mycobacterium fortuitum infections direct diagnosis approach and treatment in a patient with leg fractures. *J. Infect. Dev. Countr* 16, 1650–1654. doi:10.3855/jidc.16635

Watanabe, Y., Fujita, H., Fukushima, S., and Nakamura, I. (2022). Disseminated *Mycobacterium chelonae* infection, including discitis. *Intern. Med.* 61, 2085–2086. doi:10.2169/internalmedicine.8539-21

Wi, Y. M. (2019). Treatment of extrapulmonary nontuberculous mycobacterial diseases. *Infect. Chemother.* 51, 245–255. doi:10.3947/ic.2019.51.3.245

Wu, M. L., Aziz, D. B., Dartois, V., and Dick, T. (2018). NTM drug discovery: status, gaps and the way forward. *Drug Discov. Today* 23, 1502–1519. doi:10.1016/j.drudis.2018.04.001

Yan, M., Brode, S. K., and Marras, T. K. (2023). The other nontuberculous mycobacteria: clinical aspects of lung disease caused by less common slowly growing nontuberculous mycobacteria species. *Chest* 163, 281–291. doi:10.1016/j.chest.2022.09.025

Yang, Z., Feng, Y., Li, D., Pang, Z., Wang, S., Chen, H., et al. (2022). 5-aminolevulinic acid-photodynamic therapy ameliorates cutaneous granuloma by killing drug-resistant Mycobacterium marinum. *Photodiagn Photodyn.* 38, 102839. doi:10.1016/j.pdpdt.2022.102839

Yeo, P. M., Lee, S. X., Tan, Y. E., Sng, L. H., and Ang, C. C. (2019). Epidemiology, risk factors, and outcomes of adult cutaneous non-tuberculous mycobacterial infection over a 10-year period in Singapore. *Int. J. Dermatol* 58, 679–687. doi:10.1111/ijd.14356

Zhang, M., Feng, M., and He, J. Q. (2017). Disseminated Mycobacterium kansasii infection with cutaneous lesions in an immunocompetent patient. *Int. J. Infect. Dis.* 62, 59–63. doi:10.1016/j.ijid.2017.07.006

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Optimizing tylosin dosage for co-infection of *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* in pigs using pharmacokinetic/pharmacodynamic modeling

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Formulating a therapeutic strategy that can effectively combat concurrent infections of *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*) and *Pasteurella multocida* (*P. multocida*) can be challenging. This study aimed to 1) establish minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time kill curve, and post-antibiotic effect (PAE) of tylosin against *A. pleuropneumoniae* and *P. multocida* pig isolates and employ the MIC data for the development of epidemiological cutoff (ECOFF) values; 2) estimate the pharmacokinetics (PKs) of tylosin following its intramuscular (IM) administration (20 mg/kg) in healthy and infected pigs; and 3) establish a PK–pharmacodynamic (PD) integrated model and predict optimal dosing regimens and PK/PD cutoff values for tylosin in healthy and infected pigs. The MIC of tylosin against both 89 and 363 isolates of *A. pleuropneumoniae* and *P. multocida* strains spread widely, ranging from 1 to 256 μg/mL and from 0.5 to 128 μg/mL, respectively. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) ECOFFinder analysis ECOFF value (≤64 μg/mL), 97.75% (87 strains) of the *A. pleuropnumoniae* isolates were wild-type, whereas with the same ECOFF value (≤64 μg/mL), 99.72% (363 strains) of the *P. multicoda* isolates were considered wild-type to tylosin. Area under the concentration time curve (AUC), $T_{1/2}$, and C_{max} values were significantly greater in healthy pigs than those in infected pigs (13.33 h × μg/mL, 1.99 h, and 5.79 μg/mL vs. 10.46 h × μg/mL, 1.83 h, and 3.59 μg/mL, respectively) ($p < 0.05$). In healthy pigs, $AUC_{24\ h}$/MIC values for the bacteriostatic activity were 0.98 and 1.10 h; for the bactericidal activity, $AUC_{24\ h}$/MIC values were 1.97 and 1.99 h for *A. pleuropneumoniae* and *P. multocida*, respectively. In infected pigs, $AUC_{24\ h}$/MIC values for the bacteriostatic activity were 1.03 and 1.12 h; for bactericidal activity, $AUC_{24\ h}$/MIC values were 2.54 and 2.36 h for *A. pleuropneumoniae* and *P. multocida*, respectively. Monte Carlo simulation lead to a 2 μg/mL calculated PK/PD cutoff. Managing co-infections can present challenges, as it often demands the administration of multiple antibiotics to address diverse pathogens. However, using tylosin, which effectively targets both *A. pleuropneumoniae* and *P. multocida* in pigs, may enhance the control of bacterial burden. By employing an optimized dosage

of 11.94–15.37 mg/kg and 25.17–27.79 mg/kg of tylosin can result in achieving bacteriostatic and bactericidal effects in 90% of co-infected pigs.

KEYWORDS

dosage optimization, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, pharmacokinetic/pharmacodynamic modeling, Monte Carlo simulation

1 Introduction

Tylosin belongs to the macrolide family of antibiotics, which share a macrocyclic lactone ring structure critical for their antibacterial activity (Arsic et al., 2018). Variations in this ring structure can lead to differences in their spectrum of activity and pharmacokinetic properties (Lenz et al., 2021). It has been widely used to treat respiratory, skin, and gastrointestinal infections in pigs, effectively targeting bacterial pathogens like *Pasteurella multocida*, *Haemophilus parasuis*, and *Actinobacillus pleuropneumoniae* (Urbanová et al., 1975; DeRosa et al., 2000). Tylosin can be administered orally, intravenously, or intramuscularly, with its efficacy influenced by factors such as dosage, frequency of administration, and the specific bacterial strain being treated (Wu et al., 2019). Careful antibiotic use is essential to minimize antimicrobial resistance, and adherence to guidelines for responsible antibiotic use in livestock production is crucial (Lee et al., 2013). In addition to their antibacterial effects, macrolides, including tylosin, have demonstrated immunomodulatory and anti-inflammatory properties. They can hinder the production of pro-inflammatory cytokines, potentially contributing to therapeutic benefits in certain infections and non-infectious inflammatory conditions (Cao et al., 2006).

The typical recommended dosage for pigs is 20 mg of tylosin per kilogram of the pig's body weight (Couper et al., 2006). This dosage is commonly prescribed to treat a variety of infections affecting the respiratory system, skin, and gastrointestinal tract in pigs (Huang et al., 2021; Ronaghinia et al., 2021). By administering this dosage, the aim is to effectively combat the specific bacteria responsible for the infection while also ensuring that the risk of adverse effects is kept to a minimum. The dosage is carefully selected based on factors such as the pig's weight and the severity of the infection to ensure that it achieves optimal therapeutic outcomes without causing harm to the animal (Rizk et al., 2019).

Porcine respiratory disease complex (PRDC) is a common respiratory disease in pigs and can lead to significant economic losses in the swine industry (Eddicks et al., 2021). PRDC is caused by a combination of viral, bacterial, and environmental factors (Woeste and Grosse Beilage, 2007). Therefore, given the complexity of managing PRDC, it is common for a comprehensive and prolonged approach to be necessary. Effective antimicrobial therapy plays a crucial role in both preventing and managing multiple infections associated with PRDC (Chae, 2016).

A. pleuropneumoniae and *P. multocida* are two important pathogens that are commonly associated with PRDC (Cheong et al., 2017). *A. pleuropneumoniae* is a Gram-negative bacterium that is a significant cause of respiratory diseases in pigs. This bacterium can cause several clinical signs, including coughing, fever, weight loss, and pleurisy (Hälli et al., 2020). *A. pleuropneumoniae* is highly contagious and can rapidly spread through herds of pigs (Plasencia-Muñoz et al., 2020), which is associated with stressful conditions, including transportation, weaning, and changes in the herd (Loera-Muro et al., 2021). The bacterium can survive for long periods in the environment and is frequently present on farms without causing any clinical signs (Assavacheep and Rycroft, 2013). *P. multocida* is a Gram-negative bacterium that is commonly observed in the upper respiratory tract of pigs (Hamilton et al., 1998). *P. multocida* can cause several clinical signs, including fever, lethargy, anorexia, respiratory distress, and joint swelling (Giordano et al., 2015).

A mathematical model is created to explain how a drug behaves in the body [pharmacokinetics (PK)] and how it produces its effects [pharmacodynamics (PD)] (Derendorf et al., 2000). This model involves equations that relate the drug concentration in the body to the observed response, such as changes in blood pressure or inhibition of bacterial growth (Bauer and Bauer, 2008). To evaluate the likelihood of achieving the desired pharmacological response for a given drug dosing plan, PK/PD modeling uses the Monte Carlo Simulation, a powerful computational technique (Chua and Tam, 2022). The Probability of Target Attainment (PTA) is a vital concept in this analysis, as it helps assess the drug's effectiveness and predictability (Cristinacce et al., 2019). Once the Monte Carlo Simulation is validated and reaches convergence, it yields final dose values through statistical analysis (Shen and Kuti, 2023). These values represent the average dose and uncertainty at specific points of interest, considering the intricate interactions within the system. The management of *A. pleuropneumoniae* and *P. multocida* concurrent infections can be challenging, thereby leading to difficulties in formulating a therapeutic strategy that can effectively combat all bacteria that are simultaneously present. Therefore, it is significant to employ a multifaceted PK/PD model enabling the determination of a rational dosage regimen to optimize the efficacy of a drug for a strong therapeutic effect (Derendorf et al., 2000).

The PK/PD cutoff value (CO_{PD}) is a measure that can be useful in assessing the effectiveness of antibiotics in treating bacterial infections and is closely related to clinical efficacy (Rodríguez-Gascón et al., 2021). Establishing the CO_{PD} before approving an antimicrobial drug for clinical use is generally recommended (Derendorf et al., 2000). However, to our knowledge, there is currently no published data available regarding the CO_{PD} of tylosin against *A. pleuropneumoniae* and *P. multocida* co-infections in pigs.

Therefore, this study aimed to 1) establish the minimum inhibitory concentration (MIC), MBC, time kill curve, and post-antibiotic effect (PAE) of tylosin *in vitro* in the Brain Heart Infusion (BHI) against 89 *A. pleuropneumoniae* and 363 *P. multocida* pig isolates and employ the MIC data for the development of epidemiological cutoff (ECOFF) values; 2) evaluate the PK profile of tylosin following its intramuscular (IM) administration in healthy

and *A. pleuropneumoniae*- and *P. multocida*-infected pigs; 3) evaluate the *ex vivo* time kill curves of tylosin plasma from healthy and infected pigs against *A. pleuropneumoniae* and *P. multocida*; 4) undertake PK–PD modeling of the 125 BHI and plasma *in vitro* time kill data used as a surrogate of *in vivo* efficacy area under the concentration time curve $(AUC)_{24\ h}$/MIC ratios to establish two levels of growth inhibition; and 5) use the PK–PD-modeled data for predicting CO_{PD} and effective dosage regimens required for achieving bacterial killing of both *A. pleuropneumoniae* and *P. multocida*.

2 Materials and methods

2.1 Chemicals and reagents

Tylosin standard and nicotinamide adenine dinucleotide (NAD) was purchased from Sigma-Aldrich (St. Louis, MO, United States). Each milliliter of the tylosin injectable solution (50 mg/mL) contains 50 mg of tylosin activity (as tylosin base) in 50% propylene glycol with 4% benzyl alcohol which acts as a preservative, preventing the growth of bacteria; water for injection was obtained from Samyang Anipharm (Seoul, Korea). BHI and Mueller Hinton Broth (MHB) were purchased from BD Company (New Jersey, United States). Sheep blood defibrinated (SBD) was acquired from Kisan Bio (Seoul, Korea).

2.2 Bacterial strains

A total of 89 and 363 strains of *A. pleuropneumoniae* and *P. multocida*, respectively, were provided by the Animal and Plant Quarantine Agency (Kimchen, Korea). Quality control (QC) strains American Type Culture Collection (ATCC) 27088 and ATCC 43137 for *A. pleuropneomiae* and *P. multocida*, respectively, were purchased from ATCC (Manassas, VA, United States). Each isolate strain was cultured as described in previous research with some modifications (Dorey et al., 2017). *A. pleuropneumoniae* and *P. multocida* was subcultured over three times to reach stable growth in BHI containing 0.02% NAD for *A. pleuropneumoniae* and MHB containing 2% SBD for *P. multicoda*.

2.3 Study animals and experimental design

Fourteen clinically healthy crossbred Duroc × (Landrace × Yorkshire) male pigs, approximately 5–6 weeks of age with an average weight of 9.5 ± 1.1 kg, were obtained from Petobio (Hanam, Gyeonggi-do, Korea) and transported to the study site at Gyeongsangbuk-do Veterinary Service Laboratory (Daegu, South Korea). The animal study was approved by the Animal Ethics Committee of the Petobio Clinical Institute (PTB-2022-IACUC-013-A). Animals were acclimatized for 1 week with free access to water and food. Following proper adaptation to diet and environment, the pigs were randomly assigned into two groups, seven for the healthy (non-infected) group (Group-1) and seven for the infected group (Group-2). All 14 clinically healthy pigs were further confirmed as negative through nasal swabs (Copan Diagnostics, Murrieta, CA, United States) using polymerase chain reaction (PCR) amplification of *apxIVA* and *kmt1* genes for detecting *A. pleuropneumoniae* and *P. multocida*, respectively. The pigs in Group-2 were manually restrained using a pig snare into a dog-sitting position with front legs extended, and each pig was intranasally inoculated with 1-mL mixed suspension (0.5 mL per naris) containing 2.0×10^9 colony forming unit (CFU)/mL of *A. pleuropneumoniae* (BA2000013) (0.5 mL) and 2.0×10^9 CFU/mL of *P. multocida* (BA1700127) (0.5 mL) using a syringe upon inspiration. During the experiment, clinical respiratory disease scores, appearance/abnormal signs, and clinical signs were recorded. *A. pleuropneumoniae* and *P. multocida* infections were monitored by culturing nasal swabs and confirmed by PCR amplification of *apxIVA* and *kmt1* genes for detecting *A. pleuropneumoniae* and *P. multocida,* respectively, according to a previously described method (Schaller et al., 2001; Shalaby et al., 2021). Following 12 h of infection, the pigs in both groups received 20 mg/kg tylosin by IM administration (Couper et al., 2006). Blood samples from every pig were collected from the jugular vein at 0 (pre-dose), 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, and 24 h following IM administration in anticoagulant-containing vacutainers (BD Company, New Jersey, United States) with anticoagulants. The blood samples were centrifuged at 3,000 rpm for 10 min, and the plasma was transferred to Eppendorf tubes and stored at −70°C until analysis.

2.4 *In vitro* susceptibility study and MIC, MBC, and ECOFF determination

The MIC of tylosin for 89 strains of *A. pleuropneumoniae* (NAD-containing BHI) and 363 strains of *P. multocida* (MHB containing 2% SBD) was determined using a two-fold serial dilution method with 0.25–256 μg/mL of concentration following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). Those isolates with MIC values over 256 μg/mL were re-tested using a broader range of tylosin dilutions. Inoculated plates were kept at 37°C for 48 h. The MIC value was considered the lowest drug concentration that caused complete visible growth inhibition of bacteria in the medium. *A. pleuropneumoniae* (ATCC 27088) and *P. multocida* (ATCC 43137) susceptibility tests were simultaneously performed as QC strains to evaluate the results of the abovementioned susceptibility testing. MIC_{50} and MIC_{90}, which inhibit bacterial growth by 50% and 90% of isolates, respectively, were determined as described in a previous study (Schwarz et al., 2010). MBC was tested by inoculating the supplemented BHI plates containing 0.02% NAD and MHB containing 2% SBD with a 20-μL suspension received from three higher concentrations greater than the initial MIC detection with no distinct bacteria and were incubated for 24 h at 37°C with 5% CO_2. These were tested to determine the MBC using the spot plate technique to determine a 3log10 reduction in the inoculum count. A strain with a MIC value similar to MIC_{90} was selected for further *in vitro* susceptibility study. MIC results were used to determine ECOFF values using the iterative statistical method processed in the ECOFFinder software (version 2.1; https://www.eucast.org/mic_distributions_and_ecoffs/, EUCAST).

2.5 PAE determination

Selected isolates susceptible to tylosin with the lowest possible MIC were used for PAE determination as previously described (Luo et al., 2020). Strains were cultured in BHI containing 0.02% NAD for *A. pleuropneumonia* and MHB containing 2% SBD for *P. multicoda* at 37°C to the log phase of growth to produce a final inoculum of 1.5 × 10^7 CFU/mL. Bacteria were exposed to tylosin concentrations equal to 0.5×, 1×, or 4× MIC. Growth controls were simultaneously inoculated without antibiotics. Tubes were placed in a 37°C shaker for 2 h. At the end of the exposure period, antibiotics were washed by diluting 1: 1,000 with sterile NAD-containing BHI for *A. pleuropneumoniae* and MHB broth containing 2% SBD for *P. multicoda*. Controls were handled similarly. Following dilution of the antibiotics, tube contents were incubated at 37°C until turbidity developed. Bacterial counts were determined at 0, 1, 2, 3, 4, 5, 6, and 7 h following dilution. PAE was calculated as follows: PAE = T–C, where PAE refers to the post-antibiotic effect, T is the time required for the viable counts in bacterial suspension to increase by 1 log10 above the count following tylosin removal by dilution, and C is the concentration for the viable counts in bacterial suspension to increase by 1 log10 for controls without tylosin treatment following dilution (Luo et al., 2020).

2.6 *In vitro* and *ex vivo* time kill curves

In vitro time kill curves of tylosin against *A. pleuropneumoniae* or *P. multocida* was established using the broth dilution method following the CLIS guidelines (CLSI, 2017). Briefly, the bacteria adjusted to a final inoculum of 1.5 × 10^6 CFU/mL was exposed to various tylosin concentrations ranging from 0.5× to 4× MIC. For control growth curves, BHI and MHB without tylosin was used. Bacterial counts were performed by applying to BHI plates containing 0.02% NAD or MHB containing 2% SBD at 0, 1, 2, 4, 8, 12, and 24 h of culture following incubation for 24 h at 37°C.

Ex vivo time kill curves of tylosin were performed similarly to the above mentioned *in vitro* time kill curve using plasma obtained from healthy and infected pigs with *A. pleuropneumoniae* (BA2000013) and *P. multocida* (BA1700127) at 0-, 0.25-, 0.5-, 0.75-, 1-, 2-, 4-, 6-, 8-, 12-, and 24-h time points following IM injection of tylosin. The plasma at each time interval collected from the same pigs (n = 3) were used. Plasma samples were pre-filtered through a 0.22-μm membrane to clear any bacterial contamination. Bacteria were cultured on BHI supplemented with 0.02% NAD or MHB containing 2% SBD and incubated overnight at 37°C. 5 uL of bacterial culture in the stationary phase was introduced into 0.5 mL of plasma, resulting in a final inoculum of 1 × 10^6 CFU/mL. Subsequently, the tubes containing the bacterial-plasma mixtures were incubated at 37°C, and bacterial counts were assessed using the plate count method at 1, 2, 4, 8, 12, and 24 h (Huang et al., 2018).

2.7 HPLC procedures and PK analysis of tylosin

Frozen plasma samples were thawed at room temperature, and 245 uL of plasma was transferred to new prechilled centrifuge tubes combined with 5 uL of 0.025 μg/mL as the internal standard. For drug extraction, plasma sample aliquots were mixed with methanol (2 mL). After vortexing (15 min) and centrifugation (12,000 × g, 10 min), the supernatant was separated and filtered through a 0.22-μm nylon syringe filter, and dried in a water bath using nitrogen at 50°C. The residue was dissolved in 100-uL methanol, agitated (1 min), and centrifuged (12,000 rpm, 10 min). Drug levels in the final 70 uL were determined using the liquid chromatography-tandem mass spectrometry method (Agilent 1200 HPLC system; API 4000 triple quadrupole mass spectrometer, CA, United States). The mass spectrometer was set up with an electrospray positive ionization mode (ESI+) using a capillary voltage of 3,500 V and had optimal ESI–MS parameters, including a drying gas temperature of 350°C, a drying gas flow of 5 L/min, and a nebulizing gas pressure of 45 psi. Separations were accomplished using an Eclipse plus C18 column (2.1 × 100 mm, 3.5 μm) (Agilent Technologies, CA, United States). The mobile phase consisted of a mixture of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile solution (Eluent B) with a ratio of 30:70 (v/v) and a concentration of 1 mM. The flow rate was 0.4 mL/min, and the sample injection volume was 3 μL. The column temperature was maintained at 40°C. The monitored precursor ion for tylosin was 916.3 m/z. The validation of the assay was performed by spiked plasma samples at five different levels. The limit of detection was the concentration at which the signal-to-noise ratio was greater than three with a value of 0.017 μg/mL, whereas the limit of quantification was the concentration at which the signal-to-noise ratio was ten with a value of 0.053 μg/mL. The correlation coefficient (R) was above 0.98 in the linear range of 0.025–4 μg/mL. Inter- and intra-assay precision was determined to be all <10% and the accuracy of the assay was 101.38% ± 34.24% (Huang et al., 2018).

Tylosin time–concentration data in the plasma of individual pigs were analyzed using WinNonlin Version 8.3 software (Certara, NJ, United States) employing non-compartmental modeling. The maximal drug concentration (Cmax) was directly determined from the data with T_{max} defined as the time of the first occurrence of C_{max}. To calculate the AUC, the linear trapezoidal rule was used. Additional PK parameters, including terminal half-life ($T_{1/2}\lambda_z$) and mean residence time (MRT), were also determined.

2.8 PK/PD integration analysis

The PK/PD integration was estimated on the basis of the area under the plasma concentration curve over 24 h divided by the MIC ($AUC_{24\ h}$/MIC) (Aliabadi and Lees, 2001). The *in vitro* drug effect E) was determined by computing the log10 difference between 0- and 24-h incubation. Data were assessed using the sigmoid E_{max} model equation as shown below:

$$E = E_0 - \left[\frac{(E_{max} \times C^{\gamma})}{(C^{\gamma} + EC_{50}^{\gamma})}\right]$$

where E_0 represents drug effect, which is calculated as the change in bacterial count in the control samples after 24 h compared with the initial inoculum. E_{max} is the difference in effect between maximum growth (growth in control, E_0) and minimum growth. EC_{50} is the $AUC_{24\ h}$/MIC value producing half reduction (50%) in bacterial counts from the initial inoculum. C represents the $AUC_{24\ h}$/MIC

ratio, and γ is the Hill coefficient representing the steepness of the $AUC_{24\ h}$/MIC effect curve. T > MIC represents the time that tylosin plasma concentration is above the MIC (Turnidge, 1998).

2.9 Monte Carlo analysis for PK/PD cutoff values

The Monte Carlo simulation (MCS) analysis involved conducting 10,000 MCS trials on the basis of predetermined PK parameters and PK/PD targets ($AUC_{24\ h}$/MIC) that exhibited a bactericidal effect (E = −3) (Lei et al., 2018). The PK/PD cutoff value (CO_{PD}) was identified as the MIC level at which the probability of target at which the probability of target attainment (PTA) reached 90%, following the previously described method (Turnidge and Paterson, 2007). The daily dose was calculated using MCS in the Oracle Ball (Oracle Corporation, Redwood Shores, CA, United States) for 10,000 iterations to estimate 50% and 90% target attainment rates for bacteriostatic and bactericidal effects.

2.10 Dose estimations

The calculation of the potential optimal dosage was determined using the $AUC_{24\ h}$/MIC value at different activity levels, including the bacteriostatic activity (E = 0) and bactericidal activity (E = −3) using the following equation:

$$\text{Dose} = \frac{\left(\frac{AUC_{24hr}}{MIC}\right) \times MIC \times Cl}{F \times fu}$$

where $AUC_{24\ h}$/MIC represents the target endpoint for optimal efficacy, MIC represents the minimum inhibitory concentration in this study, Cl refers to clearance (Prats et al., 2002), F is bioavailability, and fu indicates a free fraction of tylosin in plasma (Bauer and Bauer, 2008). Relative bioavailability can serve as a substitute when intravenous administration is not available (TOUTAIN & BOUSQUET-MÉLOU, 2004). The calculation was based on previous research (Huang et al., 2018).

2.11 Statistical analysis

Data were presented as means ± standard deviations. Statistical analysis was performed with Student's t-test using GraphPad Prism software version 8.0.1 (CA, United States). $p < 0.05$ was considered statistically significant.

3 Results

3.1 MIC, MBC, and ECOFF determinations

The MIC of tylosin against 89 and 363 isolates of *A. pleuropneumoniae* and *P. multocida* strains spread widely, ranging from 1 to 256 μg/mL and from 0.5 to 128 μg/mL, respectively (Figures 1A, B), with a monomodal distribution displaying Gaussian distribution. Tylosin was active against both species isolates, with MIC_{50} and MIC_{90} of 16 μg/mL for *A. pleuropneumoniae* and MIC_{50} of 16 μg/mL and MIC_{90} of 32 μg/mL for *P. multocida*. Strains with MIC values similar to the MIC_{90} of tylosin against *A. pleuropneumoniae* (BA2000013) and *P. multocida* (BA1700127) were selected for further PD study. Both QC results were within the QC ranges specified by CLSI documents M100-S20. The MBC of tylosin against *A. pleuropneumoniae* and *P. multocida* was 32 μg/mL.

According to the EUCAST ECOFFinder analysis, the ECOFF value (≤64 μg/mL), 97.75% (87 strains) of the *A. pleuropnumoniae* isolates were wild-type, whereas with the same ECOFF value (≤64 μg/mL), 99.72% (363 strains) of the *P. multicoda* isolates were considered wild-type to tylosin using the indicated mode (Table 1).

3.2 PAE of tylosin

The PAE against *A. pleuropneumoniae* following exposure to 0.5×, 1×, and 4× MIC concentrations lasted for 0.55, 0.82, and 1.21 h, respectively, following 2-h incubation (Figure 2B). Similarly, the PAE value against *P. multocida* lasted for 0.55, 0.82, and 1.12 h for 0.5×, 1×, and 4× MIC, respectively (Figure 2D). The results indicated that PAE has a positive relationship with exposure time.

3.3 *In vitro* and *ex vivo* time kill curves

The time kill curves of tylosin against *A. pleuropneumoniae* and *P. multicoda in vitro* and *ex vivo* are illustrated in Figures 2A, C, 3. According to the profiles of curves *in vitro*, increasing drug concentrations induced more rapid and radical bactericidal effects. The bacteria growth recovery observed following exposure to 1× MIC or less of tylosin fades away with markedly decreasing bacterial CFU values (<30 CFU) (Figures 2A, C) following exposure to a greater concentration than 1× MIC of tylosin for 24 h. Bacterial CFU values were also markedly decreased (<30 CFU) in the serum from pigs of the PK experiment from the infected and control groups for samples collected between 0.025 and 8 h (Figure 3). The time kill curves *in vitro* and *ex vivo* were analogical. These findings suggest that tylosin has a concentration-dependent action against *A. pleuropneumoniae* and *P. multicoda* both *in vitro* and *ex vivo*.

3.4 PK analysis of tylosin in healthy and infected pigs

Following 12 h of infection, infected pigs showed noticeable clinical symptoms, including depression, coughing, and a slight difficulty in breathing, compared with healthy pigs, thereby confirming a successfully established a co-infected pig model. Furthermore, body temperature in healthy and infected group was 37.2°C ± 1.5°C, 40.1°C ± 1.1°C ($p < 0.001$), respectively. Furthermore, *apxIVA* and *kmt1* genes for *A. pleuropneumoniae* and *P. multocida*, respectively, were confirmed positive using PCR target gene amplification with the size of 377 bp (Supplementary Figure S1A) and 460 bp (Supplementary Figure S1B) in infected pigs.

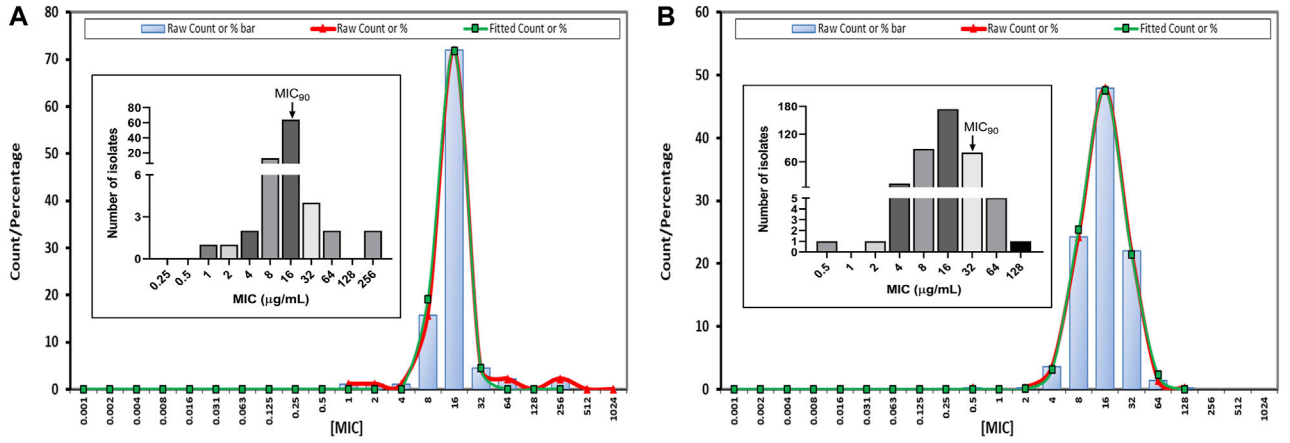

FIGURE 1
Fitted minimum inhibitory concentration (MIC) distribution for tylosin against *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*) (*n* = 89) **(A)** and *Pasteurella multocida* (*P. multocida*) (*n* = 363) **(B)** by ECOFFinder. Insert graphs represent observed MIC distribution with MIC_{90}.

TABLE 1 ***A. pleuropneumoniae*** **and** ***P. multocida modes of MIC wild-type distribution and*** **ECOFF from pig isolate and mixed origins according to the EUCAST.**

Isolates	Pig isolates from this study		Mixed origin EUCAST	
	Mode	ECOFF	Mode	ECOFF
A. pleuropneumoniae	16	64	32	64
P. multocida	16	64	32	ID[a]

[a]Insufficient data.

Tylosin plasma concentrations as a function of time profiles for the healthy and infected groups are presented in Figure 4. The outcomes achieved using blank samples were contrasted with tylosin-injected samples and no interfering peaks were detected.

The PK parameters of tylosin are presented in Table 2. The mean C_{max} of healthy and infected pigs were 5.79 and 3.59 μg/mL, respectively, with a T_{max} of 0.25 h in both groups. The AUC of healthy pigs (13.33 h μg/mL) was higher than that of infected pigs (10.46 h μg/mL) ($p < 0.05$). Differences in half-life were observed between healthy (1.99 h) and infected (1.83 h) pigs with a significant difference ($p < 0.05$).

3.5 Sigmoid E_{max} model

As shown in Table 3, several essential PK/PD parameters are taken into account, including T > MIC, AUC/MIC, and C_{max}/MIC (Nielsen et al., 2011). The bacterial killing effect of tylosin was shown through extended PAE as well as time killing curves, indicating its ability to effectively eliminate bacteria. This suggested that the ratio of AUC/MIC could be a beneficial PK-PD index to treat *A. pleuropneumoniae* and *P. multocida* infections.

Data obtained from simulating the Emax inhibitory sigmoid model for *A. pleuropneumoniae* are summarized in Figure 5; Table 3. The $AUC_{24\ h}$/MIC values for bacteriostatic and bactericidal activities in the plasma of healthy pigs were 0.98 and 1.97 h, respectively (Figure 5A). For infected pigs, 1.03 and 2.54 h were observed to produce bacteriostatic and bactericidal activities, respectively (Figure 5B). The E_{max} value of healthy pigs (7.19 ± 0.27) was slightly less than that of infected pigs (7.34 ± 0.78); however, this difference was not statistically significant.

The results of sigmoidal Emax models for *P. multocida* are summarized in Figure 5; Table 3. The $AUC_{24\ h}$/MIC values for bacteriostatic and bactericidal activities in the plasma of healthy pigs were 1.1 and 1.99 h, respectively (Figure 5C), whereas those for infected pigs were 1.12 and 2.36 h, respectively (Figure 5D). The E_{max} values were 6.98 ± 0.34 and 7.04 ± 0.26 for healthy and infected pigs, respectively, with no significant difference.

3.6 Dose estimation and CO_{PD} determination with MCS

Two levels of antibacterial efficacy were determined by computing the $AUC_{24\ h}$/MIC value using PK/PD integration and *ex vivo* distribution, through MCS in the Oracle Crystal Ball software. The calculated doses for achieving the bactericidal activity of tylosin against *A. pleuropneumoniae* over 24 h were 21.01 and 27.79 mg/kg in healthy and infected pigs, respectively, for a 90% target, according to dose equations (Figure 6; Table 4). The predicted dosages for achieving the bactericidal activity of tylosin against *P. multocida* were 21.21 and 25.17 mg/kg in healthy and infected pigs, respectively, for a 90% target (Figure 7; Table 4). These results suggest that the optimal dosage of tylosin for

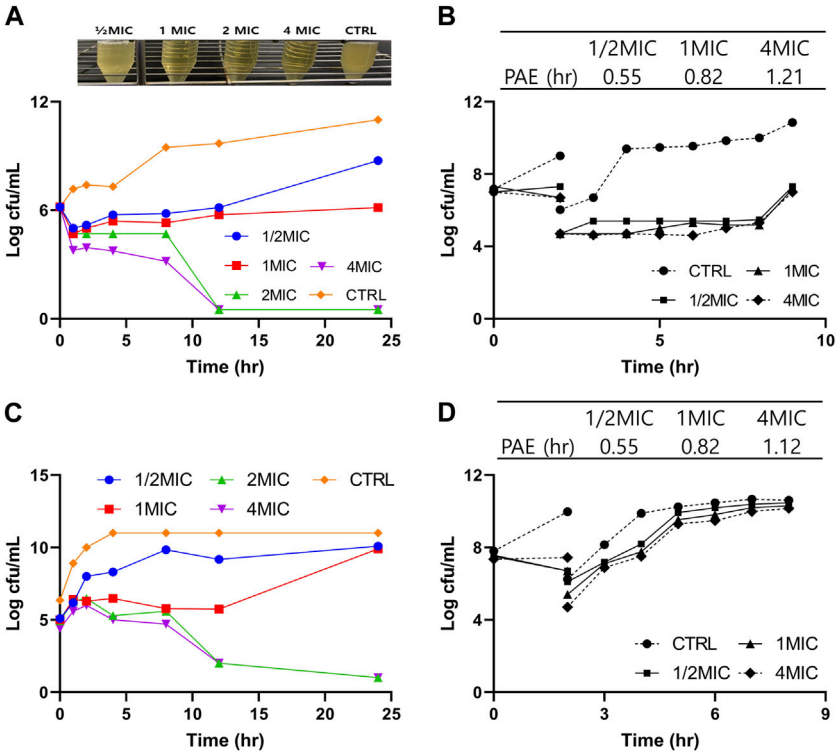

FIGURE 2
Pharmacodynamic (PD) study of tylosin against *A. pleuropneumoniae* and *P. multocida*. **(A)** *In vitro* time kill curves of tylosin against *A. pleuropneumoniae* at 0-, 1-, 2-, 4-, 8-, 12-, and 24-h time points and turbidity observations of tubes containing 1/2x, 1x, 2x, and 4x MIC of tylosin and control (without drug) following 24-h incubation, **(B)** post-antibiotic effect (PAE) following exposure to tylosin against *A. pleuropneumoniae*. **(C)** *In vitro* time kill curves of tylosin against *P. Multocida* at 0-, 1-, 2-, 4-, 8-, 12-, and 24-h time points. **(D)** PAE following exposure to tylosin against *P. multocida*.

A. pleuropneumoniae and *P. multocida* co-infections could be 25.17–27.79 mg/kg to achieve a bactericidal effect.

Furthermore, MCS was conducted for 10,000 iterations using the Oracle Crystal Ball software for determining the CO_{PD} for tylosin target achievement calculations from *ex vivo* PD and PK data (Figure 8). Regarding the CO_{PD} for tylosin against *A. pleuropneumoniae,* the PTA was 83.13% at 4 μg/mL; however, a PTA of >90% was achieved when the MIC was <2 μg/mL in healthy pigs. The PTA for infected pigs was 100% at a MIC value of 2 μg/mL (Figure 8B). A similar pattern was observed for the CO_{PD} for tylosin against *P. multocida*, achieving >90% PTA at a MIC value of 2 μg/mL when the PTA was 100% and 99.25% in healthy and infected pigs, respectively. Therefore, the CO_{PD} for tylosin against *A. pleuropneumoniae* and *P. multocida* in both healthy and infected pigs was 2 μg/mL.

4 Discussion

Antibiotics are widely used in the pig industry for different purposes, including therapeutic, metaphylactic, and prophylactic (Lekagul, Tangcharoensathien, and Yeung, 2019). However, this practice has led to an increased risk of bacteria becoming resistant to these drugs (Holmer et al., 2019). A previous study has shown that *A. pleuropneumoniae*, which causes respiratory diseases in pigs, has developed resistance to several antibiotics, including tylosin (Aarestrup et al., 1998; Vanni et al., 2012). In this study, to ensure animal health and prevent the emergence of antibiotic resistance, determining the most effective treatment and establishing an appropriate dosage regimen are significant. A rational approach to dosing antibiotics, based on PK–PD modeling, can help maximize the benefits of treatment while minimizing the risk of developing resistance (Zhang et al., 2022).

Although macrolides are traditionally classified as bacteriostatic drugs, it is crucial to recognize that bacteriostatic antibiotics do have the capacity to kill bacteria; they simply require higher concentrations than bactericidal agents to achieve specific levels of bacterial reduction (Wald-Dickler et al., 2018). The effects of antibiotics can vary depending on the pathogens they target. For example, azithromycin has been shown to exhibit bactericidal activity against *Streptococcus pyogenes* (Piscitelli et al., 1992), whereas chloramphenicol demonstrates bactericidal activity against *Streptococcus pneumoniae* but acts as a bacteriostatic agent against *Staphylococcus aureus* (Turk, 1977; Rahal and Simberkoff, 1979). Previous study indicated that tylosin effectively eliminated *Streptococcus suis* in a both time and concentration manner, suggesting that the appropriate PK-PD index for tylosin is the AUC/MIC ratio (Huang et al., 2018). Therefore, macrolide do not fit into a single distinct category (T > MIC). In this study, the *ex vivo* time killing curves and prolonged of tylosin demonstrated its bactericidal effect, suggesting AUC/MIC could be a favorable PK-PD index for tylosin against both *A. pleuropneumoniae* and *P. multocida* infections.

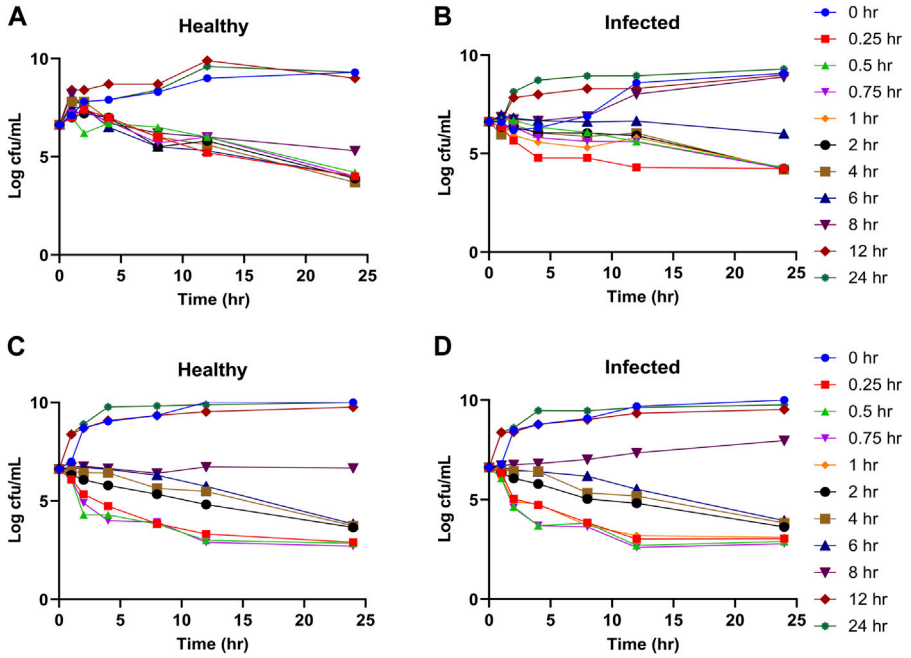

FIGURE 3
Ex vivo time kill curves of tylosin plasma against *A. pleuropneumoniae* (**(A)**, healthy; **(B)**, infected) and *P. multocida* (**(C)**, healthy; **(D)**, infected) at 0-, 1-, 2-, 4-, 8-, 12-, and 24-h time points.

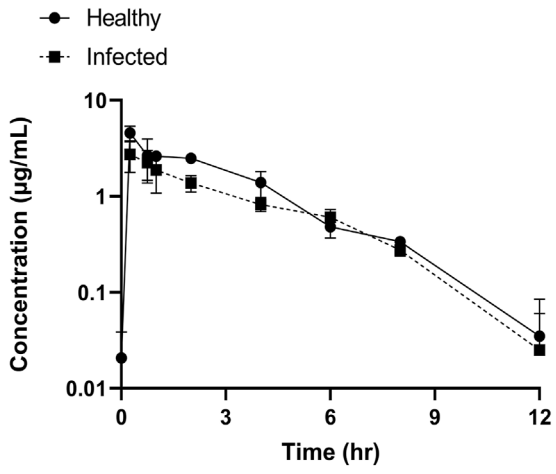

FIGURE 4
Plots of tylosin concentration–time in plasma following IM administration (20 mg/kg).

A. pleuropneumoniae and *P. multocida* are one of the major contributors to porcine respiratory diseases worldwide. The symptoms of infection can include arthritis, breathing difficulties, and lethargy in young pigs (Jensen et al., 1999; Stringer et al., 2022). The incidence rates of *A. pleuropneumoniae* infection range from 25% to 48%, with a significant mortality rate, thereby causing significant economic losses in the industrial pig breeding industry (Vangroenweghe and Thas, 2021). Tylosin is frequently used for controlling *A. pleuropneumoniae* and *P. multocida*; however, there is limited information available on its PK parameters in livestock and poultry. From these perspectives, in the current study, we have established a PD profile of tylosin against 89 and 363 pig isolates of *A. pleuropneumoniae* and *P. multocida* strains, respectively, using *in vitro* and *ex vivo* assays and the PK profile of tylosin in healthy and infected pigs. Using these PK and PD profiles in an integrated PK–PD modeling, we developed an optimized dosage that can maximize tylosin treatment benefits in pigs (Toutain and Lees, 2004).

In the PD profile, the wild-type distribution of MICs of tylosin against *A. pleuropneumoniae* and *P. multocida* strains in the current study ranges from 0.5 to 32 µg/mL, with a similar scope to a previous study (Kim et al., 2001). The MIC_{90} values of 16 and 32 µg/mL of tylosin observed in the current study for *A. pleuropneumoniae* and *P. multocida*, respectively, were higher than those previously reported MIC_{90} values of 4 and 1 µg/mL of another macrolide gamithromycin against European isolates of *A. pleuropneumoniae* and *P. multocida*, respectively (EMA, 2015). Considering the bactericidal effects of tylosin, both species show MBC values that were close to their MIC. Tylosin shows a similar activity against both species with similar MBC and time kill curves with a slight indication of *A. pleuropneumoniae* likely to be the limiting pathogen in eradicating the bacteria in co-infections *in vivo* having a slightly higher MIC range.

We studied the PK of tylosin in both healthy and infected pigs with *A. pleuropneumoniae* and *P. multocida*. In particular, closely monitoring clinical signs, including body temperature, in both healthy and infected pigs is of utmost importance for the infected animals. These parameters provide valuable insights into the physiological changes during health and disease, especially regarding drug administration (Robbins et al., 2014). Body temperature is a critical indicator of an animal's health status and helps assess the severity of infection and immune response (Teixeira et al., 2020). Additionally, measuring lung pH in both

TABLE 2 PK parameters of tylosin following IM administrations in healthy and infected pigs.

Parameters	Healthy	Infected
$T_{1/2}$ (h)	1.99 ± 0.14	1.83 ± 0.51
T_{max} (h)	0.25 ± 0.00	0.25 ± 0.00
C_{max} (μg/mL)	5.79 ± 0.76	3.59 ± 0.66
AUC (h*μg/mL)	13.33 ± 0.78	10.46 ± 1.61
Vz/F (mL/kg)	4,045.83 ± 305.73	5,019.45 ± 2,147.48
Cl/F (mL/h/kg)	1,416.19 ± 100.04	1,905.66 ± 229.96
MRT (h)	2.47 ± 0.23	2.90 ± 0.07

$T_{1/2}$ is half-life; T_{max} is the time to reach the maximum plasma concentration; C_{max} is maximum concentration in plasma; AUC_{last} is the area under the plasma concentration–time curve within 24 h; Vz/F is the apparent volume of distribution; Cl/F is clearance; MRT, is mean residence time; PK, pharmacokinetic; IM, intramuscular.

TABLE 3 Parameters of PK/PD integration for tylosin against *A. pleuropneumoniae* and *P. multocida* following IM administration in pigs.

Parameter	*A. pleuropneumoniae*		*P. multocida*	
	Healthy	Infected	Healthy	Infected
AUC/MIC (h)	1.29 ± 0.39	1.01 ± 0.26	1.29 ± 0.39	1.01 ± 0.26
C_{max}/MIC	0.30 ± 0.04	0.28 ± 0.04	0.30 ± 0.04	0.28 ± 0.04
T > MIC	13.08 ± 6.15	10.50 ± 6.51	6.54 ± 3.07	5.25 ± 3.26
E_{max}	7.19 ± 0.72	7.34 ± 0.78	6.98 ± 0.34	7.04 ± 0.26
EC_{50}	1.21 ± 0.15	1.33 ± 0.21	1.17 ± 0.05	1.06 ± 0.06
E_0	2.84 ± 0.49	2.76 ± 0.39	3.49 ± 0.26	3.41 ± 0.18
γ	2.76 ± 0.90	1.86 ± 0.46	4.78 ± 1.12	2.90 ± 0.36
E_{max}–E_o	4.35 ± 0.23	4.58 ± 0.23	3.49 ± 0.07	3.63 ± 0.07
$AUC_{24\ h}$/MIC for bacteriostatic activity	0.98	1.03	1.1	1.12
$AUC_{24\ h}$/MIC for bactericidal activity	1.97	2.54	1.99	2.36

E_{max} is the maximum difference in bacterial counts; EC_{50} is the value to produce 50% of the maximal antibacterial effect; E_0 is the maximal antibacterial effect; γ is the Hill coefficient.

healthy and infected pigs is essential for understanding the pulmonary environment and its impact on drug behavior and treatment effectiveness (Bikou et al., 2018). Infections or respiratory diseases can alter lung physiology, resulting in changes in lung pH levels, which may influence drug uptake and efficacy, particularly for drugs targeting pulmonary infections (Torres et al., 2017).

PK profiles provide significant information about what the body does to the drugs (Rodríguez-Gascón et al., 2021). This information is essential for optimizing the efficacy and safety of a drug and designing effective dosing regimens that are needed to achieve a therapeutic effect (Koiwai et al., 2021). In this study, a single IM dose of 20 mg/kg of tylosin was administered, and PK parameters in the plasma, including C_{max}, T_{max}, AUC, $T_{1/2}$, and MRT, were observed to be slightly different between healthy and infected pigs. In healthy and infected pigs, C_{max} of the drug in the bloodstream was found to be 5.79 μg/mL and 3.59 μg/mL, respectively. T_{max} was the same in both groups, occurring at 0.25 h after drug administration. However, AUC in healthy pigs (13.33 h·μg/mL) was higher compared to infected pigs (10.46 h·μg/mL), with a statistically significant difference ($p < 0.05$), indicating that healthy pigs had a greater overall exposure to the drug. Furthermore, there were differences in the drug's half-life between healthy (1.99 h) and infected (1.83 h) pigs, with a significant disparity ($p < 0.05$). Moreover, higher clearance values in infected (1,905.66 mL/h/kg) was observed compared to healthy (1,416.19 mL/h/kg). This suggests that the drug persisted for a slightly longer duration in healthy pigs compared to infected pigs. The higher apparent volume of distribution in infected pigs (4,045.83 ± 305.73 mL/kg) compared with healthy pigs (5,019.45 ± 2,147.48 mL/kg) could be attributed to the physiological changes that occur during infection, which may lead to alterations in blood flow, tissue perfusion, and permeability (Smith et al., 2015). We attributed these observed differences in PK to the physiological condition of the animals, which was influenced by the presence of infection (Blot et al., 2014). The $T_{1/2}$ value was 1.5-fold higher than that of a previous study (Huang et al., 2018) despite the dose used in this study being twice as high as in a previous study by (Huang et al., 2018) using 10 mg/kg. This suggests that tylosin has a slow clearance and sustained release, with a longer T_{max} and higher AUC. In other species, tylosin's $T_{1/2}$ value was reported as 2.88 h in ducks (Elazab et al., 2020), and 2.24 h in cattle (Saurit et al., 2002), 1.54 h in dogs (Kim et al., 2008) 0.54 h in cows (Avci and Elmas, 2014). This could be due to variances in animal physiology. Specifically tailored for time-dependent antibiotics, PK/PD integration centers on how drug

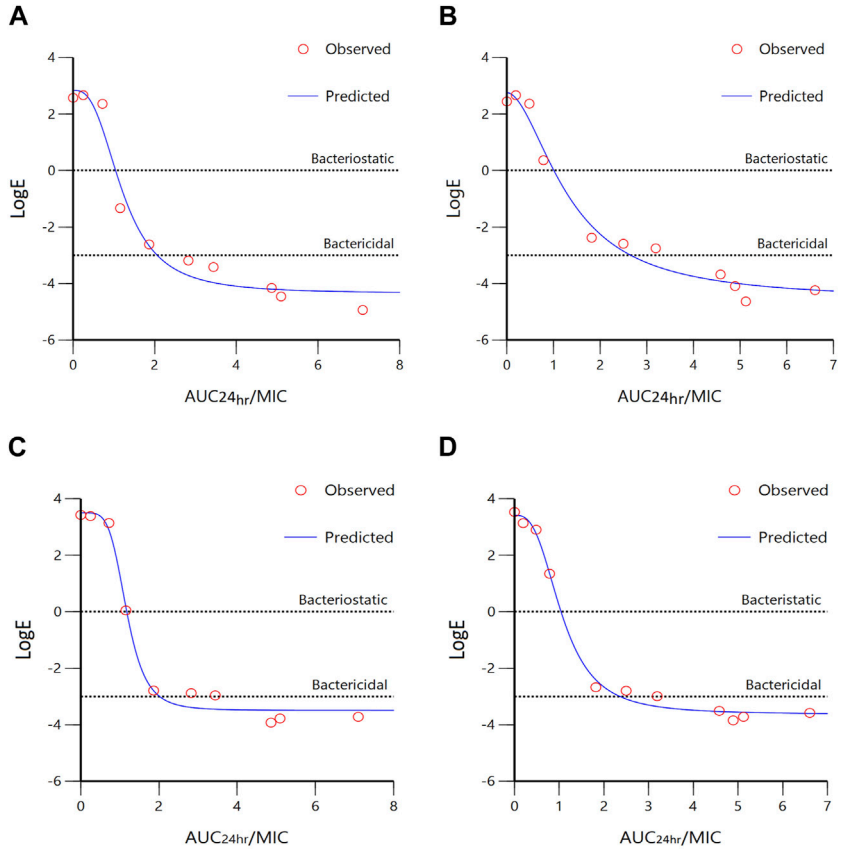

FIGURE 5
Sigmoidal Emax model of *ex vivo* $AUC_{24\ h}$/MIC ratios *versus* LogE (CFU/mL) of tylosin against *A. pleuropneumoniae* (**(A)**, healthy; **(B)**, infected) and *P. multocida* (**(C)**, healthy; **(D)**, infected) within 24 h. Dotted lines represent bacteriostatic (E = 0) and bactericidal (E = −3) activities.

concentration patterns throughout time influence the bactericidal activity against pathogens. Several essential PK/PD parameters are taken into account, including T > MIC, AUC/MIC, and C_{max}/MIC (Nielsen et al., 2011). Previous research have reported the PK of other time dependent antibiotics which correlated with T > MIC (Ahmad et al., 2015; Sjölund et al., 2020). The PK of penicillin G was studied using a 3-compartment model, which included additional tissue compartments (Li et al., 2014). The central volume of distribution and central clearance were determined to be 3.05 L and 16.9 L/h, respectively. The peripheral clearance was found to be 0.52 L/h. The majority of drugs within this category are quickly removed from the body (Smith et al., 2018). Nevertheless, tylosin sets itself apart from other time-dependent antibacterial agents in certain aspects. This research demonstrated that the clinical effectiveness of tylosin with sustained release is influenced by AUC_{24h}/MIC ratio, suggesting distinctions not just in its $T_{1/2}$ but also in how it penetrates tissues and subsequently releases the antibiotic (Van Bambeke and Tulkens, 2001).

The integration of PK/PD principles plays a vital role in comprehending the connection between drug concentration and its pharmacological effects (Zhang et al., 2022). This strategic PK/PD combination enables us to fine-tune dosing schedules and attain the most favorable therapeutic results (Rodríguez-Gascón et al., 2021). To investigate the antibacterial effects of tylosin against *A. pleuropneumoniae*, a PK/PD integration model using the inhibitory sigmoid Emax model was employed. This model demonstrated a strong correlation (R^2 = 0.99) between the observed and predicted efficacy of tylosin against *A. pleuropneumoniae*. The findings indicated that $AUC_{24\ h}$/MIC has the potential to serve as the PK-PD index for this particular model. Infected pigs had individually higher $AUC_{24\ h}$/MIC values required for bacteriostatic and bactericidal effects against *A. pleuropneumoniae* and *P. multocida* than healthy pigs. This result indicates the significance of using clinical PK parameters (PK from infected pigs) in determining the optimal dosage regimen. Based on the $AUC_{24\ h}$/MIC values for bacteriostatic (Healthy: 0.98 h, Infected: 1.03 h) and bactericidal (Healthy: 1.97 h, Infected: 2.54 h) activities against *A. pleuropneumoniae* and, bacteriostatic (Healthy: 1.1 h, Infected: 1.12 h) and bactericidal (Healthy: 1.99 h, infected: 2.36 h) activities against *P. multocida*, desirable dosage was determined. Our study showed that the accurate doses of tylosin for a PTA of ≥90% are 10.45–11.75 and 11.94–15.37 mg/kg for healthy and infected pigs, respectively, which would be sufficient for a bacteriostatic effect against *A. pleuropneumoniae* and *P. multocida* co-infections while 21.01–21.21 and 25.17–27.79 mg/kg for healthy and infected pigs could have bactericidal effect against *A. pleuropneumoniae* and *P. multocida*. However, since PK and PD data were obtained from a small sample size, these predicted daily dosages must be validated in clinical practice.

Potential adverse effects linked to the administration of high doses of drugs to animals may involve gastrointestinal irritation, resulting in symptoms such as diarrhea, vomiting, or

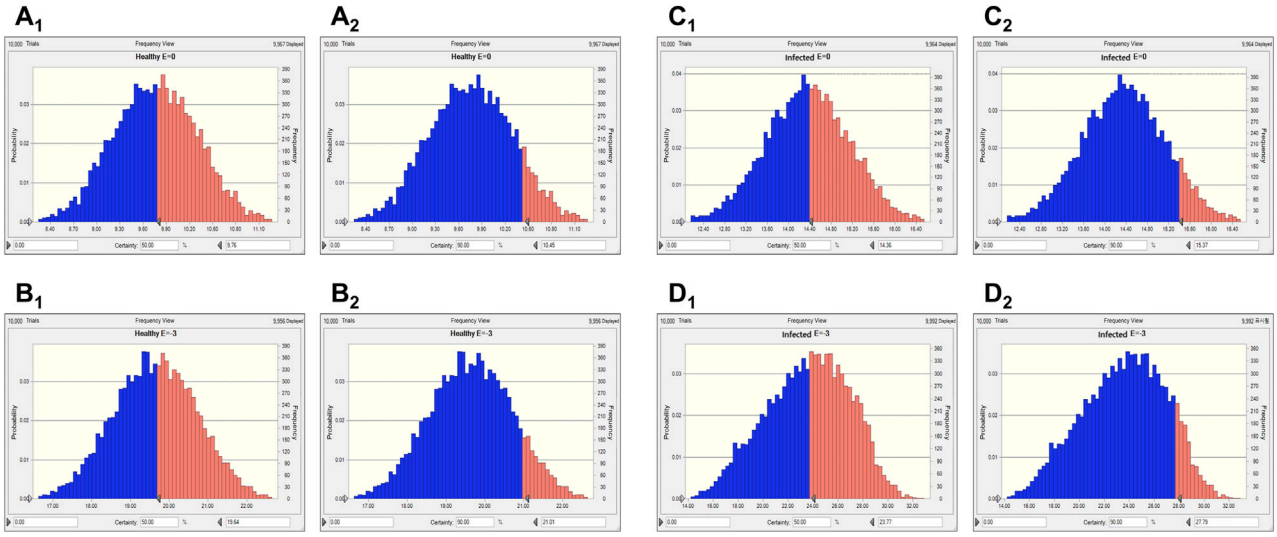

FIGURE 6
Predicted doses of tylosin for treating *A. pleuropneumoniae* at 50% and 90% target ratios in healthy and infected pigs. **(A_1)** The predicted population dose for the bacteriostatic activity at 50% target in healthy pigs; **(A_2)** the predicted population dose for the bacteriostatic activity at 90% target in healthy pigs; **(B_1)** the predicted population dose for the bactericidal activity at 50% target in healthy pigs; **(B_2)** the predicted population dose for the bactericidal activity at 90% target in healthy pigs; **(C_1)** the predicted population dose for the bacteriostatic activity at 50% target in infected pigs; **(C_2)** the predicted population dose for the bacteriostatic activity at 90% target in infected pigs; **(D_1)** the predicted population dose for the bactericidal activity at 50% target in infected pigs; **(D_2)** the predicted population dose for the bactericidal activity at 90% target in infected pigs.

TABLE 4 Predicted daily doses of tylosin for treating *A. pleuropneumoniae* and *P. multocida*.

Predicted dose (mg/kg)			Target ratios	
			50%	90%
A. pleuropneumoniae	Bacteriostatic (E = 0)	Healthy	9.76	10.45
		Infected	14.36	15.37
	Bactericidal (E = −3)	Healthy	19.64	21.01
		Infected	23.77	27.79
P. multocida	Bacteriostatic (E = 0)	Healthy	10.96	11.75
		Infected	11.15	11.94
	Bactericidal (E = −3)	Healthy	19.84	21.21
		Infected	23.48	25.17

other gastrointestinal problems (Makins and Ballinger, 2003). While no previous research on adverse effects after intramuscular administration of tylosin to pigs has been reported, studies have demonstrated that tylosin exhibited low acute oral toxicity in rats, mice and dogs. The LD50 values in rats and mice are in excess of 5,000 mg/kg, and in dogs, it is greater than 800 mg/kg. Overt signs of toxicity observed in dogs included salivation, vomiting, and defecation (CVMP, 1997). Moreover, the frequency of administration and redosing intervals when dealing with wild type strains beyond the MIC distribution can be considered for future investigations. This is especially crucial as tylosin's effectiveness can be influenced by various factors, including dosage, frequency of administration, and the specific bacterial strain being treated, as observed in previous research (Wu et al., 2019).

To determine the susceptibility and resistance of bacteria, a clinical breakpoint is frequently used (Humphries, Abbott, and Hindler, 2019). To establish this breakpoint, factors, including ECOFF, CO_{PD}, and clinical cutoff values, should be considered (Espinel-Ingroff and Turnidge, 2016). The CO_{PD} value for tylosin against *A. pleuropneumoniae* and *P. multocida* was 2 μg/mL, which is lower than the ECOFF value of *A. pleuropneumoniae* and *P. multocida*. This suggests that the current dose of 20 mg/kg may be insufficient for treating wild-type populations. While most studies (Boothe et al., 2002; Avci and Elmas, 2014; Lee et al., 2021) have primarily focused on the drug concentrations in serum/plasma for PK/PD studies, it is crucial to consider the interstitial tissue as it is commonly invaded by most bacteria. Evaluating the antibiotic concentration in the interstitial fluid of the target tissue becomes essential for assessing the antibacterial effects (Matzneller et al., 2013). PD parameters based on plasma concentration of macrolide antibiotics may not be suitable for managing respiratory infections due to much higher concentrations in the respiratory tract compared to serum/plasma (Drusano, 2005). Generally, the plasma concentrations of macrolide antibiotics in animals, even after administering recommended doses, remain notably lower than their respective MIC value (Rose et al., 2013). Therefore, measuring interstitial fluid concentration of tylosin should be considered to support the valid MIC value for clinical application.

The study has several limitations. One limitation of the study is the absence of direct measurements of lung pH. While we considered various physiological parameters, including body temperature, to gain insights into the impact of infection on drug behavior and efficacy, the lack of lung pH measurements is a potential gap in understanding the pulmonary microenvironment's influence on drug response. As mentioned earlier,

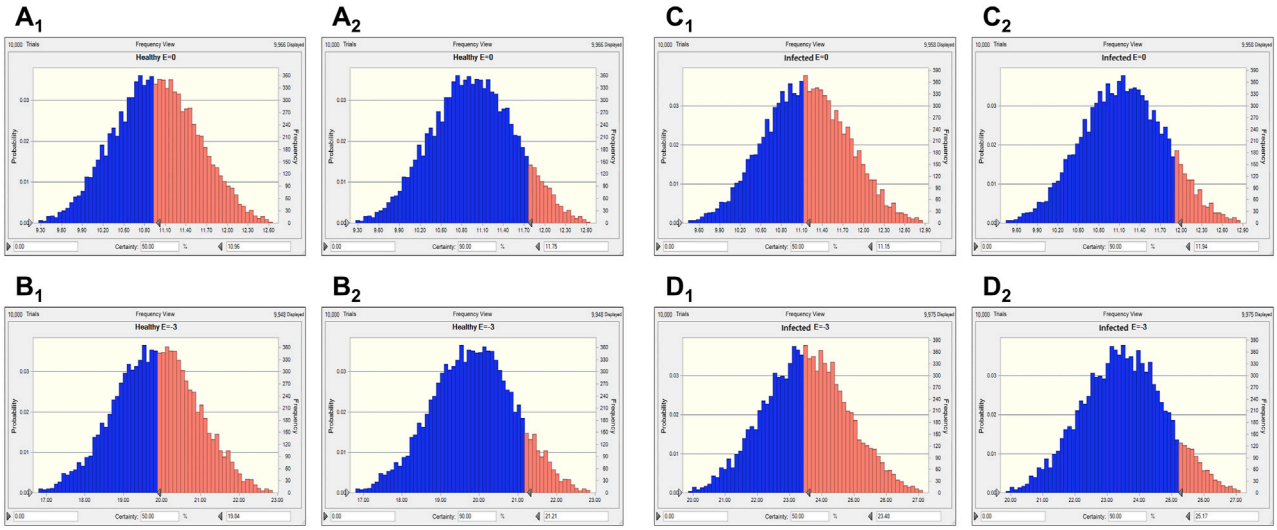

FIGURE 7
Predicted doses of tylosin for treating *P. multocida* at 50% and 90% target ratios in healthy and infected pigs. **(A_1)** The predicted population dose for the bacteriostatic activity at 50% target in healthy pigs; **(A_2)** the predicted population dose for the bacteriostatic activity at 90% target in healthy pigs,; **(B_1)** the predicted population dose for the bactericidal activity at 50% target in healthy pigs; **(B_2)** the predicted population dose for the bactericidal activity at 90% target in healthy pigs; **(C_1)** the predicted population dose for the bacteriostatic activity at 50% target in infected pigs; **(C_2)** the predicted population dose for the bacteriostatic activity at 90% target in infected pigs; **(D_1)** the predicted population dose for the bactericidal activity at 50% target in infected pigs; **(D_2)** the predicted population dose for the bactericidal activity at 90% target in infected pigs.

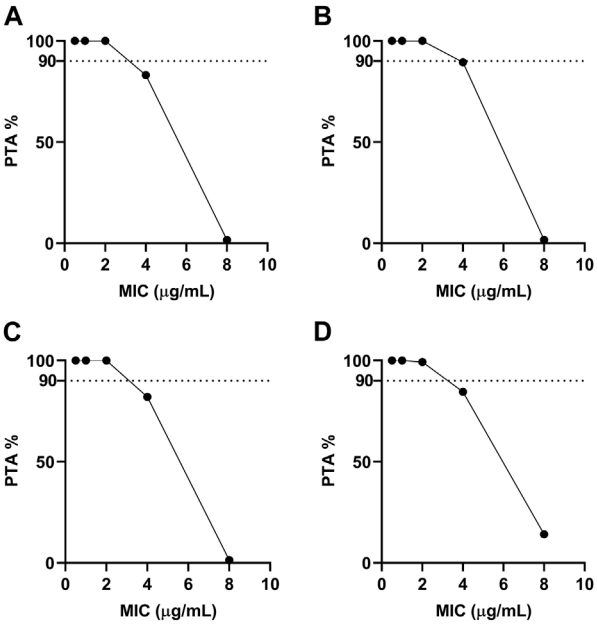

FIGURE 8
Probability of target attainment for treating with single-dose tylosin at 20 mg/kg for *A. pleuropneumoniae* (**(A)**, healthy; **(B)**, infected) and *P. multocida* (**(C)**, healthy; **(D)**, infected). Dotted lines represent 90% target.

infections or respiratory diseases can lead to changes in lung pH levels, which can significantly affect drug uptake and effectiveness, especially for drugs targeting pulmonary infections (Bikou et al., 2018). Including direct measurements of lung pH could have provided more comprehensive data and a clearer understanding of how the drug behaves in the lungs of infected animals, thus enhancing the accuracy of the dosage regimen for tylosin in clinical settings (Schanker and Less, 1977; Taburet et al., 1990).

In this study, the PAE against *A. pleuropneumoniae* was observed after exposure to 0.5×, 1×, and 4× MIC concentrations, lasting for 0.55, 0.82, and 1.21 h, respectively, following a 2-h incubation. Similarly, for *P. multocida*, the PAE durations were 0.55, 0.82, and 1.12 h at 0.5×, 1×, and 4× MIC, respectively. However, there is no data available regarding the PAE against co-inoculation of A. *pleuropneumoniae* and *P. multocida*. Bacteria engage in interactions within their own species, with different species, and sometimes across entirely different genera, families, or even domains (Weiland-Bräuer, 2021). The presence of multiple pathogens in co-infections could have a significant impact on either improving or worsening disease outcomes (Devi et al., 2021). Co-infection has the potential to either extend or shorten the PAE. It is crucial to understand how co-infections influence the PAE, as this knowledge is vital for managing complex bacterial infections and developing effective treatment strategies. Further research can explore the intricate interplay between these strains and its implications for disease severity and treatment outcomes.

The impact of the immune response and the size of the bacterial inoculum on treatment outcomes should be considered (Li et al., 2017). Mice were exposed to *Citrobacter rodentium* to investigate how tylosin influence host responses to physiological stress (Brown et al., 2016). Tylosin treatment led to a decrease in the expression of antimicrobial peptide (β-defensin 1) and helper T cell 17 cytokine (interleukin-17a) in the intestine. Conversely, it demonstrated a significant increase in the levels of interleukin-17a and regulatory T cell cytokine (interleukin-10). In addition to its direct antibacterial effects against mycoplasmosis, tylosin seems to have an additional advantage as it enhances cell-mediated immune responses in chickens (Baba et al., 1998). Taking into account that the

immune response can influence the effectiveness of tylosin treatment, conducting additional research on the immune response to tylosin could provide valuable support for the findings related to dosage optimization. *In vitro* studies have extensively examined the impact of inoculum size on antibacterial activity (Athamna et al., 2004). However, there are limited reports discussing the influence of inoculum size on the *in vivo* efficacy of antimicrobial agents (Chuang et al., 1998). Previous research indicated that the *in vitro* antimicrobial activity and *in vivo* efficacy of fluoroquinolones were minimally affected by the inoculum size, unlike carbapenems. This suggests that the reduced bactericidal activity or *in vitro* PAE of carbapenems and fluoroquinolones might be linked to their diminished *in vivo* protective effect against infections caused by high bacterial inocula of *S. aureus* or *Pseudomonas aeruginosa*. These findings could provide valuable insights into assessing the efficacy of antimicrobial agents in other animal infections. In the current study, the optimal dosage of tylosin was obtained with an inoculum size of 2.0×10^9. However, it is essential to tailor treatment strategies based on the specific bacterial burden to enhance the likelihood of successful therapeutic outcomes. Considering the bacterial inoculum size in treatment decisions can be crucial for optimizing antimicrobial efficacy and combating infections effectively in animals.

In conclusion, the present study has shown that the PK parameters of infected animals are representative of clinical conditions and can be useful in designing optimal drug dosage regimens. Therefore, the current study on the PK characteristics of tylosin in healthy and infected pigs with *A. pleuropneumoniae* and *P. multocida* is of practical significance. The CO_{PD} (μg/mL) value determined in our study holds greater importance and practical relevance in preventing the emergence of resistance compared to the ECOFF value. Furthermore, tylosin could be a valuable treatment option for effectively managing pigs co-infected with *A. pleuropneumoniae* and *P. multocida*. A carefully selected dosage regimen of 11.94–15.37 mg/kg can achieve the desired bacteriostatic activity, while a dosage of 25.17–27.79 mg/kg is determined to achieve bactericidal effect. Nevertheless, the influence of the immune response and the size of the bacterial inoculum on treatment outcomes must be taken into account (Li et al., 2017). To validate and optimize the usage of tylosin in veterinary settings, further research is necessary to evaluate the most effective dosage for treating infected pigs.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by The Animal Ethics Committee of the Petobio Clinical Institute. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

E-BL: Writing–review and editing, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing–original draft. MA: Formal Analysis, Writing–review and editing. JP: Formal Analysis, Writing–review and editing. DT: Writing–review and editing, Supervision, Validation. S-CP: Supervision, Writing–review and editing, Conceptualization, Funding acquisition, Project administration.

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Conflict of interest

JP was employed by DIVA Bio Incorporation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1258403/full#supplementary-material

References

Aarestrup, F. M., Bager, F., Jensen, N. E., Madsen, M., Meyling, A., and Wegener, H. C. (1998). Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. *APMIS* 106, 606–622. doi:10.1111/j.1699-0463.1998.tb01391.x

Ahmad, I., Hao, H., Huang, L., Sanders, P., Wang, X., Chen, D., et al. (2015). Integration of PK/PD for dose optimization of Cefquinome against *Staphylococcus aureus* causing septicemia in cattle. *Front. Microbiol.* 6, 588. doi:10.3389/fmicb.2015.00588

Aliabadi, F. S., and Lees, P. (2001). Pharmacokinetics and pharmacodynamics of danofloxacin in serum and tissue fluids of goats following intravenous and intramuscular administration. *Am. J. Vet. Res.* 62, 1979–1989. doi:10.2460/ajvr.2001.62.1979

Arsic, B., Barber, J., Čikoš, A., Mladenovic, M., Stankovic, N., and Novak, P. (2018). 16-membered macrolide antibiotics: A review. *Int. J. Antimicrob. Agents* 51, 283–298. doi:10.1016/j.ijantimicag.2017.05.020

Assavacheep, P., and Rycroft, A. N. (2013). Survival of Actinobacillus pleuropneumoniae outside the pig. *Res. Vet. Sci.* 94, 22–26. doi:10.1016/j.rvsc.2012.07.024

Athamna, A., Athamna, M., Medlej, B., Bast, D. J., and Rubinstein, E. (2004). *In vitro* post-antibiotic effect of fluoroquinolones, macrolides, β-lactams, tetracyclines, vancomycin, clindamycin, linezolid, chloramphenicol, quinupristin/dalfopristin and rifampicin on Bacillus anthracis. *J. Antimicrob. Chemother.* 53, 609–615. doi:10.1093/jac/dkh130

Avci, T., and Elmas, M. (2014). Milk and blood pharmacokinetics of tylosin and tilmicosin following parenteral administrations to cows. *Sci. World J.* 2014, 869096. doi:10.1155/2014/869096

Baba, T., Yamashita, N., Kodama, H., Mukamoto, M., Asada, M., Nakamoto, K., et al. (1998). Effect of tylosin tartrate (Tylan Soluble) on cellular immune responses in chickens. *Poult. Sci.* 77, 1306–1311. doi:10.1093/ps/77.9.1306

Bauer, L. A. (2008). "Chapter 2. Clinical pharmacokinetic equations and calculations," in *Applied clinical pharmacokinetics*. L. A. Bauer (New York, NY: The McGraw-Hill Companies).

Bikou, O., Ishikawa, K., Fish, K. M., Zarragoikoetxea, I., Hajjar, R. J., and Aguero, J. (2018). Modeling pulmonary hypertension: A pig model of postcapillary pulmonary hypertension. *Methods Mol. Biol.* 1816, 367–383. doi:10.1007/978-1-4939-8597-5_29

Blot, S. I., Pea, F., and Lipman, J. (2014). The effect of pathophysiology on pharmacokinetics in the critically ill patient — concepts appraised by the example of antimicrobial agents. *Adv. Drug Deliv. Rev.* 77, 3–11. doi:10.1016/j.addr.2014.07.006

Boothe, D. M., Boeckh, A., Boothe, H. W., Wilkie, S., and Jones, S. (2002). Plasma concentrations of enrofloxacin and its active metabolite ciprofloxacin in dogs following single oral administration of enrofloxacin at 7.5, 10, or 20 mg/kg. *Vet. Ther.* 3, 409–419.

Brown, K., Zaytsoff, S. J. M., Uwiera, R. R. E., and Inglis, G. D. (2016). Antimicrobial growth promoters modulate host responses in mice with a defined intestinal microbiota. *Sci. Rep.* 6, 38377. doi:10.1038/srep38377

Cao, X.-Y., Dong, M., Shen, J.-Z., Wu, B.-B., Wu, C.-M., Du, X.-D., et al. (2006). Tilmicosin and tylosin have anti-inflammatory properties via modulation of COX-2 and iNOS gene expression and production of cytokines in LPS-induced macrophages and monocytes. *Int. J. Antimicrob. Agents* 27, 431–438. doi:10.1016/j.ijantimicag.2005.12.010

Chae, C. (2016). Porcine respiratory disease complex: Interaction of vaccination and porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, and Mycoplasma hyopneumoniae. *Vet. J.* 212, 1–6. doi:10.1016/j.tvjl.2015.10.030

Cheong, Y., Oh, C., Lee, K., and Cho, K.-H. (2017). Survey of porcine respiratory disease complex-associated pathogens among commercial pig farms in Korea via oral fluid method. *J. Vet. Sci.* 18, 283–289. doi:10.4142/jvs.2017.18.3.283

Chua, H. C., and Tam, V. H. (2022). Optimizing clinical outcomes through rational dosing strategies: Roles of pharmacokinetic/pharmacodynamic modeling tools. *Open forum Infect. Dis.* 9, ofac626. doi:10.1093/ofid/ofac626

Chuang, Y. C., Ko, W. C., Wang, S. T., Liu, J. W., Kuo, C. F., Wu, J. J., et al. (1998). Minocycline and cefotaxime in the treatment of experimental murine Vibrio vulnificus infection. *Antimicrob. Agents Chemother.* 42, 1319–1322. doi:10.1128/AAC.42.6.1319

CLSI (2017). *Performance standards for antimicrobial susceptibility testing*. Wayne, PA: Clinical and Laboratory Standards Institute guideline M100.

Couper, A., Cromie, L., Neeve, S., Pommier, P., Keïta, A., and Pagot, E. (2006). Treatment of pneumonia in pigs with long-acting injectable tylosin. *Vet. Rec.* 159, 805–807.

Cristinacce, A., Wright, J. G., Stone, G. G., Hammond, J., McFadyen, L., and Raber, S. (2019). A retrospective analysis of probability of target attainment in community-acquired pneumonia: Ceftaroline fosamil versus comparators. *Infect. Dis. Ther.* 8, 185–198. doi:10.1007/s40121-019-0243-4

CVMP, (1997). *Tylosin summary report EMEA MRL/205/97-Final*. London, UK: European Medicines Agency.

Derendorf, H., Lesko, L. J., Chaikin, P., Colburn, W. A., Lee, P., Miller, R., et al. (2000). Pharmacokinetic/pharmacodynamic modeling in drug research and development. *J. Clin. Pharmacol.* 40, 1399–1418. doi:10.1177/009127000004001211

DeRosa, D. C., Veenhuizen, M. F., Bade, D. J., and Shryock, T. R. (2000). *In vitro* susceptibility of porcine respiratory pathogens to tilmicosin. *J. Vet. Diagn. Investig. Off. Publ. Am. Assoc. Vet. Lab. Diagn. Inc.* 12, 541–546. doi:10.1177/104063870001200608

Devi, P., Khan, A., Chattopadhyay, P., Mehta, P., Sahni, S., Sharma, S., et al. (2021). Co-Infections as modulators of disease outcome: Minor players or major players? *Front. Microbiol.* 12, 664386. doi:10.3389/fmicb.2021.664386

Dorey, L., Hobson, S., and Lees, P. (2017). Potency of marbofloxacin for pig pneumonia pathogens Actinobacillus pleuropneumoniae and Pasteurella multocida: Comparison of growth media. *Res. Vet. Sci.* 111, 43–48. doi:10.1016/j.rvsc.2016.11.001

Drusano, G. L. (2005). Infection site concentrations: Their therapeutic importance and the macrolide and macrolide-like class of antibiotics. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* 25, 150S-158S–158S. doi:10.1592/phco.2005.25.12part2.150S

Eddicks, M., Eddicks, L., Stadler, J., Hermanns, W., and Ritzmann, M. (2021). The porcine respiratory disease complex (PRDC) - a clinical review. *Tierarztl Prax. Ausg G. Grosstiere Nutztiere* 49, 120–132. doi:10.1055/a-1403-1976

Elazab, S. T., Elshater, N. S., Hashem, Y. H., Park, S.-C., and Hsu, W. H. (2020). Pharmacokinetics, tissue residues, and *ex vivo* pharmacodynamics of tylosin against Mycoplasma anatis in ducks. *J. Vet. Pharmacol. Ther.* 43, 57–66. doi:10.1111/jvp.12819

Ema, (2015). "CVMP assessment report for ZACTRAN for pigs (EMEA/V/C/000129/X/0027)," in *The European agency for the evaluation of medicinal products*. [WWW Document]. Eur Med Agency. Available at: https://www.ema.europa.eu/en/documents/variation-report/zactran-v-c-129-x-0027-epar-assessment-report-variation_en.pdf.

Espinel-Ingroff, A., and Turnidge, J. (2016). The role of epidemiological cutoff values (ECVs/ECOFFs) in antifungal susceptibility testing and interpretation for uncommon yeasts and moulds. *Rev. Iberoam. Micol.* 33, 63–75. doi:10.1016/j.riam.2016.04.001

Giordano, A., Dincman, T., Clyburn, B. E., Steed, L. L., and Rockey, D. C. (2015). Clinical features and outcomes of Pasteurella multocida infection. *Med. Baltim.* 94, e1285. doi:10.1097/MD.0000000000001285

Hälli, O., Haimi-Hakala, M., Oliviero, C., and Heinonen, M. (2020). Herd-level risk factors for chronic pleurisy in finishing pigs: A case-control study. *Porc. Heal Manag.* 6, 21. doi:10.1186/s40813-020-00156-0

Hamilton, T. D., Roe, J. M., Hayes, C. M., and Webster, A. J. (1998). Effects of ammonia inhalation and acetic acid pretreatment on colonization kinetics of toxigenic Pasteurella multocida within upper respiratory tracts of swine. *J. Clin. Microbiol.* 36, 1260–1265. doi:10.1128/JCM.36.5.1260-1265.1998

Holmer, I., Salomonsen, C. M., Jorsal, S. E., Astrup, L. B., Jensen, V. F., Høg, B. B., et al. (2019). Antibiotic resistance in porcine pathogenic bacteria and relation to antibiotic usage. *BMC Vet. Res.* 15, 449. doi:10.1186/s12917-019-2162-8

Huang, A., Wang, S., Guo, J., Gu, Y., Li, J., Huang, L., et al. (2021). Prudent use of tylosin for treatment of mycoplasma gallisepticum based on its clinical breakpoint and lung microbiota shift. *Front. Microbiol.* 12, 712473. doi:10.3389/fmicb.2021.712473

Huang, L., Zhang, H., Li, M., Ahmad, I., Wang, Y., and Yuan, Z. (2018). Pharmacokinetic-pharmacodynamic modeling of tylosin against Streptococcus suis in pigs. *BMC Vet. Res.* 14, 319. doi:10.1186/s12917-018-1645-3

Humphries, R. M., Abbott, A. N., and Hindler, J. A. (2019). Understanding and addressing CLSI breakpoint revisions: A primer for clinical laboratories. *J. Clin. Microbiol.* 57, e00203-19. doi:10.1128/JCM.00203-19

Jensen, T. K., Boye, M., Hagedorn-Olsen, T., Riising, H. J., and Angen, O. (1999). Actinobacillus pleuropneumoniae osteomyelitis in pigs demonstrated by fluorescent *in situ* hybridization. *Vet. Pathol.* 36, 258–261. doi:10.1354/vp.36-3-258

Kim, B., Min, K., Choi, C., Cho, W. S., Cheon, D. S., Kwon, D., et al. (2001). Antimicrobial susceptibility of Actinobacillus pleuropneumoniae isolated from pigs in Korea using new standardized procedures. *J. Vet. Med. Sci.* 63, 341–342. doi:10.1292/jvms.63.341

Kim, M.-H., Gebru, E., Chang, Z.-Q., Choi, J., Hwang, M., Kang, E.-H., et al. (2008). Comparative pharmacokinetics of tylosin or florfenicol after a single intramuscular administration at two different doses of tylosin-florfenicol combination in pigs. *J. Vet. Med. Sci.* 70, 99–102. doi:10.1292/jvms.70.99

Koiwai, K., El-Cheikh, R., Thai, H.-T., Brillac, C., Fau, J.-B., Veyrat-Follet, C., et al. (2021). PK/PD modeling analysis for dosing regimen selection of isatuximab as single agent and in combination therapy in patients with multiple myeloma. *CPT pharmacometrics Syst. Pharmacol.* 10, 928–940. doi:10.1002/psp4.12666

Lee, C.-R., Cho, I. H., Jeong, B. C., and Lee, S. H. (2013). Strategies to minimize antibiotic resistance. *Int. J. Environ. Res. Public Health* 10, 4274–4305. doi:10.3390/ijerph10094274

Lee, J.-H., Kim, G. W., Kwon, M.-G., and Seo, J. S. (2021). Pharmacokinetic-pharmacodynamic profile, bioavailability, and withdrawal time of tylosin tartrate following a single intramuscular administration in olive flounder (*Paralichthys olivaceus*). *Anim open access J MDPI* 11, 2468. doi:10.3390/ani11082468

Lei, Z., Liu, Q., Yang, S., Yang, B., Khaliq, H., Li, K., et al. (2018). PK-PD integration modeling and cutoff value of florfenicol against Streptococcus suis in pigs. *Front. Pharmacol.* 9, 2. doi:10.3389/fphar.2018.00002

Lekagul, A., Tangcharoensathien, V., and Yeung, S. (2019). Patterns of antibiotic use in global pig production: A systematic review. *Vet. Anim. Sci.* 7, 100058. doi:10.1016/j.vas.2019.100058

Lenz, K. D., Klosterman, K. E., Mukundan, H., and Kubicek-Sutherland, J. Z. (2021). Macrolides: From toxins to therapeutics. *Toxins (Basel)* 13, 347. doi:10.3390/toxins13050347

Li, J., Xie, S., Ahmed, S., Wang, F., Gu, Y., Zhang, C., et al. (2017). Antimicrobial activity and resistance: Influencing factors. *Front. Pharmacol.* 8, 364. doi:10.3389/fphar.2017.00364

Li, M., Gehring, R., Tell, L., Baynes, R., Huang, Q., and Riviere, J. E. (2014). Interspecies mixed-effect pharmacokinetic modeling of penicillin G in cattle and swine. *Antimicrob. Agents Chemother.* 58, 4495–4503. doi:10.1128/AAC.02806-14

Loera-Muro, A., Ramírez-Castillo, F. Y., Moreno-Flores, A. C., Martin, E. M., Avelar-González, F. J., and Guerrero-Barrera, A. L. (2021). Actinobacillus pleuropneumoniae surviving on environmental multi-species biofilms in swine farms. *Front. Vet. Sci.* 8, 722683. doi:10.3389/fvets.2021.722683

Luo, W., Qin, H., Chen, D., Wu, M., Meng, K., Zhang, A., et al. (2020). The dose regimen formulation of tilmicosin against Lawsonia intracellularis in pigs by pharmacokinetic-pharmacodynamic (PK-PD) model. *Microb. Pathog.* 147, 104389. doi:10.1016/j.micpath.2020.104389

Makins, R., and Ballinger, A. (2003). Gastrointestinal side effects of drugs. *Expert Opin. Drug Saf.* 2, 421–429. doi:10.1517/14740338.2.4.421

Matzneller, P., Krasniqi, S., Kinzig, M., Sörgel, F., Hüttner, S., Lackner, E., et al. (2013). Blood, tissue, and intracellular concentrations of azithromycin during and after end of therapy. *Antimicrob. Agents Chemother.* 57, 1736–1742. doi:10.1128/AAC.02011-12

Nielsen, E. I., Cars, O., and Friberg, L. E. (2011). Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: A step toward model-based dose optimization. *Antimicrob. Agents Chemother.* 55, 4619–4630. doi:10.1128/AAC.00182-11

Piscitelli, S. C., Danziger, L. H., and Rodvold, K. A. (1992). Clarithromycin and azithromycin: New macrolide antibiotics. *Clin. Pharm.* 11, 137–152.

Plasencia-Muñoz, B., Avelar-González, F. J., De la Garza, M., Jacques, M., Moreno-Flores, A., and Guerrero-Barrera, A. L. (2020). Actinobacillus pleuropneumoniae interaction with swine endothelial cells. *Front. Vet. Sci.* 7, 569370. doi:10.3389/fvets.2020.569370

Prats, C., El Korchi, G., Francesch, R., Arboix, M., and Pérez, B. (2002). Disposition kinetics of tylosin administered intravenously and intramuscularly to pigs. *Res. Vet. Sci.* 73, 141–144. doi:10.1016/S0034-5288(02)00036-X

Rahal, J. J. J., and Simberkoff, M. S. (1979). Bactericidal and bacteriostatic action of chloramphenicol against memingeal pathogens. *Antimicrob. Agents Chemother.* 16, 13–18. doi:10.1128/AAC.16.1.13

Rizk, M. L., Bhavnani, S. M., Drusano, G., Dane, A., Eakin, A. E., Guina, T., et al. (2019). Considerations for dose selection and clinical pharmacokinetics/pharmacodynamics for the development of antibacterial agents. *Antimicrob. Agents Chemother.* 63, e02309-18. doi:10.1128/AAC.02309-18

Robbins, R. C., Almond, G., and Byers, E. (2014). Swine diseases and disorders. *Encycl. Agric. Food Syst.*, 261–276. doi:10.1016/B978-0-444-52512-3.00134-0

Rodríguez-Gascón, A., Solinís, M. Á., and Isla, A. (2021). The role of PK/PD analysis in the development and evaluation of antimicrobials. *Pharmaceutics* 13, 833. doi:10.3390/pharmaceutics13060833

Ronaghinia, A. A., Birch, J. M., Frandsen, H. L., Toutain, P.-L., Damborg, P., and Struve, T. (2021). Evaluating a tylosin dosage regimen for treatment of Staphylococcus delphini infection in mink (neovison vison): A pharmacokinetic-pharmacodynamic approach. *Vet. Res.* 52, 34. doi:10.1186/s13567-021-00906-0

Rose, M., Menge, M., Bohland, C., Zschiesche, E., Wilhelm, C., Kilp, S., et al. (2013). Pharmacokinetics of tildipirosin in porcine plasma, lung tissue, and bronchial fluid and effects of test conditions on *in vitro* activity against reference strains and field isolates of Actinobacillus pleuropneumoniae. *J. Vet. Pharmacol. Ther.* 36, 140–153. doi:10.1111/j.1365-2885.2012.01397.x

Saurit, A. R., Rubio, M., Baroni, E., San Andrés, M., Sánchez, S., and Boggio, J. C. (2002). Some comparative aspects of the pharmacokinetics of tylosin in buffaloes and cattle. *Vet. Res. Commun.* 26, 49–54. doi:10.1023/A:1013305721813

Schaller, A., Djordjevic, S. P., Eamens, G. J., Forbes, W. A., Kuhn, R., Kuhnert, P., et al. (2001). Identification and detection of Actinobacillus pleuropneumoniae by PCR based on the gene apxIVA. *Vet. Microbiol.* 79, 47–62. doi:10.1016/s0378-1135(00)00345-x

Schanker, L. S., and Less, M. J. (1977). Lung pH and pulmonary absorption of nonvolatile drugs in the rat. *Drug Metab. Dispos.* 5, 174–178.

Schwarz, S., Silley, P., Simjee, S., Woodford, N., van Duijkeren, E., Johnson, A. P., et al. (2010). Editorial: Assessing the antimicrobial susceptibility of bacteria obtained from animals. *J. Antimicrob. Chemother.* 65, 601–604. doi:10.1093/jac/dkq037

Shalaby, A., Bakry, N., and El-Demerdash, A. (2021). Virulence attitude estimation of Pasteurella multocida isolates in embryonated chicken eggs. *Arch. Microbiol.* 203, 6153–6162. doi:10.1007/s00203-021-02579-x

Shen, Y., and Kuti, J. L. (2023). Optimizing antibiotic dosing regimens for nosocomial pneumonia: A window of opportunity for pharmacokinetic and pharmacodynamic modeling. *Expert Opin. Drug Metab. Toxicol.* 19, 13–25. doi:10.1080/17425255.2023.2178896

Sjölund, M., Ekstrand, C., Wallgren, P., Bondesson, U., Pringle, M., and Bengtsson, B. (2020). Exposure to benzylpenicillin after different dosage regimens in growing pigs. *Acta Vet. Scand.* 62, 55. doi:10.1186/s13028-020-00552-0

Smith, D. A., Beaumont, K., Maurer, T. S., and Di, L. (2015). Volume of distribution in drug design. *J. Med. Chem.* 58, 5691–5698. doi:10.1021/acs.jmedchem.5b00201

Smith, P. W., Zuccotto, F., Bates, R. H., Martinez-Martinez, M. S., Read, K. D., Peet, C., et al. (2018). Pharmacokinetics of β-lactam antibiotics: Clues from the past to help discover long-acting oral drugs in the future. *ACS Infect. Dis.* 4, 1439–1447. doi:10.1021/acsinfecdis.8b00160

Stringer, O. W., Li, Y., Bossé, J. T., Forrest, M. S., Hernandez-Garcia, J., Tucker, A. W., et al. (2022). Rapid detection of Actinobacillus pleuropneumoniae from clinical samples using recombinase polymerase amplification. *Front. Vet. Sci.* 9, 805382. doi:10.3389/fvets.2022.805382

Taburet, A. M., Tollier, C., and Richard, C. (1990). The effect of respiratory disorders on clinical pharmacokinetic variables. *Clin. Pharmacokinet.* 19, 462–490. doi:10.2165/00003088-199019060-00004

Teixeira, D. L., Boyle, L. A., and Enríquez-Hidalgo, D. (2020). Skin temperature of slaughter pigs with tail lesions. *Front. Vet. Sci.* 7, 198. doi:10.3389/fvets.2020.00198

Torres, I. M., Demirdjian, S., Vargas, J., Goodale, B. C., and Berwin, B. (2017). Acidosis increases the susceptibility of respiratory epithelial cells to Pseudomonas aeruginosa-induced cytotoxicity. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 313, L126-L137–L137. doi:10.1152/ajplung.00524.2016

Toutain, P. L., and Bousquet-Mélou, A. (2004). Bioavailability and its assessment. *J. Vet. Pharmacol. Ther.* 27, 455–466. doi:10.1111/j.1365-2885.2004.00604.x

Toutain, P. L., and Lees, P. (2004). Integration and modelling of pharmacokinetic and pharmacodynamic data to optimize dosage regimens in veterinary medicine. *J. Vet. Pharmacol. Ther.* 27, 467–477. doi:10.1111/j.1365-2885.2004.00613.x

Turk, D. C. (1977). A comparison of chloramphenicol and ampicillin as bactericidal agents for Haemophilus influenzae type B. *J. Med. Microbiol.* 10, 127–131. doi:10.1099/00222615-10-1-127

Turnidge, J. D. (1998). The pharmacodynamics of beta-lactams. *Clin Infect Dis Off Publ Infect Dis Soc Am* 27, 10–22. doi:10.1086/514622

Turnidge, J., and Paterson, D. L. (2007). Setting and revising antibacterial susceptibility breakpoints. *Clin. Microbiol. Rev.* 20, 391–408. table of contents. doi:10.1128/CMR.00047-06

Urbanová, Z., Zahradníková, M., Schovánek, V., Polák, L., Rabas, P., Sechser, T., et al. (1975). Effect of tylosin in pigs. *Vet. Med. (Praha)* 20, 251–260.

Van Bambeke, F., and Tulkens, P. M. (2001). Macrolides: Pharmacokinetics and pharmacodynamics. *Int. J. Antimicrob. Agents* 18, 17–23. doi:10.1016/S0924-8579(01)00406-X

Vangroenweghe, F. A. C. J., and Thas, O. (2021). *Seasonal variation in prevalence of mycoplasma hyopneumoniae and other respiratory pathogens in peri-weaned, post-weaned, and fattening pigs with clinical signs of respiratory diseases in Belgian and Dutch pig herds, using a tracheobronchial swab sampling technique, and their associations with local weather conditions*. Basel, Switzerland: Pathog, 10. doi:10.3390/pathogens10091202

Vanni, M., Merenda, M., Barigazzi, G., Garbarino, C., Luppi, A., Tognetti, R., et al. (2012). Antimicrobial resistance of Actinobacillus pleuropneumoniae isolated from swine. *Vet. Microbiol.* 156, 172–177. doi:10.1016/j.vetmic.2011.10.022

Wald-Dickler, N., Holtom, P., and Spellberg, B. (2018). Busting the myth of "static vs cidal": A systemic literature review. *Clin Infect Dis Off Publ Infect Dis Soc Am* 66, 1470–1474. doi:10.1093/cid/cix1127

Weiland-Bräuer, N. (2021). Friends or foes-microbial interactions in nature. *Biol. (Basel)* 10, 496. doi:10.3390/biology10060496

Woeste, K., and Grosse Beilage, E. (2007). Transmission of agents of the porcine respiratory disease complex (PRDC) between swine herds: A review. Part 1--diagnosis, transmission by animal contact. *Dtsch. Tierarztl Wochenschr* 114 (324-326), 324–326, 328-337.

Wu, F., Tokach, M. D., DeRouchey, J. M., Dritz, S. S., Woodworth, J. C., Goodband, R. D., et al. (2019). Effects of tylosin administration routes on the prevalence of antimicrobial resistance among fecal enterococci of finishing swine. *Foodborne Pathog. Dis.* 16, 309–316. doi:10.1089/fpd.2018.2551

Zhang, L., Xie, H., Wang, Y., Wang, H., Hu, J., and Zhang, G. (2022). Pharmacodynamic parameters of pharmacokinetic/pharmacodynamic (PK/PD) integration models. *Front. Vet. Sci.* 9, 860472. doi:10.3389/fvets.2022.860472

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Analysis of drug resistance in pulmonary tuberculosis patients with positive sputum tuberculosis culture in Northeast China

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Objective: The objective of this study is to determine the drug resistance status of pulmonary tuberculosis patients in Jilin Province.

Methods: A retrospective survey was conducted on 395 sputum culture TB-positive patients admitted to the tuberculosis hospital in Jilin Province in 2019. Sputum samples were cultured in acidic Roche medium. Drug sensitivity testing was conducted using the proportional method. Sensitivity was reported if the percentage of drug resistance was less than 1%, and resistance was reported if the percentage was ≥1%. Statistical analysis was performed using SPSS 22.0.

Results: 395 tuberculosis patients with positive sputum tuberculosis culture were included in the study, with 102 being initially treated and 293 being retreated. The study population consisted of 283 males and 112 females. Sex, age, nationality, occupation, marital status, diabetes comorbidity, initial treatment, normal health status, BCG vaccine vaccination, smoking, and alcohol consumption were considered as factors that may affect the rate of multidrug resistance. And only the history of treatment (initial treatment) was associated with multidrug resistance (p = 0.032). This indicates that retreatment is the most significant risk factor for the occurrence of multidrug resistance in tuberculosis. The multidrug resistance rate in retreated patients is 3.764 times higher than that in initially treated patients.

Conclusion: The prevalence of multidrug-resistant is higher in retreated patients compared to initially treated patients in the study population. Multidrug resistance is only associated with the treatment history (initial retreatment) and not with other factors.

KEYWORDS

pulmonary tuberculosis, drug resistant, multidrug-resistant, retreated, initially treated

Introduction

Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (Mtb). The respiratory system is usually the first to be affected, but it can also cause damage to other tissues (Holmes et al., 2017). The gastrointestinal system, lymphatic network, skin, central nervous system, musculoskeletal system, and reproductive system are among the most frequently involved organ systems (Adigun and Singh, 2023). According to a report from the World Health Organization (WHO) in 2017, the global estimated incidence rate of

tuberculosis had been decreasing by 1.5% annually since 2000 (Jilani et al., 2023). It was predicted that in 2021, there will be 780,000 new tuberculosis patients in China (compared to 842,000 in 2020 and 833,000 in 2019), and the incidence rate of tuberculosis had been declining in China since 2000 (Bagcchi, 2023) Among the 30 countries with a high burden of tuberculosis, China's estimated incidence rate (780,000) was lower than that of India (2.95 million) and Indonesia (969,000), ranking second in 2020 and third in 2021 (Bagcchi, 2023). However, despite global efforts to eradicate tuberculosis, the incidence rate and mortality of the disease remained high worldwide. Factors such as the increasing elderly population, the AIDS epidemic, and the influx of people in certain regions have contributed to the high infectivity, drug resistance, and regional differences associated with tuberculosis (Chakaya et al., 2021; Jali et al., 2022). Drug-resistant tuberculosis was not only a significant public health issue but also a serious social problem (Alame Emane et al., 2021; da Silva et al., 2022). Since the onset of the COVID-19 pandemic in 2019, the human immune system has been negatively impacted, resulting in a higher occurrence and reoccurrence of tuberculosis (Tadolini et al., 2020).

Research has shown that there is widespread resistance to anti-tuberculosis drugs. The prevalence rate of resistance to Isoniazid (INH) and Rifampicin (RIF) is significantly higher than previously reported, and there is still a high proportion of newly diagnosed cases with multidrug-resistant tuberculosis (Reta et al., 2022). Drug-resistant tuberculosis has increased the burden of global antimicrobial drug resistance, resulting in significant medical care expenditure and resource consumption in affected countries (Liang et al., 2022; Liebenberg et al., 2022). The world is currently facing severe challenges such as the dual infection of TB bacteria and the coronavirus, as well as HIV. Therefore, the prevention and control of tuberculosis remains a crucial task (Shariq et al., 2022; Yang et al., 2022).

Being one of the three northeastern provinces in China, Jilin Province was also grappling with respiratory diseases. This study aims to analyze the drug resistance of sputum culture-positive patients in the tuberculosis hospitals of Jilin Province. It will observe and analyze the drug resistance of pulmonary tuberculosis in northern China and investigate the factors related to drug-resistant bacteria. The findings will serve as a foundation for rational drug use in clinical settings and the development of tuberculosis prevention and control strategies.

Methods

Materials and methods

Data sources and experimental methods

Data sources

A total of 395 sputum culture-positive TB patients admitted to the tuberculosis Hospital in Jilin Province were selected in 2019. Among them, 102 cases were initially treated and 293 cases were retreated, with 283 males and 112 females. Test Methods According to the requirements of the Bacteriology test procedure for tuberculosis diagnosis issued by the China Anti-TB Association, sputum samples were inoculated into acidic Roche medium for culture. Drug sensitivity testing was performed using the proportional method (Habimana-Mucyo et al., 2023). If the drug resistance percentage is less than 1%, it is reported as sensitive (S); if the percentage is ≥1%, it is reported as resistant (R). The drug resistance percentage is calculated as follows: (number of colonies growing on drug-containing medium/number of colonies growing on control medium) × 100% (Getnet et al., 2017).

Related definitions

Determination of Initial and Secondary Treatment, as well as Resistance to Initial and Secondary Treatment:

(1) Initially treated patients: patients who have not undergone anti-tuberculosis treatment or have been treated for less than 1 month.
(2) Retreated patients: patients who have been on anti-tuberculosis treatment for more than 1 month (Zhang et al., 2016).
(3) Initial treatment/initial drug resistance: tuberculosis patients who have not received anti-tuberculosis treatment in the past or have been treated for less than 1 month, and the tuberculosis bacteria they are infected with are resistant to at least one anti-tuberculosis drug.
(4) Retreatment case: a patient who had been treated for any form of TB before but has initiated treatment again following relapse or default or failure to cure of the 1st regimen (Getnet et al., 2017).

Patients who had received multiple treatments with anti-tuberculosis drugs were excluded. These drugs primarily consist of the 16 medications mentioned in this study.

Drug resistance determination

According to the WHO definition of drug-resistant tuberculosis, drug resistance patterns are classified as follows (Song et al., 2019; Shibabaw et al., 2020): Monoresistance refers to tuberculosis bacteria infected by patients that are resistant to only one anti-tuberculosis drug; Multiresistance refers to the resistance of tuberculosis bacteria to more than one anti-tuberculosis drug, excluding simultaneous resistance to Isoniazid and Rifampicin; Multidrug resistance (MDR) refers to resistance to Isoniazid and Rifampicin at the same time; Extensively drug-resistant (XDR) refers to MDR that is also resistant to at least one of fluoroquinolones and second-line anti-tuberculosis injections (kanamycin, capreomycin, amikacin). According to expert consensus and treatment guidelines (Chinese Medic al Association, 2023), this study involves 4 first-line oral anti-tuberculosis drugs and 12 other anti-tuberculosis drugs.

Statistical methods

A database was established using Epidata 3.1, and statistical analysis was performed using SPSS 22.0. Econometric data was represented using M (QL, QU), and comparisons between groups were made using the rank sum test. Counting data was expressed in

TABLE 1 Comparison of general conditions of the two groups.

Items		Initially treated patients (n = 102)	Retreated patients (n = 293)	Z/t/χ^2	p
Sex	Male	76 (74.51%)	207 (70.65%)	0.55	0.456
	Female	26 (25.49%)	86 (29.35%)		
Age (years)		44.0 ± 25.0	47.0 ± 17.5	−1.897	0.058
BMI		22.35 ± 2.70	22.29 ± 2.90	−0.094	0.411
Smoking	Yes	29 (28.43%)	67 (22.87%)	1.273	0.259
	No	73 (71.57%)	226 (77.13%)		

TABLE 2 Comparison of drug resistance among patients.

Drug resistance types	Initially treated patients (n = 102)	Retreated patients (n = 293)	χ^2	p
Monoresistance	26 (25.5%)	85 (29.0%)	0.464	0.496
Multiresistance	44 (43.1%)	148 (50.5%)	1.647	0.199
MDR	3 (2.9%)	3010.2 (%)	4.939	0.026
XDR	2 (2.0%)	5 (1.7%)	0.028	0.867

terms of rate or composition ratio, and comparisons between groups were made using the χ^2-test. Univariate analysis of influencing factors was conducted using unconditional logistic regression.

Quality control

Culture and identification of strains were carried out in strict accordance with the "tuberculosis Diagnostic Laboratory Test Procedures". The sensitivity test was uniformly conducted using the proportional method. The drug resistance rate was calculated based on the first sputum culture isolation of the patient after admission. Operators received unified training, and a dual entry system was used for data entry.

Results

In this study, 102 patients were initially treated, while 293 patients underwent re-treatment. Out of these patients, there were 283 males and 112 females. The age distribution does not follow a normal distribution, with the minimum age being 18 years old, the maximum age being 80 years old, and the average age being (47.0 ± 19.0) years old. There were 96 smokers and 299 non-smokers included in the study. No statistically significant differences were found in terms of gender, age, and smoking status between the initial and recurrent patients ($p > 0.05$) (Table 1).

When comparing the rates of Monoresistance, Multiresistance, MDR and XDR between initial and recurrent patients, the results showed that only the difference in MDR rate was statistically significant ($\chi^2 = 4.939$, $p = 0.026$). The MDR rate of patients in the retreatment group (10.2%) was significantly higher than that of patients in the initial treatment group (2.9%) (Table 2).

Among the first-line oral antituberculosis drugs, the drug resistance rate in the first treatment group was highest for Isoniazid (15.7%), followed by Rifampicin (6.9%) and Ethambutol and Rifabutin (3.9%). In the retreatment group, the highest resistance rates were observed for Isoniazid (24.6%), Rifampicin (17.4%), Rifabutin (11.3%), and Ethambutol (6.5%). The drug resistance rate to Rifampicin and Rifabutin was significantly higher in retreated patients compared to untreated patients ($p = 0.010$, $p = 0.028$). Among second-line oral antituberculosis drugs, the resistance rate to propafenicotinide was higher in retreated patients (63.1%) compared to initially treated patients (46.1%), with a statistically significant difference ($p = 0.003$). There was no statistically significant difference ($p > 0.05$) in the resistance of initially treated patients to other anti-tuberculosis drugs (Table 3).

The factors examined in this study to determine their impact on the rate of multidrug resistance were sex, age, nationality, occupation, marital status, presence of diabetes, initial treatment and retreatment history, general health status, BCG vaccine vaccination, smoking, and alcohol consumption. Results from the single factor logistic regression analysis revealed that only the treatment history (i.e., initial treatment and retreatment) was found to be significantly associated with multidrug resistance ($p = 0.032$). The odds ratio (OR) and 95% confidence interval (CI) were calculated to be 3.764 (1.123, 12.612), and the 95% CI did not include the value 1. This suggests that retreatment is a risk factor for the development of multidrug resistance in patients. Furthermore, patients who undergo retreatment are 3.764 times more likely to develop multidrug resistance compared to those who receive initial treatment (Table 4).

Discussions

The 2021 WHO Global Tuberculosis Report provides a grim assessment of the global tuberculosis incidence. The annual decline in tuberculosis incidence rates has stalled or even reversed, and the

TABLE 3 16 types of anti-tuberculosis drug resistance in two groups of patients.

Drug sensitivity	Initially treated patient (n = 102)		Retreated patients (n = 293)		Total (%)	χ^2	*P*
	Drug resistance	Rate (%)	Drug resistance	Rate (%)			
Ciprofloxacin	8	7.8	29	9.9	37 (9.4)	0.376	0.054
Amikacin	1	1.0	3	1.0	4 (1.0)	0.000	1.000
Capreomycin	2	2.0	4	1.4	6 (1.5)	0.000	1.000
Propylthioisoniazid	47	46.1	185	63.1	232 (58.7)	9.087	0.003
Isoniazid Aminosalicylate Tablets	16	15.7	26	8.9	42 (10.6)	3.695	0.055
Moxifloxacin	17	16.7	68	23.2	85 (21.5)	1.917	0.166
Clarithromycin	11	10.8	27	9.2	38 (9.6)	0.242	0.623
Rifabutin	4	3.9	33	11.3	37 (9.4)	4.803	0.028
Ethambutol	4	3.9	19	6.5	23 (5.8)	0.906	0.341
Isoniazid	16	15.7	72	24.6	88 (22.3)	3.451	0.063
Rifampicin	7	6.9	51	17.4	58 (14.7)	6.714	0.010
Streptomycin	19	18.6	74	25.3	93 (23.5)	1.847	0.174
Levofloxacin	9	8.8	26	8.9	35 (8.9)	0	0.988
Ofloxacin	14	13.7	61	20.8	75 (19.0)	2.475	0.116
Linezolid	6	5.9	22	7.5	28 (7.1)	0.303	0.582
Gatifloxacin	7	6.9	16	5.5	23 (5.8)	0.271	0.603

estimated death toll from tuberculosis has increased (Chakaya et al., 2022). Although there are differences in prevention and treatment models both domestically and internationally, drug-resistant tuberculosis clearly imposes a heavy economic burden on patients' families and increases the risk factors for social stability (Devoid et al., 2022; Jarde et al., 2022; Trauer, 2023). The plan to eradicate tuberculosis still requires significant efforts (Aia et al., 2022). Through an analysis of drug resistance in tuberculosis patients admitted to Jilin Provincial Tuberculosis Hospital in 2023, several findings were made. Comparing the rates of single drug resistance, multi-drug resistance, and broad drug resistance between patients undergoing initial and retreatment, it was found that the multi-drug resistance rate among retreatment patients (10.2%) was significantly higher than that among patients receiving initial treatment (2.9%). These findings align with results reported in other parts of the world. For instance, a meta-analysis of 18,908 tuberculosis patients across 24 studies showed a slight variation in drug resistance rates between initial treatment (2.64%) and retreatment (11.54%) (Reta et al., 2022). In a study of 207 tuberculosis patients in Osun State (Oyedeji et al., 2020), Nigeria, the prevalence rates of multi-drug resistant tuberculosis in previously treated and new cases were 7.0% and 3.5%, respectively. Similarly, the first national survey of tuberculosis drug resistance in Lao People's Democratic Republic revealed a retreatment multi-drug resistance rate of 2.3%, whereas the rate for initial treatment was 0.5% (Iem et al., 2019). Another study conducted in Basra, Iraq, involving 2,542 new and old patients found that the drug resistance rate among retreatment patients was significantly higher than that among initially diagnosed patients (20.3% vs. 2.4%) (Mohammed et al., 2022). These findings suggest that retreatment typically results in higher drug resistance rates in underdeveloped regions, which is consistent with the conclusions of this study. Comparing these results with those from domestic studies, the retreatment multi-drug resistance rate among 236 tuberculosis cases in Huairou District of Beijing was found to be higher at 34.5%, as opposed to 6.8% for initial treatment. This discrepancy may be attributed to differences in population density and drug use, as the capital city has a much higher population density than Jilin Province (Zhang et al., 2021). Finally, comparing the results to Dalian City in Liaoning Province, another city in northeastern China, this study found lower rates of initial multidrug resistance (2.9%) and retreatment multidrug resistance (10.2%) compared to Dalian City's rates of 5.8% and 17.7%, respectively. This indicates that Jilin Province has a lower drug resistance rate and demonstrates effective control measures (Ganapathi et al., 2017; Wang et al., 2019). Overall, the drug resistance rates of both initially diagnosed and retreatment cases have decreased in recent years, but the decrease is more significant in initially diagnosed cases, while the drug resistance rate among retreatment cases continues to rise (Duan et al., 2016). Insufficient public health resources, lack of public attention, and noncompliance with treatment regulations have contributed to the difficulty in treating tuberculosis, and retreatment cases are more likely to develop into multidrug-resistant cases.

Four first-line oral antituberculosis drugs have been identified, with the following drug resistance rates: Isoniazid (22.3%), Rifampicin (14.7%), Rifabutin (9.4%), and Ethambutol (5.8%). The success of TB prevention efforts in Jilin Province in recent

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TABLE 4 Univariate logistic regression analysis of multidrug resistance.

Variables		PR (%)	Waldχ^2	P	OR	95% CI for OR	
						Lower	Upper
Sex	Male	71.6	0.067	0.795	1.000		
	Female	28.4			1.108	0.510	2.410
Age	<40	31.6	4.575	0.102	1.000		
	40–49	30.4			0.487	0.200	1.183
	≥50	38.0			0.435	0.185	1.022
BMI			4.082	0.167	0.822	0.156	4.335
Nationality	Han	93.7	3.026	0.388	1.000		
	Manchu	3.8			1.809	0.389	8.406
	Korean	1.5			2.352	0.266	20.809
	Other	0.8			5.879	0.517	66.796
Occupation	Farmer	58.0	4.736	0.192	1.000		
	Worker	2.5			1.479	0.176	12.416
	Unemployed	29.9			1.939	0.923	4.074
	Other	9.6			0.360	0.046	2.795
Marital status	Unmarried	9.1	5.339	0.149	1.000		
	Married	70.9			0.784	0.257	2.398
	Widow	18.0			0.232	0.040	1.332
	Divorced	2.0			2.667	0.396	17.977
Diabetes	No	89.4	0.766	0.381	1.000		
	Yes	10.6			0.519	0.120	2.252
TB treatment	Initial	25.8	4.617	0.032	1.000		
	Retreated	74.2			3.764	1.123	12.612
Health condition	Good	11.6	5.000	0.082	1.000		
	Common	75.7			0.588	0.209	1.652
	Poor	12.7			1.562	0.472	5.172
BCG vaccination	No	84.3	2.291	0.130	1.000		
	Yes	15.7			3.079	0.718	13.214
Smoking	No	75.7	0	0.993	1.000		
	Yes	24.3			0.996	0.434	2.289
Drinking	No	76.7	0.981	0.322	1.000		
	Yes	23.3			1.485	0.679	3.246

years means that the survey results can inform the selection of clinical drugs in the area. It also provides a foundation for treating patients in the region and controlling drug-resistant tuberculosis. The drug resistance rates for Rifampicin and Rifabutin were significantly higher among retreated patients compared to untreated patients (p = 0.010, p = 0.028, respectively). This suggests that Rifampicin and Rifabutin may be more suitable for treating newly diagnosed pulmonary tuberculosis patients. The resistance rate of retreated patients to propafenamide, a second-line oral antituberculosis drug, was significantly higher than that of newly diagnosed patients (63.1% vs. 46.1%). This indicates that it may be advisable to avoid using highly resistant drugs in future clinical treatments for retreated patients. This study confirms that treatment history, including initial and recurrent treatment, is the only factor associated with multidrug resistance when using univariate logistic regression analysis. Recurrent patients are

3.764 times more likely to have multidrug resistance compared to initial treatment patients, regardless of gender, age, or smoking status. Other studies have shown that factors such as age<30 years, unemployment rate, economic status, residence, lifestyle, and previous treatment of tuberculosis are also related to the occurrence of multidrug-resistant tuberculosis (MDR-TB) (Ali et al., 2019; Chakaya et al., 2021; Lecai et al., 2021). The development of initially treated drug-resistant tuberculosis into retreatment drug-resistant tuberculosis can be influenced by various factors, including long treatment cycles, improper use of antituberculosis drugs, drug side effects, poor patient compliance, economic difficulties, and other reasons. Additionally, the production of drug-resistant strains, low patient immunity, and comorbidities such as diabetes or other pulmonary infections are also factors that cannot be ignored (Shivekar et al., 2020; Antonio-Arques et al., 2021; Antimicrobial Resistance Collaborators, 2022; Williams et al., 2022). However, this study did not find an association between BMI and binding resistance, while previous studies have shown that higher BMI was negatively associated with being a relapse/defaulter/treatment-failure cases (Goswami et al., 2014; Sharma et al., 2019). Improved nutritional status, which can positively influence immunity and treatment outcome, could be cited as a possible explanation; but their findings could also have been an artifact of reverse causation, as implicated by deterioration of general health among relapse/defaulter/treatment-failure cases. This study failed to establish a correlation between BMI and tuberculosis resistance, potentially due to the absence of long-term BMI changes in patients. Hence, additional research is required to ascertain the relationship between BMI and tuberculosis resistance.

To sum up, timely treatment and standardized management of newly diagnosed tuberculosis patients are crucial in preventing the occurrence of drug-resistant tuberculosis. Medical institutions should improve relevant regulations and systems (Pontali et al., 2019; Williams et al., 2022), establish a new tuberculosis monitoring system (Jiang et al., 2021), and collaborate with relevant departments to develop social and economic models and programs. Furthermore, collective efforts are needed to increase awareness of tuberculosis (Katiyar and Katiyar, 2019; Long et al., 2020). A program tailored to our socio-economic conditions is necessary to enhance tuberculosis management by private sectors, promote unity in treatment, provide better treatment outcomes, and help prevent the spread of the disease within the community while inhibiting the development of drug resistance.

Conclusion

The prevalence of multidrug-resistant is higher in retreated patients compared to initially treated patients in the study population. Multidrug resistance is only associated with the treatment history (initial retreatment) and not with other factors.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the First Hospital of Jilin University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LS: Conceptualization, Formal Analysis, Methodology, Writing–original draft, Writing–review and editing. WR: Conceptualization, Investigation, Methodology, Software, Writing–original draft, Writing–review and editing. YY: Investigation, Writing–original draft. LX: Data curation, Formal Analysis, Investigation, Writing–review and editing. CY: Funding acquisition, Resources, Visualization, Writing–review and editing. TZ: Conceptualization, Funding acquisition, Resources, Writing–original draft, Writing–review and editing. CH: Conceptualization, Funding acquisition, Project administration, Resources, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Adigun, R., and Singh, R. (2023). "Tuberculosis," in *StatPearls* (Treasure Island (FL): StatPearls Publishing).

Aia, P., Viney, K., Kal, M., Kisomb, J., Yasi, R., Wangchuk, L. Z., et al. (2022). The economic burden of TB faced by patients and affected families in Papua New Guinea. *Int. J. Tuberc. Lung Dis.* 26 (10), 934–941. doi:10.5588/ijtld.21.0664

Alame Emane, A. K., Guo, X., Takiff, H. E., and Liu, S. (2021). Drug resistance, fitness and compensatory mutations in *Mycobacterium tuberculosis*. *Mycobacterium Tuberc. Tuberc. (Edinb).* 129, 102091. doi:10.1016/j.tube.2021.102091

Ali, M. H., Alrasheedy, A. A., Hassali, M. A., Kibuule, D., and Godman, B. (2019). Predictors of multidrug-resistant tuberculosis (MDR-TB) in Sudan. *Antibiot. (Basel)* 8 (3), 90. doi:10.3390/antibiotics8030090

Antimicrobial Resistance Collaborators (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet* 399 (10325), 629–655. doi:10.1016/S0140-6736(21)02724-0

Antonio-Arques, V., Franch-Nadal, J., and Caylà, J. A. (2021). Diabetes and tuberculosis: A syndemic complicated by COVID-19. *Med. Clin. Barc.* 157 (6), 288–293. doi:10.1016/j.medcle.2021.04.006

Bagcchi, S. (2023). WHO's global tuberculosis report 2022. *Lancet Microbe* 4 (1), e20. doi:10.1016/S2666-5247(22)00359-7

Chakaya, J., Khan, M., Ntoumi, F., Aklillu, E., Fatima, R., Mwaba, P., et al. (2021). Global tuberculosis report 2020 - reflections on the global TB burden, treatment and prevention efforts. *Int. J. Infect. Dis.* 113, S7–s12. doi:10.1016/j.ijid.2021.02.107

Chakaya, J., Petersen, E., Nantanda, R., Mungai, B. N., Migliori, G. B., Amanullah, F., et al. (2022). The WHO Global Tuberculosis 2021 Report - not so good news and turning the tide back to End TB. *Int. J. Infect. Dis.* 124 (1), S26–S29. doi:10.1016/j.ijid.2022.03.011

Chinese Medical Association (2023). Expert consensus on surgical treatment of multidrug-resistant and rifampicin-resistant pulmonary tuberculosis in China. *Zhonghua Jie He He Hu Xi Za Zhi* 46 (2), 111–120. doi:10.3760/cma.j.cn112147-20221222-00986

da Silva, E. H., Lima, E., Dos Santos, T. R., and Padoveze, M. C. (2022). Prevalence and incidence of tuberculosis in health workers: A systematic review of the literature. *Am. J. Infect. Control* 50 (7), 820–827. doi:10.1016/j.ajic.2022.01.021

Devoid, I., Sillah, A. K., Sutherland, J., Owolabi, O., Ivanova, O., Govathson, C., et al. (2022). The household economic burden of drug-susceptible TB diagnosis and treatment in the Gambia. *Int. J. Tuberc. Lung Dis.* 26 (12), 1162–1169. doi:10.5588/ijtld.22.0091

Duan, Q., Chen, Z., Chen, C., Zhang, Z., Lu, Z., Yang, Y., et al. (2016). The prevalence of drug-resistant tuberculosis in mainland China: An updated systematic review and meta-analysis. *PLoS One* 11 (2), e0148041. doi:10.1371/journal.pone.0148041

Ganapathi, A. M., Mulvihill, M. S., Englum, B. R., Speicher, P. J., Gulack, B. C., Osho, A. A., et al. (2017). Transplant size mismatch in restrictive lung disease. *Transpl. Int.* 30 (4), 378–387. doi:10.1111/tri.12913

Getnet, F., Sileshi, H., Seifu, W., Yirga, S., and Alemu, A. S. (2017). Do retreatment tuberculosis patients need special treatment response follow-up beyond the standard regimen? Finding of five-year retrospective study in pastoralist setting. *BMC Infect. Dis.* 17 (1), 762. doi:10.1186/s12879-017-2882-y

Goswami, A., Chakraborty, U., Mahapatra, T., Mahapatra, S., Mukherjee, T., Das, S., et al. (2014). Correlates of treatment outcomes and drug resistance among pulmonary tuberculosis patients attending tertiary care hospitals of Kolkata, India. *PLoS One* 9 (10), e109563. doi:10.1371/journal.pone.0109563

Habimana-Mucyo, Y., Dushime, A., Migambi, P., Habiyambere, I., Semuto Ngabonziza, J. C., and Decroo, T. (2023). Continuous surveillance of drug-resistant TB burden in Rwanda: A retrospective cross-sectional study. *Int. Health* 15 (4), 357–364. doi:10.1093/inthealth/ihac039

K. K. Holmes, S. Bertozzi, B. R. Bloom, and P. Jha (Editors) (2017). *Major infectious diseases*. 3rd ed. (Washington (DC): The International Bank for Reconstruction and Development/The World Bank).

Iem, V., Dean, A., Zignol, M., Vongvichit, P., Inthavong, D., Siphanthong, S., et al. (2019). Low prevalence of MDR-TB in Lao PDR: Results from the first national anti-tuberculosis drug resistance survey. *Trop. Med. Int. Health* 24 (4), 421–431. doi:10.1111/tmi.13205

Jali, M. V., Kavital, A., and Hiremath, M. B. (2022). Challenges of diabetes in elderly TB patients. *Indian J. Tuberc.* 69 (2), S264–S266. doi:10.1016/j.ijtb.2022.10.017

Jarde, A., Siqueira, N., Afaq, S., Naz, F., Irfan, M., Tufail, P., et al. (2022). Addressing TB multimorbidity in policy and practice: An exploratory survey of TB providers in 27 high-TB burden countries. *PLOS Glob. Public Health* 2 (12), e0001205. doi:10.1371/journal.pgph.0001205

Jiang, W. X., Huang, F., Tang, S. L., Wang, N., Du, X., Zhang, H., et al. (2021). Implementing a new tuberculosis surveillance system in zhejiang, Jilin and ningxia: Improvements, challenges and implications for China's national health information system. *Infect. Dis. Poverty* 10 (1), 22. doi:10.1186/s40249-021-00811-w

Jilani, T. N., Avula, A., Zafar Gondal, A., and Siddiqui, A. H. (2023). "Active tuberculosis," in *StatPearls* (Treasure Island (FL): StatPearls Publishing).

Katiyar, S. K., and Katiyar, S. (2019). Protocol for the management of newly diagnosed cases of tuberculosis. *Indian J. Tuberc.* 66 (4), 507–515. doi:10.1016/j.ijtb.2019.11.003

Lecai, J., Mijiti, P., Chuangyue, H., Mingzhen, L., Qian, G., Weiguo, T., et al. (2021). Predictors and trends of MDR/RR-TB in shenzhen China: A retrospective 2012-2020 period analysis. *Infect. Drug Resist* 14, 4481–4491. doi:10.2147/IDR.S335329

Liang, S., Ma, J., Wang, G., Shao, J., Li, J., Deng, H., et al. (2022). The application of artificial intelligence in the diagnosis and drug resistance prediction of pulmonary tuberculosis. *Front. Med. (Lausanne).* 9, 935080. doi:10.3389/fmed.2022.935080

Liebenberg, D., Gordhan, B. G., and Kana, B. D. (2022). Drug resistant tuberculosis: Implications for transmission, diagnosis, and disease management. *Front. Cell Infect. Microbiol.* 12, 943545. doi:10.3389/fcimb.2022.943545

Long, Q., Jiang, W., Dong, D., Chen, J., Xiang, L., Li, Q., et al. (2020). A new financing model for tuberculosis (TB) care in China: Challenges of policy development and lessons learned from the implementation. *Int. J. Environ. Res. Public Health* 17 (4), 1400. doi:10.3390/ijerph17041400

Mohammed, K. A. S., Khudhair, G. S., and Al-Rabeai, D. B. (2022). Prevalence and drug resistance pattern of *Mycobacterium tuberculosis* isolated from tuberculosis patients in Basra, Iraq. *Pol. J. Microbiol.* 71 (2), 205–215. doi:10.33073/pjm-2022-018

Oyedeji, G. J., Adeyemo, C., Dissou, A., Abiodun, T., Alli, O. A. T., Onaolapo, O. J., et al. (2020). Prevalence of multi-drug resistant tuberculosis among tuberculosis patients attending chest clinics in osun-state, Nigeria. *Curr. Pharm. Biotechnol.* 21 (10), 939–947. doi:10.2174/1389201021666200226100242

Pontali, E., Raviglione, M. C., and Migliori, G. B. (2019). Regimens to treat multidrug-resistant tuberculosis: Past, present and future perspectives. *Eur. Respir. Rev.* 28 (152), 190035. doi:10.1183/16000617.0035-2019

Reta, M. A., Tamene, B. A., Abate, B. B., Mensah, E., Maningi, N. E., and Fourie, P. B. (2022). *Mycobacterium tuberculosis* drug resistance in Ethiopia: An updated systematic review and meta-analysis. *Trop. Med. Infect. Dis.* 7 (10), 300. doi:10.3390/tropicalmed7100300

Shariq, M., Sheikh, J. A., Quadir, N., Sharma, N., Hasnain, S. E., and Ehtesham, N. Z. (2022). COVID-19 and tuberculosis: The double whammy of respiratory pathogens. *Eur. Respir. Rev.* 31 (164), 210264. doi:10.1183/16000617.0264-2021

Sharma, M., Roy, N., Banerjee, R., Kishore, J., and Jakhar, A. (2019). Determinants of drug resistance in previously-treated pulmonary tuberculosis patients registered at a chest clinic in south Delhi, India. *Cureus* 11 (8), e5541. doi:10.7759/cureus.5541

Shibabaw, A., Gelaw, B., Gebreyes, W., Robinson, R., Wang, S. H., and Tessema, B. (2020). The burden of pre-extensively and extensively drug-resistant tuberculosis among MDR-TB patients in the Amhara region, Ethiopia. *PLoS One* 15 (2), e0229040. doi:10.1371/journal.pone.0229040

Shivekar, S. S., Kaliaperumal, V., Brammacharry, U., Sakkaravarthy, A., Raj, C. K. V., Alagappan, C., et al. (2020). Prevalence and factors associated with multidrug-resistant tuberculosis in South India. *Sci. Rep.* 10 (1), 17552. doi:10.1038/s41598-020-74432-y

Song, W. M., Li, Y. F., Ma, X. B., Liu, J. Y., Tao, N. N., Liu, Y., et al. (2019). Primary drug resistance of mycobacterium tuberculosis in Shandong, China, 2004-2018. *Respir. Res.* 20 (1), 223. doi:10.1186/s12931-019-1199-3

Tadolini, M., García-García, J. M., Blanc, F. X., Borisov, S., Goletti, D., Motta, I., et al. (2020). On tuberculosis and COVID-19 co-infection. *Eur. Respir. J.* 56 (2), 2002328. doi:10.1183/13993003.02328-2020

Trauer, J. M. (2023). Targeted TB control in migrants to low-burden countries. *Int. J. Tuberc. Lung Dis.* 27 (3), 169–170. doi:10.5588/ijtld.23.0014

Wang, Z., Jiang, W., Liu, Y., Zhang, L., Zhu, A., Tang, S., et al. (2019). Transforming tuberculosis (TB) service delivery model in China: Issues and challenges for health workforce. *Hum. Resour. Health* 17 (1), 83. doi:10.1186/s12960-019-0420-2

Williams, V., Vos, A., Otwombe, K., Grobbee, D. E., and Klipstein-Grobusch, K. (2022). Epidemiology and control of diabetes - tuberculosis comorbidity in eswatini: Protocol for the prospective study of tuberculosis patients on predictive factors, treatment outcomes and patient management practices. *BMJ Open* 12 (6), e059254. doi:10.1136/bmjopen-2021-059254

Yang, Q., Han, J., Shen, J., Peng, X., Zhou, L., and Yin, X. (2022). Diagnosis and treatment of tuberculosis in adults with HIV. *Med. Baltim.* 101 (35), e30405. doi:10.1097/MD.0000000000030405

Zhang, J., Gou, H., Hu, X., Hu, X., Shang, M., Zhou, J., et al. (2016). Status of drug-resistant tuberculosis in China: A systematic review and meta-analysis. *Am. J. Infect. Control* 44 (6), 671–676. doi:10.1016/j.ajic.2015.12.042

Zhang, J., Ren, Y., Pan, L., Yi, J., Guan, T., Yang, X., et al. (2021). Analysis of drug resistance and mutation profiles in *Mycobacterium tuberculosis* isolates in a surveillance site in Beijing, China. *J. Int. Med. Res.* 49 (1), 300060520984932. doi:10.1177/0300060520984932

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Head-to-head comparison of azvudine and nirmatrelvir/ritonavir for the hospitalized patients with COVID-19: a real-world retrospective cohort study with propensity score matching

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Background: Nirmatrelvir/ritonavir and azvudine have been approved for the early treatment of COVID-19 in China, however, limited real-world data exists regarding their effectiveness and safety.

Methods: We conducted a retrospective cohort study involving the hospitalized COVID-19 patients in China between December 2022 and January 2023. Demographic, clinical, and safety variables were recorded.

Results: Among the 6,616 hospitalized COVID-19 patients, we included a total of 725 patients including azvudine recipients (N = 461) and nirmatrelvir/ritonavir (N = 264) recipients after exclusions and propensity score matching (1:2). There was no significant difference in the composite disease progression events between azvudine (98, 21.26%) and nirmatrelvir/ritonavir (72, 27.27%) groups (p = 0.066). Azvudine was associated with a significant reduction in secondary outcomes, including the percentage of intensive care unit admission (p = 0.038) and the need for invasive mechanical ventilation (p = 0.035), while the in-hospital death event did not significantly differ (p = 0.991). As for safety outcomes, 33 out of 461 patients (7.16%) in azvudine group and 22 out of 264 patients (8.33%) in nirmatrelvir/ritonavir group experienced drug-related adverse events between the day of admission (p = 0.565).

Conclusion: In our real-world setting, azvudine treatment demonstrated similar safety compared to nirmatrelvir/ritonavir in hospitalized COVID-19 patients. Additionally, it showed slightly better clinical benefits in this population. However, further confirmation through additional clinical trials is necessary.

KEYWORDS

COVID-19, azvudine, nirmatrelvir/ritonavir, real-world, effectiveness, safety

Background

The coronavirus disease 2019 (COVID-19) continues to pose a significant threat to global health. It is crucial to have early and appropriate antiviral agents to treat patients at risk for severe COVID-19 or death (Singh and De Wit, 2022). This is important not only to decrease morbidities and mortalities, but also to restore healthcare capacities and facilitate a return to the new normal (Singh and De Wit, 2022). Currently, antiviral therapy for COVID-19 includes the use of neutralizing monoclonal antibodies (mAbs) and direct antiviral agents (Chen et al., 2023). Neutralizing mAbs specifically target the spike protein of severe acute respiratory syndrome coronavirus 2 (SASR-CoV-2), and their neutralizing activities against viruses and preventing viral entry into human cells contributes to therapeutic effects (Miljanovic et al., 2023). Clinical utilization of either single mAbs or combinations of two or more mAbs has proven effective in reducing the frequency of hospitalization, severe forms of COVID-19, and mortality (Singh and De Wit, 2022; Miljanovic et al., 2023). Direct antiviral agents, on the other hand, are designed to target the viral encoded enzymes essential for viral replication. Specifically, the SARS-CoV-2 3CL protease and RNA-dependent RNA polymerase are two key enzymes, and corresponding three antiviral agents, including remdesivir, nirmatrelvir/ritonavir and molnupiravir were recommended by the World Health Organization (WHO) for patients with mild and moderate COVID-19 (Singh and De Wit, 2022; Murakami et al., 2023). Up to date, the clinical effectiveness of COVID-19 antiviral agents in reduction of hospitalization for those at risk for disease progression fluctuates between 30% and 90% (Singh and De Wit, 2022). However, the current evidence regarding the effectiveness and safety of antiviral agents remains inadequate.

In China, several direct antiviral drugs, including nirmatrelvir/ritonavir, azvudine, remdesivir, lopinavir/ritonavir, and molnupiravir have been approved for the treatment of COVID-19 patients (Singh and De Wit, 2022; Murakami et al., 2023; Mazzitelli et al., 2023b). It is worth noting that, except for remdesivir, which is administered intravenously, the others are oral drugs (Marzi et al., 2022). Among them, oral nirmatrelvir/ritonavir was the first to be granted approval for treating mild to moderate COVID-19 in both adult and paediatric patients who were at high risk of developing severe disease within 5 days of symptom onset. Nirmatrelvir is r is a potent and selective inhibitor of the SARS-CoV-2 3CL protease, while ritonavir is an HIV-1 protease inhibitor and CYP3A inhibitor. By Inhibiting the SARS-CoV-2 3CL protease, viral replication can be prevented by blocking the processing of polyprotein precursors (Hammond et al., 2022; Marzi et al., 2022; Amani and Amani, 2023). Up to date, numerous clinical trials and real-world studies have been conducted to evaluate the effectiveness and safety of nirmatrelvir/ritonavir. The majority of these studies have consistently shown that nirmatrelvir/ritonavir significantly reduces the severity of COVID-19 and mortality (Wong et al., 2022a; Wong et al., 2022b; Wen et al., 2022; Zhou et al., 2022; Cheema et al., 2023; Zheng et al., 2023). Azvudine, on the other hand, is the first double-target nucleoside drug and has demonstrated significant and broad-spectrum antiviral effects *in vitro* (Wang et al., 2014; Zhang et al., 2021). An phase three multicenter randomized clinical study further suggested that azvudine significantly shorten the symptom improvement time and increase the proportion of mild and common COVID-19 patients with improved clinical symptoms (Yu and Chang, 2022). Real-world studies have also confirmed the substantial clinical benefits of azvudine treatment in hospitalized COVID-19 patients (Ren et al., 2020; Shen et al., 2023). As a result, the National Medical Products Administration (NMPA) granted conditional authorization for the use of azvudine in the treatment of COVID-19 on 25 July 2022. In China, both nirmatrelvir/ritonavir and azvudine were approved by the National Healthcare Security Administration on 12 August 2022 for inclusion in the medical reimbursement list.

While current guidelines prioritize the use of direct antiviral drugs in COVID-19 patients, there is still a need for more clinical data on their real-world effectiveness and safety. In this retrospective cohort study, we aimed to conduct a head-to-head comparison of the clinical effectiveness and safety of nirmatrelvir/ritonavir and azvudine in hospitalized COVID-19 patients at the Tongji Hospital, as the largest hospital in the central region of China and the main treatment facility for acute or critical COVID-19 patients during a specific pandemic wave.

Methods

Patient population and data elements

We conducted a single-center, retrospective cohort study involving the hospitalized adult patients (aged ≥18 years) with COVID-19 (confirmed by RT-PCR), who were given azvudine or nirmatrelvir/ritonavir plus standard treatment at Tongji hospital of Huazhong University of Science and Technology, during the period from 1 Dec 2022 to 31 Jan 2023. This study was approved by the institutional review board of Tongji hospital (TJ-IRB20230202). Patient data were extracted from the hospital's Electronic Medical Records (EMRs) and anonymized to ensure patient privacy. The EMRs information including demographic characteristics, admission data, diagnoses, clinical categories, prescription and drug dispensing records, procedures, laboratory tests, and discharge or death dates were analyzed. The different clinical categories of COVID-19 were defined according to the Chinese Diagnosis and Treatment Program for Novel Coronavirus Pneumonia (10th Edition). We also considered comorbidities such as diabetes mellitus, cancer, hypertension, cardiovascular disease, cerebral infarction, chronic kidney disease, chronic obstructive pulmonary disease, and chronic liver disease. Additionally, we evaluated the impact of co-medications, including baricitinib, systemic steroid and tocilizumab. Baseline laboratory parameters and changes in values over time were collected from the EMRs, encompassing complete blood cell count, electrolyte levels, renal function, hepatic function, and coagulation function.

Outcome definition

The primary outcome of our study was defined as a composite of disease progression events, including the intensive care unit admission, the need for invasive mechanical ventilation, and in-hospital death. Additionally, we also analyzed each of these events

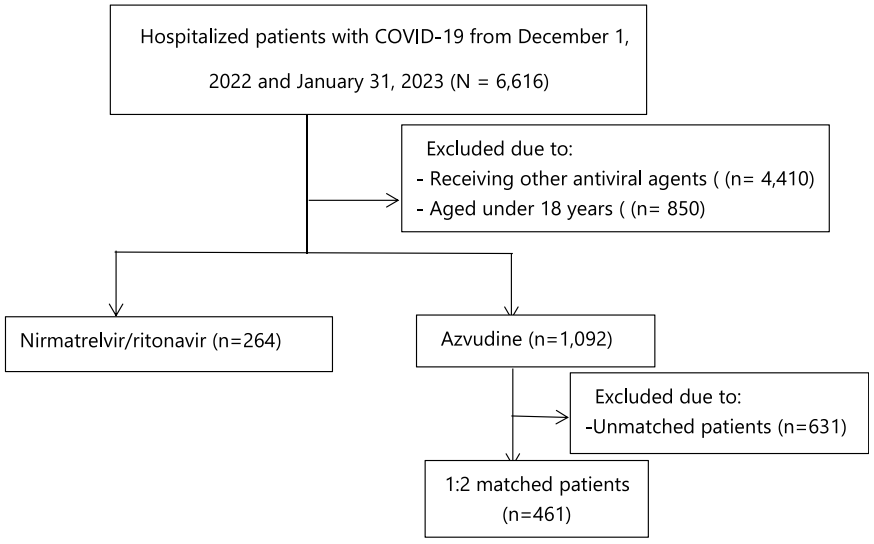

FIGURE 1
Identification of azvudine recipients and nirmatrelvir-ritonavir recipients among the hospitalized COVID-19 patients.

individually as secondary outcomes. In terms of safety outcomes, we assessed the incidence of adverse events and categorized them based on various organ systems.

Propensity matching

To account for potential confounding factors, propensity score (PS) models were employed in our study. Baseline covariates and laboratory parameters on admission of patients, such as age, gender, BMI, comorbidities, severity of COVID-19 on admission, concomitant treatments initiated at admission were included to be analyzed. We used PS models conditional on the aforementioned baseline covariates, which was performed with a 1:2 match between two groups with a calliper width of 0.02 without replacement. All baseline variables in the PS-matched cohort were descriptively analyzed, and then the standard mean differences (SMDs) were used to assess the balance of each baseline covariate between the groups before and after PS- matching. Subgroup analyses were performed at each level of the baseline covariates above to assess the robustness of the estimates.

Statistical analysis

Quantitative variables were summarized using medians with interquartile ranges (IQRs), while qualitative variables were presented as absolute and percentage frequencies. Baseline characteristics were compared between patients using appropriate statistical tests. Student's *t*-test for near-normal continuous variables, the Mann-Whitney *U*-test for other continuous variables, and the chi-square test (or Fisher's exact test when appropriate) for categorical variable. Missing data were not imputed for any of the baseline variables. All p values were two-sided and $p < 0.05$ was considered statistically significant. The statistical analyses were performed using R version 3.6.3 and python version 3.7.

Results

Patient characteristics

As shown in Figure 1, a total of 6,616 patients with COVID-19 were admitted to Tongji Hospital. After excluding patients under 18 years old and those who receiving other treatments, the final database included 1,356 patients, with 1,092 in azvudine group and 264 in nirmatrelvir/ritonavir group. Supplementary Table S1 provides details of the missing baseline laboratory data. Table 1 presents the baseline characteristics of the 1,356 patients before PS-matching. Patients in azvudine group were older [median age 65 (IQR, 54–77) vs. 70 (60–77) years, $p = 0.004$], but there were no significant differences in terms of sex ($p = 0.943$) and BMI ($p = 0.108$). The azvudine group had a lower proportion of patients with chronic kidney disease (14.74% vs. 20.83%, $p = 0.015$), and a higher proportion with cardiovascular disease (17.86% vs. 10.23%, $p = 0.003$) and hypertension (33.61% vs. 11.74%, $p < 0.001$). A lower proportion of patients with moderate clinical categories on admission (25.64% vs. 41.67%, $p < 0.001$) and receiving systemic steroids (62.64% vs. 82.58%, $p < 0.001$) was observed in azvudine group. Other comorbidities (diabetes mellitus, cancer, cerebral infarction, chronic obstructive pulmonary disease, and chronic liver disease) and co-medications (baricitinib and tocilizumab) did not significantly differ between the two groups. In terms of laboratory parameters, there were significant differences in the values of red blood cell count (RBC), hemoglobin (Hg), platelet count (PLT), and total bilirubin (TB). Patients treated with nirmatrelvir/ritonavir had significantly lower values of RBC (3.78 ± 0.89 vs. 3.99 ± 0.72 $*10^{12}$/L, $p < 0.001$), Hg (114.11 ± 26.15 vs. 121.61 ± 21.35 g/L, $p < 0.001$), PLT (181.03 ± 106.75 vs. 221.89 ± 249.30) $*10^{9}$/L, $p = 0.01$), and TB (9.66 ± 6.52 vs. 10.68 ± 7.31 umol/L, $p = 0.04$), whereas there were no significant difference in values of white blood cell count (WBC), absolute neutrophil count (NEU), neutrophil percentage (NEUP), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP),

TABLE 1 Baseline characteristics of the participants before and after propensity score matching.

Characteristics	unmatched (*n* = 1356)			matched (*n* = 725)		
	Azvudine (*n* = 1092)	nirmatrelvir-ritonavir (*n* = 264)	*p*	Azvudine (*n* = 461)	nirmatrelvir-ritonavir (*n* = 264)	*p*
Gender,n(%)			0.943			0.754
Male	378 (34.62)	92 (34.85)		295 (63.99)	172 (65.15)	
Female	714 (65.39)	172 (65.15)		166 (36.01)	92 (34.85)	
BMI(kg/m^2), mean(±SD)	24.04 ± 3.74	23.54 ± 3.60	0.108	23.67 ± 3.80	23.54 ± 3.60	0.713
Age(yr), median[IQR]	70 [60,77]	65 [54,77]	0.004	68 [57,76]	65 [54,77]	0.389
Comorbidities, n(%)						
Diabetes mellitus	292 (26.74)	66 (25.00)	0.565	111 (24.08)	66 (25.00)	0.781
Cancer	135 (12.36)	44 (16.67)	0.064	75 (16.27)	44 (16.67)	0.889
Hypertension	367 (33.61)	31 (11.74)	<0.001	61 (13.23)	31 (11.74)	0.562
Cardiovascular disease	195 (17.86)	27 (10.23)	0.003	71 (15.40)	27 (10.23)	0.053
Cerebral infarction	112 (10.26)	21 (7.96)	0.259	38 (8.24)	21 (7.96)	0.891
Chronic kidney disease	161 (14.74)	55 (20.83)	0.015	93 (20.17)	55 (20.83)	0.832
Chronic obstructive pulmonary disease	39 (3.57)	7 (2.65)	0.459	14 (3.04)	7 (2.65)	0.766
Chronic liver disease	36 (3.30)	7 (2.65)	0.591	16 (3.47)	7 (2.65)	0.545
Clinical categories, n (%)			<0.001			0.363
Moderate	280 (25.64)	110 (41.67)		129 (27.98)	87 (32.96)	
Severe	456 (41.76)	92 (34.85)		217 (47.07)	114 (43.18)	
Critical	356 (32.60)	62 (23.49)		115 (24.94)	63 (23.86)	
Co-medications, n(%)						
Baricitinib,n (%)	14 (1.28)	8 (3.03)	0.044	8 (1.74)	8 (3.03)	0.253
Systemic steroid, n (%)	684 (62.64)	218 (82.58)	<0.001	370 (80.26)	218 (82.58)	0.443
Tocilizumab, n (%)	10 (0.92)	7 (2.65)	0.023	7 (1.52)	7 (2.65)	0.286
Laboratory maker, mean(±SD)						
RBC(*10^{12}/L)	3.99 ± 0.72	3.78 ± 0.89	<0.001	3.88 ± 0.77	3.78 ± 0.89	0.138
WBC(*10^9/L)	7.45 ± 5.48	8.20 ± 26.28	0.391	7.00 ± 6.00	6.62 ± 4.43	0.38
Hg (g/L)	121.61 ± 21.35	114.11 ± 26.15	<0.001	117.34 ± 22.21	114.22 ± 26.02	0.106
PLT (*10^9/L)	221.89 ± 249.30	181.03 ± 106.75	0.01	193.78 ± 93.27	181.68 ± 106.32	0.115
NEU(*10^9/L)	5.81 ± 4.15	6.63 ± 23.29	0.572	5.49 ± 3.79	6.63 ± 23.29	0.308
NEUP(%)	75.59 ± 14.60	74.25 ± 15.89	0.192	74.96 ± 15.14	74.26 ± 15.98	0.568
AST (U/L)	48.97 ± 209.26	39.53 ± 85.40	0.476	53.99 ± 283.75	39.68 ± 86.05	0.433
ALT (U/L)	34.79 ± 82.00	34.12 ± 88.77	0.907	36.76 ± 115.66	33.76 ± 89.24	0.722
ALP(U/L)	81.00 ± 47.45	82.61 ± 43.77	0.618	81.69 ± 45.06	82.11 ± 43.59	0.905
LDH(U/L)	322.37 ± 184.24	303.80 ± 142.07	0.129	315.41 ± 174.09	300.92 ± 127.94	0.248
TB (umol/L)	10.68 ± 7.31	9.66 ± 6.52	0.04	10.26 ± 7.30	9.66 ± 6.52	0.278
eGFR (mL/min/1.73m^2)	71.05 ± 28.16	71.96 ± 31.40	0.674	70.09 ± 30.24	71.31 ± 31.15	0.617
CCR(umol/L)	130.08 ± 196.79	125.35 ± 141.48	0.715	144.36 ± 240.60	125.35 ± 141.48	0.246

(Continued on following page)

TABLE 1 (*Continued*) Baseline characteristics of the participants before and after propensity score matching.

Characteristics	unmatched (n = 1356)			matched (n = 725)		
	Azvudine (n = 1092)	nirmatrelvir-ritonavir (n = 264)	p	Azvudine (n = 461)	nirmatrelvir-ritonavir (n = 264)	p
U (mmol/L)	8.92 ± 7.72	8.79 ± 7.19	0.815	9.41 ± 8.90	8.87 ± 7.22	0.41
UA (mmol/L)	295.89 ± 138.53	292.77 ± 123.80	0.74	296.27 ± 143.61	293.28 ± 124.49	0.782
NA (mmol/L)	137.16 ± 4.93	136.91 ± 4.48	0.466	137.23 ± 4.19	136.88 ± 4.49	0.305
K (mmol/L)	4.16 ± 0.60	4.20 ± 0.55	0.299	4.19 ± 0.63	4.21 ± 0.55	0.626
CL (mmol/L)	101.62 ± 5.25	101.56 ± 4.83	0.882	101.71 ± 4.60	101.54 ± 4.86	0.647
TT(s)	18.32 ± 10.28	17.76 ± 2.03	0.376	17.92 ± 3.21	17.76 ± 2.04	0.428
FBG (g/L)	4.69 ± 1.38	4.65 ± 1.42	0.643	4.58 ± 1.40	4.65 ± 1.43	0.51
APTT(s)	37.65 ± 9.82	38.19 ± 7.24	0.408	38.49 ± 10.81	38.18 ± 7.28	0.686
PT(s)	13.53 ± 2.45	13.50 ± 1.75	0.818	13.66 ± 3.05	13.49 ± 1.75	0.42

lactate dehydrogenase (LDH), estimated glomerular filtration rate (eGFR), serum creatinine (CCR), urea (U), uric acid (UA), sodium (NA), potassium (K), chloride (CL), Thrombin time (TT), fibrinogen (FBG), activation of partial thromboplastin time (APTT), and prothrombin time (PT). After 1:2 propensity score matching, a total of 725 patients were included to be analysis. Supplementary Figure S1 shows the distributions of covariates before and after PS matching. As shown in Table 1, demographics and comorbidities did not significantly differ between the PS-matched groups.

Clinical outcomes of PS-matched cohort

Table 2 presents the clinical and safety outcomes observed in the PS-matched cohort. The composite outcome did not show a significant difference between azvudine and nirmatrelvir/ritonavir groups (p = 0.066). The composite disease progression events occurred in 98 (21.26%) patients treated with azvudine and 72 (27.27%) patients treated with nirmatrelvir/ritonavir. However, we did observe a significant reduction in secondary outcome measures associated with azvudine treatment, including the percentage of intensive care unit admission (p = 0.038) and the need for invasive mechanical ventilation (p = 0.035), while the event of in-hospital death did not significantly differ (p = 0.991).

As for safety outcomes, a total of 33 out of 461 patients (7.16%) in azvudine group and 22 out of 264 patients (8.33%) in nirmatrelvir/ritonavir group were observed the drug-related adverse events during their hospital stay. There was no significant difference between two groups (p = 0.565). The most common adverse events in azvudine group were constipation (9), aypnia (7), dizziness (4), diarrhea (4), stomachache (3), headache (3), vomiting (2), drowsiness (1), nausea (1), and melena (1). In the nirmatrelvir/ritonavir group, a total of 22 patients experienced drug-related adverse events, including diarrhea (3), abnormal urination (2), aypnia (2), edema (2), eruption (2), dysphoria (2), stomachache (1), fever (1), feeble (1), arrhythmia (1), bleeding (1), and headache (1). Meanwhile, the change values of laboratory parameters (Table 3), such as the blood cell count, electrolytes, renal function, hepatic function, and coagulation function did not significantly differ between the groups, except for eGFR (p = 0.007). In further analysis, no significant difference was observed in the percentage of eGFR decline greater than 10% (p = 0.151), 30% (p = 0.471) or 50% (p = 0.581).

The subgroup analyses of outcomes were performed, and the results were presented in Table 3 and Supplementary Tables S2–S6. Patients with diabetes in azvudine group had a lower risk of composite disease progression events compared to the nirmatrelvir/ritonavir group [OR = 2.404, 95%CI (1.095, 5.277), p = 0.029]. However, no significant differences were observed in other subgroups. A similar lower risk of needing invasive mechanical ventilation for patients with diabetes in the azvudine group was also found [OR = 2.771, 95%CI (1.321, 5.812), p = 0.007]. Greater benefits associated with azvudine treatment were observed in patients with a moderate clinical category on admission, those without concomitant cardiovascular disease and chronic obstructive pulmonary disease, and those who did not receive tocilizumab and baricitinib. Additionally, patients with chronic kidney disease [OR = 9.20, 95%CI (1.046, 80.937), p = 0.045] or without cancer [OR = 2.631, 95%CI (1.106, 6.258), p = 0.029] had a relatively lower incidence of intensive care unit admission in azvudine group.

Discussion

To the best of our knowledge, this retrospective cohort study is the first to directly compare both clinical effectiveness and safety of these oral antiviral agents in China. There was no statistically significant difference in the clinical effectiveness, in terms of reducing disease progression, and safety between nirmatrelvir/ritonavir and azvudine in hospitalized COVID-19 patients. However, azvudine showed potential clinical benefits in secondary outcomes for specific subgroups, including patients with diabetes, chronic kidney disease, and those with a moderate clinical category on admission. It is important to note that these findings should be further confirmed through clinical trials with larger sample sizes to establish more robust evidence.

TABLE 2 Clinical effectiveness and safety outcomes among azvudine *versus* nirmatrelvir-ritonavir recipients.

Outcomes	Azvudine (*n* = 461)	Nirmatrelvir-ritonavir (*n* = 264)	*p*-value
Primary outcome	98 (21.26)	72 (27.27)	0.066
Secondary outcomes			
Intensive care unit admission	11(2.39)	14(5.30)	0.038
Need for invasive mechanical ventilation	77(16.70)	61(23.11)	0.035
In-hospital death	63 (13.67)	36 (13.64)	0.991
Safety outcome			
All drug-related adverse events	33 (7.16)	22 (8.33)	0.565
Change of laboratory maker, mean(±SD)			
RBC(*10^{12}/L)	0.36 ± 0.36	0.38 ± 0.33	0.541
WBC(*10^9/L)	6.52 ± 8.74	7.80 ± 11.79	0.152
Hemoglobin (g/L)	10.84 ± 9.99	11.83 ± 10.07	0.374
Platelet count (*10^9/L)	115.56 ± 297.07	120.49 ± 352.49	0.866
NEU (*10^9/L)	5.76 ± 5.84	6.82 ± 6.72	0.058
NEUP(%)	13.44 ± 12.61	14.75 ± 13.05	0.276
AST (U/L)	100.67 ± 516.37	173.67 ± 759.55	0.301
ALT (U/L)	43.34 ± 105.89	72.14 ± 174.16	0.056
ALP(U/L)	38.79 ± 56.84	60.45 ± 144.61	0.052
LDH(U/L)	205.38 ± 319.45	214.42 ± 324.78	0.798
TB (umol/L)	7.87 ± 14.93	8.41 ± 12.76	0.714
eGFR (mL/min/1.73m^2)	13.81 ± 14.07	17.76 ± 17.65	0.007
CCR(umol/L)	61.61 ± 114.77	62.82 ± 105.91	0.926
U (mmol/L)	6.79 ± 10.49	8.12 ± 12.05	0.213
UA (mmol/L)	110.59 ± 142.35	100.85 ± 102.08	0.501
NA (mmol/L)	6.25 ± 5.66	7.12 ± 6.09	0.094
K (mmol/L)	0.70 ± 0.69	0.79 ± 0.65	0.184
CL (mmol/L)	6.267 ± 5.36	6.96 ± 5.84	0.185
TT(s)	7.56 ± 30.40	19.92 ± 60.32	0.028
FBG (g/L)	1.22 ± 1.12	1.21 ± 1.03	0.926
APTT(s)	9.78 ± 17.40	22.01 ± 48.67	0.021
PT(s)	3.93 ± 8.36	3.46 ± 7.94	0.616
Percentage of eGFR Change			
Decline of eGFR>10%	205 (44.47)	132 (50.00)	0.151
Decline of eGFR>30%	101 (21.91)	64 (24.24)	0.471
Decline of eGFR>50%	63 (13.66)	40 (15.15)	0.581

Our findings regarding the clinical effectiveness differ from a previous study conducted at Xiangya Hospital in China (Deng et al., 2023). Deng et al. observed a lower incidence rate of composite disease progression outcome and all-cause death in azvudine recipients, especially in patients aged <65 years, those with a history of disease, those with severe COVID-19 at admission, and those receiving antibiotics. The whole conclusion suggests that azvudine treatment may be more effective compared to nirmatrelvir/ritonavir in terms of composite disease progression outcome. However, our study did not find a significant difference in the composite disease progression outcome between azvudine and nirmatrelvir/ritonavir, except for certain subgroups such as patients

TABLE 3 Subgroup analysis of clinical effectiveness and safety outcomes.

Outcomes	Covariates	Subgroup	N	OR	95%CI	*p*-value
Composite disease progression outcome	Overall		725	1.102	[0.743,1.634]	0.629
	Diabetes mellitus	No	548	0.845	[0.531,1.345]	0.478
		Yes	177	2.404	[1.095,5.277]	0.029
Intensive care unit admission	Overall		725	2.291	[1.025,5.122]	0.043
	Cancer	No	606	2.631	[1.106,6.258]	0.029
		Yes	119	0.849	[0.075,9.640]	0.895
	Chronic kidney disease	No	577	1.611	[0.644,4.030]	0.308
		Yes	148	9.2	[1.046,80.937]	0.045
Need for invasive mechanical ventilation	Overall		725	1.499	[1.028,2.184]	0.035
	Diabetes mellitus	No	548	1.199	[0.770,1.869]	0.422
		Yes	177	2.771	[1.321,5.812]	0.007
	Cardiovascular disease	No	627	1.823	[1.189,2.794]	0.006
		Yes	98	1.018	[0.406,2.550]	0.969
	Chronic obstructive pulmonary disease	No	704	1.502	[1.024,2.203]	0.037
		Yes	21	1.467	[0.184,11.718]	0.718
	Clinical categories	Moderate	216	2.89	[1.254,6.661]	0.013
		Severe	331	1.516	[0.837,2.749]	0.17
		Critical	178	1.097	[0.570,2.113]	0.78ll1
	Tocilizumab	No	711	1.607	[1.093,2.364]	0.016
		Yes	14	0.067	[0.005,0.970]	0.047
	Baricitinib	No	709	1.509	[1.030,2.212]	0.035
		Yes	16	1	[0.104,9.614]	1

OR, odds ratio; CI, confidence interval.

with diabetes. It is known that nirmatrelvir/ritonavir has been reported to reduce hospitalization rate and mortality by 88% when initiated within 5 days of symptom onset in high-risk patients in a phase III clinical trial, leading to its authorization for the treatment of high-risk patients with mild to moderate COVID-19 (Arbel et al., 2022). A real-world study conducted in China showed that patients who received nirmatrelvir/ritonavir had more rapid virus suppression in the early stages of hospitalization compared to those who received azvudine (Gao et al., 2023). On the other hand, azvudine was mainly approved to treat all patients with common and severe COVID-19 (Gentile et al., 2022). Though the results of the phase III study have not been officially released, both of Shen et al. (2023) and Deng et al. (2023) suggested that azvudine treatment is associated with significantly lower risks of composite disease progression outcome and all-cause death in real-world studies. Therefore, we tried to speculate that initiating treatment with nirmatrelvir/ritonavir as early as possible may provide clinical benefits, while azvudine with a longer course (14 days) of antiviral treatment appears to yield a better therapeutic effect for specific COVID-19 patients. However, there is no doubt that further studies with larger sample sizes are needed to validate these findings. In addition to assessing the composite disease progression outcome, our study also examined other disease progression indicators, such as in-hospital death, admission to the intensive care unit, and the need for invasive mechanical ventilation. We observed a relative advantage of azvudine over nirmatrelvir/ritonavir in terms of the proportion of patients admitted to the intensive care unit and the need for invasive mechanical ventilation.

In our study, the safety of both azvudine and nirmatrelvir/ritonavir was also analyzed. The total incidences of adverse events in azvudine and nirmatrelvir/ritonavir groups were 7.16% and 8.33%, respectively. No serious adverse events were observed, and there was no significant difference in the change values of laboratory parameters. These findings suggest that both azvudine and nirmatrelvir/ritonavir have an overall favorable safety profile for the treatment of COVID-19 patients, which aligns with the results of a recent meta-analysis involving 2,143 patients (Amani and Amani, 2023). From the incidence of adverse events perspective, our data, albeit limited, suggested an overall lower frequency of adverse events compared to clinical trials or other cohort studies (Gentile et al., 2022; Amani and Amani, 2023; Mazzitelli et al., 2023a; Cheema et al., 2023; Gao et al., 2023). For example, in a phase 2–3 double-blind, randomized, controlled trial, the incidence of any adverse events during the treatment period was higher with nirmatrelvir/

ritonavir compared to placebo (22.6% vs. 23.9%), while serious adverse events were 1.6% vs. 6.6% (Amani and Amani, 2023). Dysgeusia (5.6% vs. 0.3%) and diarrhea (3.1% vs. 1.6%) occurred more frequently with nirmatrelvir/ritonavir than with placebo (Miljanovic et al., 2023). Another real-life study reported adverse events in 12.1% of patients receiving nirmatrelvir/ritonavir treatment, mainly dysgeusia, diarrhea, and nausea. When compared with molnupiravir, 19.1% of patients experienced adverse events following nirmatrelvir/ritonavir intake (Najjar-Debbiny et al., 2023). In our study, the most common adverse events in azvudine group were related to gastrointestinal disorders, psychiatric/nervous system disorders, including constipation, aypnia, dizziness, diarrhea, stomachache, and headache, which was basically in accordance with the instructions and clinical trials. Adverse events associated with nirmatrelvir/ritonavir included diarrhea, abnormal urination, aypnia, edema, eruption, and dysphoria. We observed a potential influence on renal function, as measured by value of eGFR, which appeared to be higher in the nirmatrelvir/ritonavir group compared to the azvudine group. However, there was no significant difference in further analysis. It's important to note that ritonavir, as a strong CYP3A4 inhibitor, may affect the metabolism of various drugs used for arrhythmia, diabetes, and neurological diseases, even when nirmatrelvir/ritonavir is used for a short duration (Loos et al., 2022). Therefore, more clinical trials are needed to investigate in further. Overall, the safety profiles of both azvudine and nirmatrelvir/ritonavir were good, and no serious drug-related adverse events were observed.

There were several limitations in our study. Firstly, the included the hospitalized COVID-19 patients were mainly from Hubei province in China during a specific pandemic wave, thus the results may only be representative of this specific population and cannot be generalized to all COVID-19 patients or other countries. Secondly, the timing of symptom onset and vaccination status were not recorded for some missing data, and the relatively small sample size might have influenced the statistical power of our subgroup analyses. Thirdly, despite our efforts to collect data consecutively and adjust for a wide range of confounders using the propensity score model, we can not completely rule out the possibility of selection bias or confounding by indication in this retrospective cohort study. Randomized controlled trials would provide more rigorous evidence in this regard. Lastly, the incidence of adverse events was determined by pharmacists based on causal criteria, which it subjective to some extent, and there were inevitably the possible of omissions and misstatements for retrospective study. Future prospective studies with standardized protocols for adverse event assessment would provide more accurate and reliable data.

Conclusion

In conclusion, our study indicates that both azvudine and nirmatrelvir/ritonavir have comparable safety profiles in hospitalized COVID-19 patients. Azvudine showed slightly better clinical benefits in this population, although further clinical trials are necessary to confirm these findings. It is important to continue research efforts to gather more evidence and validate these results obtained in our study.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the institutional review board of Tongji hospital (TJ-IRB20230202). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because Surveys and observational study.

Author contributions

A-HW: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing–original draft, Writing–review and editing. LZ: Data curation, Formal Analysis, Methodology, Writing–review and editing. LW: Formal Analysis, Methodology, Writing–original draft. LG: Investigation, Writing–review and editing. W-TZ: Investigation, Project administration, Writing–review and editing. X-PG: Project administration, Writing–review and editing. JL: Supervision, Writing–review and editing. DL: Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1274294/full#supplementary-material

References

Amani, B., and Amani, B. (2023). Efficacy and safety of nirmatrelvir/ritonavir (Paxlovid) for COVID-19: a rapid review and meta-analysis. *J. Med. virology* 95 (2), e28441. doi:10.1002/jmv.28441

Arbel, R., Wolff Sagy, Y., Hoshen, M., Battat, E., Lavie, G., Sergienko, R., et al. (2022). Nirmatrelvir use and severe covid-19 outcomes during the omicron surge. *N. Engl. J. Med.* 387 (9), 790–798. doi:10.1056/NEJMoa2204919

Cheema, H. A., Jafar, U., Sohail, A., Shahid, A., Sahra, S., Ehsan, M., et al. (2023). Nirmatrelvir-ritonavir for the treatment of COVID-19 patients: a systematic review and meta-analysis. *J. Med. virology* 95 (2), e28471. doi:10.1002/jmv.28471

Chen, P. Y., Wang, J. T., and Chang, S. C. (2023). Antiviral therapy of coronavirus disease 2019 (COVID-19). *J. Formos. Med. Assoc. = Taiwan yi zhi.* doi:10.1016/j.jfma.2023.08.029

Deng, G., Li, D., Sun, Y., Jin, L., Zhou, Q., Xiao, C., et al. (2023). Real-world effectiveness of Azvudine versus nirmatrelvir-ritonavir in hospitalized patients with COVID-19: a retrospective cohort study. *J. Med. virology* 95 (4), e28756. doi:10.1002/jmv.28756

Gao, Y., Luo, Z., Ren, S., Duan, Z., Han, Y., Liu, H., et al. (2023). Antiviral effect of azvudine and nirmatrelvir-ritonavir among hospitalized patients with COVID-19. *J. Infect.* 86 (6), e158–e160. doi:10.1016/j.jinf.2023.03.023

Gentile, I., Scotto, R., Schiano Moriello, N., Pinchera, B., Villari, R., Trucillo, E., et al. (2022). Nirmatrelvir/ritonavir and molnupiravir in the treatment of mild/moderate COVID-19: results of a real-life study. *Vaccines* 10 (10), 1731. doi:10.3390/vaccines10101731

Hammond, J., Leister-Tebbe, H., Gardner, A., Abreu, P., Bao, W., Wisemandle, W., et al. (2022). Oral nirmatrelvir for high-risk, nonhospitalized adults with covid-19. *N. Engl. J. Med.* 386 (15), 1397–1408. doi:10.1056/NEJMoa2118542

Loos, N. H. C., Beijnen, J. H., and Schinkel, A. H. (2022). The mechanism-based inactivation of CYP3A4 by ritonavir: what mechanism? *Int. J. Mol. Sci.* 23 (17), 9866. doi:10.3390/ijms23179866

Marzi, M., Vakil, M. K., Bahmanyar, M., and Zarenezhad, E. (2022). Paxlovid: mechanism of action, synthesis, and *in silico* study. *BioMed Res. Int.* 2022, 7341493. doi:10.1155/2022/7341493

Mazzitelli, M., Trunfio, M., Sasset, L., Scaglione, V., Ferrari, A., Mengato, D., et al. (2023a). Risk of hospitalization and sequelae in patients with COVID-19 treated with 3-day early remdesivir vs. controls in the vaccine and Omicron era: a real-life cohort study. *J. Med. virology* 95 (3), e28660. doi:10.1002/jmv.28660

Mazzitelli, M., Mengato, D., Sasset, L., Ferrari, A., Gardin, S., Scaglione, V., et al. (2023b). Molnupiravir and nirmatrelvir/ritonavir: tolerability, safety, and adherence in a retrospective cohort study. *Viruses* 15 (2), 384. doi:10.3390/v15020384

Miljanovic, D., Cirkovic, A., Lazarevic, I., Knezevic, A., Cupic, M., and Banko, A. (2023). Clinical efficacy of anti-SARS-CoV-2 monoclonal antibodies in preventing hospitalisation and mortality among patients infected with Omicron variants: a systematic review and meta-analysis. *Rev. Med. virology* 33 (4), e2439. doi:10.1002/rmv.2439

Murakami, N., Hayden, R., Hills, T., Al-Samkari, H., Casey, J., Del Sorbo, L., et al. (2023). Therapeutic advances in COVID-19. *Nat. Rev. Nephrol.* 19 (1), 38–52. doi:10.1038/s41581-022-00642-4

Najjar-Debbiny, R., Gronich, N., Weber, G., Khoury, J., Amar, M., Stein, N., et al. (2023). Effectiveness of paxlovid in reducing severe coronavirus disease 2019 and mortality in high-risk patients. *Clin. Infect. Dis.* 76 (3), e342–e349. an official publication of the Infectious Diseases Society of America. doi:10.1093/cid/ciac443

Ren, Z., Luo, H., Yu, Z., Song, J., Liang, L., Wang, L., et al. (2020). A randomized, open-label, controlled clinical trial of azvudine tablets in the treatment of mild and common COVID-19, a pilot study. *Adv. Sci. (Weinheim, Baden-Wurttemberg, Ger.* 7 (19), e2001435. doi:10.1002/advs.202001435

Shen, M., Xiao, C., Sun, Y., Li, D., Wu, P., Jin, L., et al. (2023). Real-world effectiveness of Azvudine in hospitalized patients with COVID-19: a retrospective cohort study. medRxiv. doi:10.1101/2023.01.23.23284899

Singh, M., and De Wit, E. (2022). Antiviral agents for the treatment of COVID-19: progress and challenges. *Cell. Rep. Med.* 3 (3), 100549. doi:10.1016/j.xcrm.2022.100549

Wang, R. R., Yang, Q. H., Luo, R. H., Peng, Y. M., Dai, S. X., Zhang, X. J., et al. (2014). Azvudine, a novel nucleoside reverse transcriptase inhibitor showed good drug combination features and better inhibition on drug-resistant strains than lamivudine *in vitro*. *PloS one* 9 (8), e105617. doi:10.1371/journal.pone.0105617

Wen, W., Chen, C., Tang, J., Wang, C., Zhou, M., Cheng, Y., et al. (2022). Efficacy and safety of three new oral antiviral treatment (molnupiravir, fluvoxamine and Paxlovid) for COVID-19: a meta-analysis. *Ann. Med.* 54 (1), 516–523. doi:10.1080/07853890.2022.2034936

Wong, C. K. H., Au, I. C. H., Lau, K. T. K., Lau, E. H. Y., Cowling, B. J., and Leung, G. M. (2022a). Real-world effectiveness of molnupiravir and nirmatrelvir plus ritonavir against mortality, hospitalisation, and in-hospital outcomes among community-dwelling, ambulatory patients with confirmed SARS-CoV-2 infection during the omicron wave in Hong Kong: an observational study. *Lancet (London, Engl.* 400 (10359), 1213–1222. doi:10.1016/S0140-6736(22)01586-0

Wong, C. K. H., Au, I. C. H., Lau, K. T. K., Lau, E. H. Y., Cowling, B. J., and Leung, G. M. (2022b). Real-world effectiveness of early molnupiravir or nirmatrelvir-ritonavir in hospitalised patients with COVID-19 without supplemental oxygen requirement on admission during Hong Kong's omicron BA.2 wave: a retrospective cohort study. *Lancet. Infect. Dis.* 22 (12), 1681–1693. doi:10.1016/S1473-3099(22)00507-2

Yu, B., and Chang, J. (2022). The first Chinese oral anti-COVID-19 drug Azvudine launched. *Innov. Camb. (Mass.))* 3 (6), 100321. doi:10.1016/j.xinn.2022.100321

Zhang, J. L., Li, Y. H., Wang, L. L., Liu, H. Q., Lu, S. Y., Liu, Y., et al. (2021). Azvudine is a thymus-homing anti-SARS-CoV-2 drug effective in treating COVID-19 patients. *Signal Transduct. Target. Ther.* 6 (1), 414. doi:10.1038/s41392-021-00835-6

Zheng, Q., Ma, P., Wang, M., Cheng, Y., Zhou, M., Ye, L., et al. (2023). Efficacy and safety of Paxlovid for COVID-19:a meta-analysis. *J. Infect.* 86 (1), 66–117. doi:10.1016/j.jinf.2022.09.027

Zhou, X., Kelly, S. P., Liang, C., Li, L., Shen, R., Leister-Tebbe, H. K., et al. (2022). Real-world effectiveness of nirmatrelvir/ritonavir in preventing hospitalization among patients with COVID-19 at high risk for severe disease in the United States: a nationwide population-based cohort study. medRxiv. doi:10.1101/2022.09.13.22279908

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The effects of combination-therapy of tocilizumab and baricitinib on the management of severe COVID-19 cases: a randomized open-label clinical trial

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Background: Tocilizumab and baricitinib are considered standard treatments for hospitalized COVID-19 patients with an inflammatory status. However, the effects of co-administering these medications aiming for more rapid patient recovery are controversial among practitioners. The potential benefits include the rapid improvement of patients and regulation of the immune system, and the potential risks include the increased chance of serious adverse events, including infections. This study aimed to investigate the effects of co-administering these two medications on the 28-day mortality rate, other efficacy parameters, and safety issues.

Methods: In this randomized open-label trial, 68 patients were recruited. The study was conducted at Dr. Masih Daneshvari Hospital during 6 months (from 21 March 2022 to 23 August 2022). Severely ill patients aged between 18 and 100 years old with confirmed COVID-19 were enrolled. The primary outcomes included the need for invasive mechanical ventilation and a 28-day mortality rate. Secondary outcomes included the need for non-invasive mechanical ventilation, the need for admission to the intensive care unit (ICU), the length of hospital stay, and the need for a second dose of tocilizumab. Safety assessments were also performed for 28 days. The data were collected from the patients' medical records, which included age, gender, and comorbidities.

Results: The 28-day mortality rate or the need for mechanical ventilation was not statistically different among the two groups (p-value = 0.49 for both outcomes). The need for non-invasive mechanical ventilation, the need for admission to the ICU, or the need for a second dose of tocilizumab and the length of hospital stay was not affected either (p-value = 1; 0.1; 0.49 and 0.9, respectively). One patient developed thrombosis in the combination group. No adverse events related to infectious complications were recorded in any groups.

Conclusion: This study showed no beneficial effects of combining tocilizumab and baricitinib in managing severe COVID-19 cases. However, the need for ICU admission was meaningfully lower in the combination group. Studies with larger sample sizes are needed to confirm these results.

Clinical Trial Registration: Identifier: RCT20151227025726N30M

KEYWORDS

baricitinib, COVID-19, inflammation, pneumonia, SARS-CoV-2, tocilizumab

1 Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a profound and lasting impact on global health and society. Since its emergence in Wuhan, China, in late 2019, COVID-19 has spread relentlessly across the globe, challenging healthcare systems, economies, and daily life as we know it. As of September 2023, the global rate of SARS-CoV-2 infections remains high, with seroprevalence studies showing that> 80% of the population of countries such as South Africa have already been infected (Hajissa et al., 2022). The treatment strategies for COVID-19 depend on its stage and should be tailored to the specific stage of the disease that a patient is experiencing (Stasi et al., 2020; Wang et al., 2023).

Inflammatory markers play a crucial role in the pathogenesis of COVID-19, contributing to the disease's severity and complications. One of the hallmark features of severe COVID-19 is a hyperinflammatory response often referred to as a "cytokine storm" (Mehta et al., 2020). This involves the overproduction of pro-inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α). This excessive cytokine release can lead to acute respiratory distress syndrome (ARDS) and multi-organ dysfunction (Dastan et al., 2020; Mehta et al., 2020; Rezaei et al., 2021; Rubin et al., 2021). Until now, many antiviral drugs and monoclonal antibodies (bebtelovimab, sotrovimab and etc.) have been used for the treatment of COVID-19. One of the most promising drugs is remdesivir which is an adenosine analogue and has antiviral activity (Li et al., 2023; Mengato et al., 2023). Tocilizumab is an interleukin-6 (IL-6) receptor blocker currently approved for hospitalized patients with high inflammatory status (Samaee et al., 2020). It promotes B and T cells' proliferation, which may trigger further immune activation (Uciechowski and Dempke, 2020). COVID-19 patients treated with tocilizumab have a lower mortality rate and a lower need for mechanical ventilation (Wei et al., 2021). Other interleukin blockers including anakinra which inhibit interleukin-1 have been used in the management of COVID-19 before. Some studies have suggested potential benefits of this medication, including improved oxygenation and reduced need for mechanical ventilation, in patients with severe COVID-19 and signs of hyperinflammation (Cavalli et al., 2020).

The Janus kinase (JAK) inhibitor, baricitinib, inhibits the passage and intracellular assembly of SARS-CoV-2 into target cells through the ACE2 receptor (Keystone et al., 2015). Moreover, this medication suppresses both JAK1/JAK2, which leads to inhibiting the proinflammatory signal of several cytokines, including IL-6, IL-12, IL-23, and IFN-γ (Keystone et al., 2015).

The indication for tocilizumab or baricitinib is almost similar in COVID-19 patients. Patients with a rapid disease progression, high oxygen needs on admission, or patients with risk factors for poor outcomes, including age or medical history plus elevated C-reactive protein (CRP) plus low-flow oxygen, did receive tocilizumab or baricitinib (Karolyi et al., 2022). Unlike tocilizumab, baricitinib could be prescribed at any CRP level, while tocilizumab required a CRP of 75 mg/L (Karolyi et al., 2022).

The effects and outcomes of co-administering these two medications have yet to be assessed in well-designed trials. The potential benefits include the rapid improvement of patients and regulation of the immune system. The potential risks include the increased chance of infections due to over-suppression of the immune system and further immune issues. However, the short duration of administration may reduce the importance of this concern. This study aimed to investigate the effects of co-administering these two medications on the 28-day mortality rate, the need for mechanical ventilation, along with other efficacy parameters and safety issues.

2 Materials and methods

2.1 Setting

This study was a randomized, open-labeled, parallel-group, two-armed, single-center clinical trial conducted on 68 COVID-19 patients at Dr. Masih Daneshvari Hospital, affiliated with Shahid Beheshti University of Medical Sciences (SBMU), a referral centre for COVID-19 patients in Tehran, Iran. A block randomization method was used to allocate patients to the tocilizumab group and tocilizumab + baricitinib group.

2.2 Patients

Severely ill patients aged between 18 and 100 years old with COVID-19, confirmed based on the reports of Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and who have also signed the study consent form, were allowed to be recruited. Severely ill patients were defined as any of the following: respiratory rate of 30 or more breaths per minute; heart rate at or exceeding 125 beats per minute; oxygen saturation at 93% or less while the participant was breathing ambient air at sea; acute respiratory distress syndrome and evidence of shock (Baden et al., 2021).

The exclusion criteria were as follows: patients who denied signing the consent form; an acute or chronic kidney disease

defined as a rise in serum creatinine greater than 0.3 mg/dL within 48 h or a lower glomerular filtration rate than 30 mL/min; the history of liver failure (Child-Pugh stage C or D or more than five times the upper limit of normal in liver function tests or three times in patients with liver failure symptoms); patients with a history of an allergic reaction to the study medications or any history of anaphylaxis to any medication; mildly ill patients; patients needing mechanical ventilation at admission, and pregnancy or breastfeeding.

2.3 Interventions

Patients were randomized to two equal sample sizes. The intervention group received a single intravenous dose of 400 mg tocilizumab (Temziva®, AryoGen Pharmed, Iran) via slow intravenous infusion plus 4 mg baricitinib (Intyma®, Nanoalvand, Iran) daily for 14 days or until discharge. The control group received a single intravenous dose of 400 mg tocilizumab (Temziva®, AryoGen Pharmed, Iran) via slow intravenous infusion. Patients in both groups received oxygen and fluid support, remdesivir 200 mg stat followed by 100 mg daily intravenously for 5 days, and dexamethasone 6 mg intravenously daily for 10 days or until discharge. The concomitant medications were the same in the both groups.

2.4 Outcomes

The primary outcomes included the need for invasive mechanical ventilation and the 28-day mortality rate. Secondary outcomes included the need for non-invasive mechanical ventilation, the need for admission to the intensive care unit (ICU), the length of hospital stay, and the need for second dose of tocilizumab. The data were collected from the patients' medical records, which included age, gender, underlying diseases, and laboratory test results.

Safety assessments were performed for 28 days after the first day of the intervention. These statements were made based on physicians' reports in the hospital. After discharge, safety assessments were performed based on follow-up telephone calls, and these assessments were patient-reported.

2.5 Statistical analysis

Overall, 68 participants were enrolled in the study. This sample size was not determined based on any power calculation and was chosen according to the available COVID-19 patients needing tocilizumab at admission.

Missing data were not imputed. No multiplicity adjustments were made in this study. Based on the central limit theorem, the normality tests were not performed, as this theorem believes that the sampling distribution of the mean will always be normally distributed, as long as the sample size is large enough (Kwak and Kim, 2017).

Categorical variables were expressed as frequency (%) and were compared using the Chi-Square or Fisher exact test. The risk difference was calculated as a proper effect size for the primary outcome. Continuous variables were expressed as mean ± standard deviations. To compare the differences in the quantitative variables of both groups, the student-t test was carried out. p-values <0.05 were considered significant.

All the statistical analyses were performed using SPSS software for Windows (Version 23.0; SPSS Inc., Chicago, IL, United States) and STATA 14.

2.6 Ethical considerations

This trial was conducted according to the declaration of Helsinki and was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.PHARMACY.REC.1400.296). Written informed consent was obtained from all the participants before recruitment.

Moreover, the trial was registered in Iranian Registry of Clinical Trials with the registration code IRCT20151227025726N30.

3 Results

The screening and randomization process of the patients are provided in the CONSORT diagram in Figure 1.

Table 1 demonstrates the demographics and past medical histories of the subjects.

The results regarding the 28-day mortality rate, the need for invasive or non-invasive mechanical ventilation, and the need for the second tocilizumab dose or admission in the ICU are provided in Table 2. As the Table shows, there were no statistical differences in terms of the mentioned parameters between the two groups (Table 2).

3.1 Safety outcomes

The lab tests regarding the assessments of the patients' inflammatory status and safety assessments are provided in Table 3.

One patient in the tocilizumab + baricitinib group developed thrombosis. No other adverse events related to the study treatments were reported.

4 Discussion

The results of this study showed no beneficial effects of combining tocilizumab and baricitinib on the management of severe COVID-19 cases. The health outcomes, including the need for mechanical or non-invasive mechanical ventilation, and the mortality rate were not different between the groups receiving the combination of these two medications and the group receiving a single dose of tocilizumab. The days of hospital stay or the need for second dose of tocilizumab was not affected either.

It is noteworthy that the risk difference in the need for admission to the ICU is calculated to be 0.15. Based on this calculation, the number need to treat (NNT) to prevent one person from admitting to the ICU is 7, which is considered a medium effect size (Rahlfs and Zimmermann, 2019). Hence, combination therapy may have

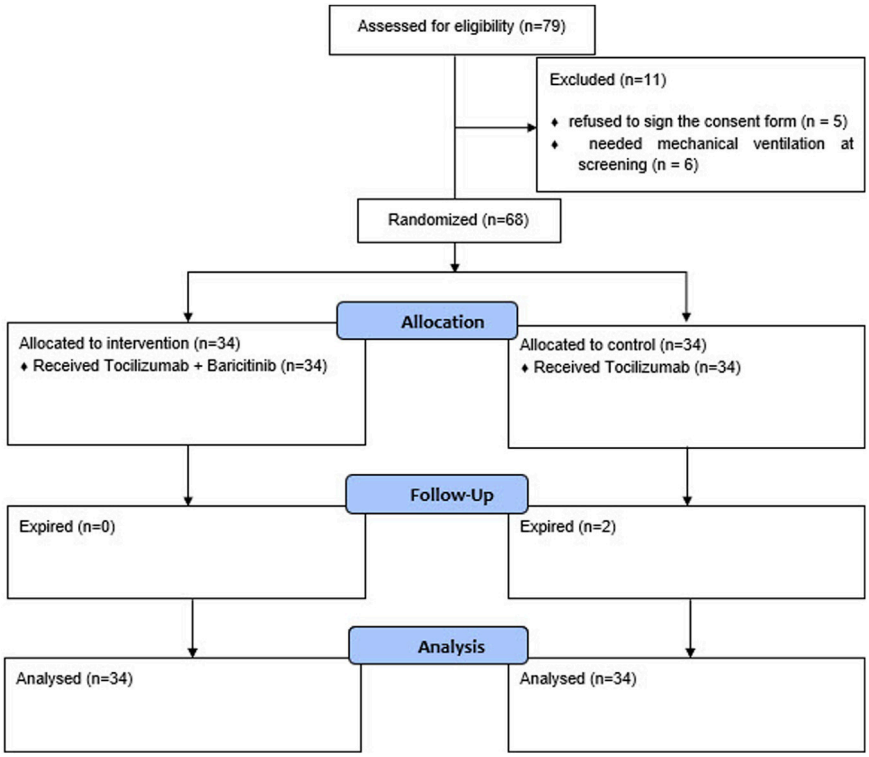

FIGURE 1
Screening and randomization process of the patients.

TABLE 1 Demographics and baseline characteristics of the patients.

Characteristics	Tocilizumab (N = 34)	Tocilizumab + baricitinib (N = 34)	*p*-value
Sex–n (%) Male	24 (70.58)	22 (64.7)	0.6
Age (year)–mean ± SD	61.32 ± 17.1	69.21 ± 16.62	0.058
Smoking (yes)	6 (17.64)	2 (5.88)	0.25
Asthma (yes)	1 (2.94)	1 (2.94)	1
Hypertension (yes)	15 (44.11)	17 (50)	0.62
Diabetes mellitus (yes)	8 (23.52)	9 (26.47)	0.77
Chronic kidney diseases (yes)	3 (8.82)	1 (2.94)	0.61
Chronic obstructive pulmonary disease (yes)	2 (5.88)	0 (0)	0.49
Rheumatoid arthritis (yes)	1 (2.94)	0 (0)	1
Malignancy (yes)	5 (14.7)	4 (11.76)	1

beneficial effects on the prevention from ICU admission. However, the sample size needed to be larger to detect that beneficial effect.

Baricitinib became the first immunomodulatory treatment for COVID-19 to receive FDA approval. This approval was supported by two phase 3, randomized, double-blind placebo-controlled trials (Rubin, 2022). Based on a review article, baricitinib was effective in decreasing respiratory failure and the use of mechanical ventilation, also preventing symptoms deterioration. Furthermore, baricitinib as a single agent or combined with other drugs, improved the peripheral capillary oxygen saturation (SpO2)/fraction of inspired oxygen (FiO2) ratio (Dupuis et al., 2022).

Both baricitinib and tocilizumab are considered a standard of care in managing severe COVID-19 patients with inflammatory status based on COVID-19 treatment guidelines (Bhimraj et al., 2020). Some other studies have also considered the co-administration of these two medications, which may lead to more rapid recovery of the patients from the inflammatory status as they both suppress the immune system (Rosas et al., 2020). The authors of that study believed that the

TABLE 2 Results of the study outcomes.

Outcomes	Tocilizumab (N = 34)	Tocilizumab + baricitinib (N = 34)	*p*-value
28-day mortality rate	2 (5.88%)	0 (0%)	0.18
The need for mechanical ventilation	2 (5.88%)	0 (0%)	0.18
The need for non-invasive mechanical ventilation	5 (14.7%)	4 (11.76%)	1
The need for tocilizumab second dose	10 (29.41%)	10 (29.41%)	0.42
The need for admission to the ICU	6 (17.64%)	1 (2.94%)	0.1
Length of hospital stay (Day)	10.55 (6.34)	10.41 (5.37)	0.9

Data are presented as n (%); ICU, intensive care unit.

TABLE 3 Comparison of the lab tests between the two groups.

Lab tests	Tocilizumab	Total	Tocilizumab + baricitinib	Total	*p*-value
Cr before Intervention (mg/dL)	1.51 ± 0.49	21	1.33 ± 0.36	24	0.18
Cr 5 days after Intervention (mg/dL)	1.44 ± 0.35	17	1.24 ± 0.33	20	0.09
AST before Intervention (IU/L)	44.72 ± 34.82	18	45.7 ± 25.81	17	0.92
AST 5 days after Intervention (IU/L)	41 ± 22.42	15	33.46 ± 12.92	13	0.29
ALT before Intervention (IU/L)	45 ± 48.32	18	46.47 ± 45.32	17	0.92
ALT 5 days after Intervention (IU/L)	81 ± 99.02	15	41.07 ± 23.35	13	0.15
CRP before Intervention (mg/dL)	46.26 ± 27.09	17	56.34 ± 19.63	20	0.21
CRP 5 days after Intervention (mg/dL)	12.61 ± 17.61	11	14.4 ± 11.15	14	0.75
D-Dimer before Intervention (mg/L)	1,197.44 ± 1,060.95	34	771.5 ± 905.43	30	0.09
D-Dimer 3 days after Intervention (mg/L)	1,255.77 ± 12.08.03	27	1,034.86 ± 1,105.24	23	0.5

Data are presented as Mean ± SD; Cr, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-Reactive protein; IL-6, Interleukin-6.

combination therapy of these medications could be considered in COVID-19 patients with impaired PaO_2/PaFi. No serious adverse events were reported in their study (Rosas et al., 2020). In one study, authors concluded that using baricitinib along with standard of care treatments was associated with mortality reduction in hospitalized COVID-19 patients (Banga et al., 2023).

Both tocilizumab and baricitinib have anti-inflammatory properties. Combining these medications may result in a more comprehensive suppression of the hyperinflammatory response often seen in severe COVID-19 cases (Bryus et al., 2022).

In our study, one patient developed thrombosis. The relation of this event to the study intervention may not be conclusive as COVID-19 is a risk factor for developing thrombotic events itself. However, it should be noted that the use of baricitinib also may increase this risk. No infectious-related complications or any issues related to over-suppression of the immune system were noted.

In another observational cohort study performed on microbiologically-confirmed COVID-19 hospitalizations, the addition of baricitinib to the standard of care, including tocilizumab did not reduce the mortality rate in hospitalized COVID-19 patients, which is in line with our results (Masiá et al., 2021). Moreover, no difference in the thromboembolic events or infection rates was detected in that study (Masiá et al., 2021). While some studies have shown promise, the evidence regarding the combined use of tocilizumab and baricitinib for COVID-19 is still evolving. More research is needed to establish the safety and efficacy of this combination definitively. It is noteworthy that both tocilizumab and baricitinib are expensive medications, and using them together may significantly increase the overall treatment cost. The decision to combine these medications should be made on a case-by-case basis, taking into account the patient's overall health, comorbidities, and individual response to treatment.

The results of the lab tests, demonstrates no significant and meaningful differences regarding the safety issues or the inflammation reduction between the two groups.

While some studies have shown promise, the evidence regarding the combined use of tocilizumab and baricitinib for COVID-19 is still evolving. More research is needed to establish the safety and efficacy of this combination definitively.

This study had some limitations. The main limitation of our study was the absence of a power calculation for sample size determination. Nevertheless, given the calculated risk difference of 0.05 in the primary outcomes, which represents a trivial effect size, it appears that increasing the sample size would not alter the results. In addition, the open-label design of the study could have affected the results which must be taken into account.

Moreover, according to the participants' baseline characteristics, the patients' smoking status might be considered a covariate in this study. However, the low number of the events related to the primary outcomes was a limitation for performing adjustment analysis based on regression models.

5 Conclusion

The combination therapy of tocilizumab plus baricitinib did not have any beneficial effects on managing severe COVID-19 cases. However, the need for ICU admission was meaningfully lower in the combination group.

Data availability statement

The raw data supporting the conclusion of this article will be made available upon a reasonable request to the corresponding author.

Ethics statement

The studies involving humans were approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.PHARMACY.REC.1400.296). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

FD: Data curation, Investigation, Methodology, Project administration, Supervision, Writing–original draft, Writing–review and editing. HJ: Conceptualization, Formal Analysis, Methodology, Validation, Visualization, Writing–original draft, Writing–review and editing. SB: Investigation, Methodology, Validation, Visualization, Writing–original draft, Writing–review and editing. SV: Data curation, Methodology, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. SY: Data curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing–original draft, Writing–review and editing. EN: Data curation, Formal Analysis, Writing–original draft, Writing–review and editing. PT: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Baden, L. R., El Sahly, H. M., Essink, B., Follmann, D., Neuzil, K. M., August, A., et al. (2021). Phase 3 trial of mRNA-1273 during the delta-variant surge. *N. Engl. J. Med.* 385 (26), 2485–2487. doi:10.1056/NEJMc2115597

Banga, A., Bansal, V., Iqbal, k, Rathore, S., Amal, T., Patnaik, H., et al. (2023). 356: therapeutic use of baricitinib in covid-19: a systematic review and meta-analysis. *Crit. care Med.* 51 (1), 164. doi:10.1097/01.ccm.0000907152.30646.d6

Bhimraj, A., Morgan, R. L., Shumaker, A. H., Lavergne, V., Baden, L., Cheng, V. C., et al. (2020). Infectious diseases society of America guidelines on the treatment and management of patients with coronavirus disease 2019 (COVID-19). *Clin. Infect. Dis.*, ciaa478. doi:10.1093/cid/ciaa478

Bryushkova, E. A., Skatova, V. D., Mutovina, Z. Y., Zagrebneva, A. I., Fomina, D. S., Kruglova, T. S., et al. (2022). Tocilizumab, netakimab, and baricitinib in patients with mild-to-moderate COVID-19: an observational study. *PLoS One* 17 (8), e0273340. doi:10.1371/journal.pone.0273340

Cavalli, G., De Luca, G., Campochiaro, C., Della-Torre, E., Ripa, M., Canetti, D., et al. (2020). Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol.* 2 (6), e325–e331. doi:10.1016/S2665-9913(20)30127-2

Dastan, F., Saffaei, A., Haseli, S., Marjani, M., Moniri, A., Abtahian, Z., et al. (2020). Promising effects of tocilizumab in COVID-19: a non-controlled, prospective clinical trial. *Int. Immunopharmacol.* 88, 106869. doi:10.1016/j.intimp.2020.106869

Dupuis, D., Fritz, K., Ike, E., Arogundade, O., Adewara, E. O., Monday, E. O., et al. (2022). Current use of baricitinib in COVID-19 treatment and its future: an updated literature review. *Cureus* 14 (9), e28680. doi:10.7759/cureus.28680

Hajissa, K., Islam, M. A., Hassan, S. A., Zaidah, A. R., Ismail, N., and Mohamed, Z. (2022). Seroprevalence of SARS-CoV-2 antibodies in Africa: a systematic review and meta-analysis. *Int. J. Environ. Res. Public Health* 19 (12), 7257. doi:10.3390/ijerph19127257

Karolyi, M., Gruebl, A., Omid, S., Saak, M., Pawelka, E., Hoepler, W., et al. (2022). Tocilizumab vs. baricitinib in hospitalized severe COVID-19 patients: results from a real-world cohort. *Infection* 51, 851–858. doi:10.1007/s15010-022-01915-7

Keystone, E. C., Taylor, P. C., Drescher, E., Schlichting, D. E., Beattie, S. D., Berclaz, P. Y., et al. (2015). Safety and efficacy of baricitinib at 24 weeks in patients with rheumatoid arthritis who have had an inadequate response to methotrexate. *Ann. Rheum. Dis.* 74 (2), 333–340. doi:10.1136/annrheumdis-2014-206478

Kwak, S. G., and Kim, J. H. (2017). Central limit theorem: the cornerstone of modern statistics. *Korean J. Anesthesiol.* 70 (2), 144–156. doi:10.4097/kjae.2017.70.2.144

Li, G., Hilgenfeld, R., Whitley, R., and De Clercq, E. (2023). Therapeutic strategies for COVID-19: progress and lessons learned. *Nat. Rev. Drug Discov.* 22 (6), 449–475. doi:10.1038/s41573-023-00672-y

Masiá, M., Padilla, S., García, J. A., García-Abellán, J., Navarro, A., Guillén, L., et al. (2021). Impact of the addition of baricitinib to standard of care including tocilizumab and corticosteroids on mortality and safety in severe COVID-19. *Front. Med. (Lausanne)* 8, 749657. doi:10.3389/fmed.2021.749657

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., et al. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395 (10229), 1033–1034. doi:10.1016/S0140-6736(20)30628-0

Mengato, D., Mazzitelli, M., Francavilla, A., Bettio, M., Sasset, L., Presa, N., et al. (2023). Changing patterns and clinical outcomes of hospitalized patients with COVID-19 severe pneumonia treated with remdesivir according to vaccination status: results from a real-world retrospective study. *Clin. Exp. Med.* 23, 2749–2756. doi:10.1007/s10238-023-01036-x

Rahlfs, V., and Zimmermann, H. (2019). Effect size measures and their benchmark values for quantifying benefit or risk of medicinal products. *Biometrical J. Biometrische Zeitschrift* 61 (4), 973–982. doi:10.1002/bimj.201800107

Rezaei, S., Fatemi, B., Karimi Majd, Z., Minaei, H., Peikanpour, M., Anjidani, N., et al. (2021). Efficacy and safety of tocilizumab in severe and critical COVID-19: a systematic review and meta-analysis. *Expert Rev. Clin. Immunol.* 17 (5), 499–511. doi:10.1080/1744666X.2021.1908128

Rosas, J., Liaño, F. P., Cantó, M. L., Barea, J. M. C., Beser, A. R., Rabasa, J. T. A., et al. (2020). Experience with the use of baricitinib and tocilizumab monotherapy or combined, in patients with interstitial pneumonia secondary to coronavirus COVID19: a real-world study. *Reumatol. Clin. Engl. Ed.* 18 (3), 150–156. doi:10.1016/j.reumae.2020.10.006

Rubin, E. J., Longo, D. L., and Baden, L. R. (2021). Interleukin-6 receptor inhibition in covid-19 - cooling the inflammatory soup. *N. Engl. J. Med.* 384 (16), 1564–1565. doi:10.1056/NEJMe2103108

Rubin, R. (2022). Baricitinib is first approved COVID-19 immunomodulatory treatment. *JAMA* 327 (23), 2281. doi:10.1001/jama.2022.9846

Samaee, H., Mohsenzadegan, M., Ala, S., Maroufi, S. S., and Moradimajd, P. (2020). Tocilizumab for treatment patients with COVID-19: recommended medication for novel disease. *Int. Immunopharmacol.* 89, 107018. doi:10.1016/j.intimp.2020.107018

Stasi, C., Fallani, S., Voller, F., and Silvestri, C. (2020). Treatment for COVID-19: an overview. *Eur. J. Pharmacol.* 889, 173644. doi:10.1016/j.ejphar.2020.173644

Uciechowski, P., and Dempke, W. C. M. (2020). Interleukin-6: a masterplayer in the cytokine network. *Oncology* 98 (3), 131–137. doi:10.1159/000505099

Wang, X., Wang, H., Yin, G., and Zhang, Y. D. (2023). Network-based drug repurposing for the treatment of COVID-19 patients in different clinical stages. *Heliyon* 9 (3), e14059. doi:10.1016/j.heliyon.2023.e14059

Wei, Q., Lin, H., Wei, R.-G., Chen, N., He, F., Zou, D.-H., et al. (2021). Tocilizumab treatment for COVID-19 patients: a systematic review and meta-analysis. *Infect. Dis. Poverty* 10 (1), 71. doi:10.1186/s40249-021-00857-w

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Efficacy of adjunctive topical liposomal clarithromycin on systemic Glucantime in Old World cutaneous leishmaniasis: a pilot clinical study

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Aim: This study aimed to investigate the effects of topical liposomal clarithromycin in combination with *meglumine antimoniate* (Glucantime®) on cutaneous leishmaniasis (CL) lesions.

Methods: This pilot, randomized, double-blinded clinical trial was conducted on patients with CL lesions. Patients were randomly assigned to two groups: the first group received liposomal clarithromycin in combination with Glucantime for 28 days, while the second group received Glucantime and a placebo. Afterward, patients were followed up at 1.5, 3, and 6 months after treatment initiation and were evaluated for recovery time, induration, and size of the lesions.

Results: Sixty patients with CL lesions were divided into two separate groups with 30 members each and were examined. Within-group analysis revealed that recovery time in the clarithromycin group was 26.65 ± 5.12 days, while in the placebo group, it was 32.84 ± 24.43, which was statistically insignificant ($p = 0.18$). Lesion size comparison in the first and last follow-ups reduced in both groups: 7.73 ± 4.31 to 0.48 ± 0.50 in the clarithromycin group ($p = 0.006$) and 5.47 ± 5.83 to 0.76 ± 0.88 in the placebo group ($p = 0.03$). Moreover, the size of lesions in the intervention group was significantly reduced compared to that in the placebo group ($p = 0.02$). Recognizable induration reduction was observed in the clarithromycin group (2.60 ± 0.77 to 1.0 ± 0.00). No adverse effects attributable to clarithromycin were reported.

Conclusion: The administration of liposomal clarithromycin in combination with systemic Glucantime had a significant beneficial effect on reducing lesion size in leishmaniasis. Further studies on larger populations are recommended.

Systematic Review Registration: https://www.irct.ir/trial/46611.

KEYWORDS

leishmaniasis, cutaneous leishmaniasis, Glucantime, liposomal clarithromycin, clarithromycin

1 Introduction

Cutaneous leishmaniasis (CL) has been re-emerging for the past two decades. Despite its incidence increasing worldwide, the lack of interest from financial donors, public health authorities, and professionals is largely due to the fact that it is rarely fatal and mainly affects people with low socioeconomic conditions. Accordingly, CL has become one of the so-called neglected diseases. Most of these lesions heal automatically with scar formation after a few months (Tiuman et al., 2011; Alvar et al., 2012; Hailu et al., 2016). However, treatment is necessary for several reasons, including weight loss, cosmetic importance, and the risk of presenting as a severe form, such as lupoid CL (Sundar and Chakravarty, 2013; Ponte-Sucre et al., 2017).

Pentavalent antimoniate compounds have traditionally been used to treat leishmaniasis and are the primary treatment for CL. Other available medications include paromomycin, miltefosine, amphotericin B, and allopurinol. However, prescription of these drugs is widely challenging due to their severe side effects, poor tolerability, narrow therapeutic window and risk of toxicity, long-term therapy, lack of efficacy, increasing rate of resistance, and high expense of specific formulation (Tiuman et al., 2011; Sazgarnia et al., 2012; Scott and Novais, 2016; Ponte-Sucre et al., 2017; Wolf Nassif et al., 2017).

Azithromycin and clarithromycin are macrolides and are suggested to have efficacy against intracellular microbes, including *Mycobacterium avium complex*, *Legionella*, *Toxoplasma gondii*, *Cryptosporidium parvum*, *Pneumocystis carinii*, *Plasmodium falciparum*, and *Leishmania* promastigotes (Balcioğlu et al., 2012; Sazgarnia et al., 2012). Topical liposomal clarithromycin has been indicated to have beneficial therapeutic effects on sensitive microorganisms and could be a suitable alternative to traditional medications for patients with mild lesions (Sazgarnia et al., 2012; Alhajlan et al., 2013).

However, the effects of liposomal clarithromycin on CL lesions have not been evaluated. Therefore, in this study, we aim to investigate the effects and complications of liposomal clarithromycin in combination with meglumine antimoniate (Glucantime®).

2 Methods and materials

2.1 Trial design and participants

The current study was a single-center, pilot, randomized, double-blinded clinical trial performed in 2020, from April to October, on patients with CL lesions in educational dermatology or infectious diseases clinics or leishmaniasis centers in Isfahan, Iran. The trial was approved by the Ethics Committee of the Isfahan University of Medical Science (Grant No. IR.MUI.MED.REC.1398.396). The study was conducted in accordance with the Declaration of Helsinki and subsequent revisions and was registered at the Iranian clinical trials (www.irct.ir; IRCTID: IRCT20171230038142N17). Written informed consent was obtained from all subjects before the initiation of the study.

The inclusion criteria were as follows: 1) patients aged 2–65 years, 2) diagnosed as CL cases by an expert dermatologist, 3) positive parasitology for leishmaniasis, including a positive smear of Leishman body PCR or skin biopsy, 4) lesion diameter up to 5 cm, 5) lesion count up to 5, and 6) no joint or mucosal membrane involvement. Patients were excluded if they 1) had a previous history of leishmaniasis, 2) were currently pregnant or lactating, 3) had a history of cardiac, renal, or liver problems, 4) were taking any medication that interferes with clarithromycin, 5) had any contraindication for the use of clarithromycin such as hypersensitivity to macrolide or ketolide antibiotics, and 6) declined to participate in the study. During the follow-up phase, subjects were excluded if they did not show up for follow-up visits, did not take the medication according to the study protocol, or experienced non-tolerable side effects. Choosing the criteria was based on similar studies evaluating topical liposomal medication's efficacy on CL (Abtahi-Naeini et al., 2021; Khamesipour et al., 2022).

2.2 Study protocol and outcome assessment

Patients were randomized into two groups using Random Allocation software for parallel-group randomized trials introduced by Saghaei (2004). The first group received liposomal clarithromycin in combination with Glucantime, and the second group was administered Glucantime and a placebo.

All patients were followed up at three different time points during this study: 6 weeks, 3 months, and 6 months after treatment initiation. The main outcome measure was the difference in lesion size (the extent of re-epithelialization in ulcerative lesions) and lesion induration from the baseline. During the follow-up sessions, patients were examined, and re-epithelialization and lesion size were measured using a photography technique. Photographs of pre- and post-treatment were evaluated by two dermatologists who were blinded to the types of treatments. The details about any side effects or complications associated with the drug were also inquired about and collected.

2.3 Medications

The Glucantime treatment was based on the national standard treatment (Khamesipour et al., 2022), consisting of intralesional administration of ampules ranging from 0.2 to 2 ccs, depending on the size of the lesion, and was delivered subcutaneously and inside the lesion. This medication was administered weekly for 8 weeks to all patients. Furthermore, the first arm was instructed to apply 2 cc of liposomal clarithromycin lotion (1% clarithromycin) nightly for

28 consecutive days. The second group received equivalent volumes of normal saline as a placebo.

For this purpose, liposomal formulations of topical clarithromycin were prepared using the dehydration–rehydration technique. It was prepared in the laboratory of the School of Pharmacy at Isfahan University of Medical Sciences using the following method: 114 mg of *dipalmitoyl phosphatidylcholine* (DPPC) and 10 mg of cholesterol taken at a ratio of 6:1 M were used. They were added to a round-bottomed balloon and dissolved in a sufficient amount of chloroform–methanol (2:1). The solution dried in the rotary evaporator and turned into a thin film. Then, 1 mg of clarithromycin was dissolved in 1 mL of phosphate buffer at pH 7.4, and the aqueous solution was used to hydrate the lipid film. The resulting suspension was vortexed for 5 min and then exposed to ultrasonic waves at a frequency of 45 Hz for 2 min (in cycles of 45 s on and 10 s off). The resulting suspension was frozen and stored in the refrigerator until subsequent use. To rehydrate the suspension, 100 µL of phosphate was added, and the mixture was vortexed for 5 min at 40°C. This step is repeated three times, and finally, we added 700 µL of buffer until the final volume reached 1 mL. The free drug was separated using an ultracentrifuge. The trapped drug in liposomes was measured spectrophotometrically at 208 nm, or microbiologically, and by *Bacillus subtilis* by agar diffusion. If the measurement was to be performed using the microbial method, the bacterium was cultured overnight in cation-adjusted *Mueller–Hinton broth* (CAMHB), and a solution of 0.5 *McFarland bacterium* (1.5 × l08 CFU/mL) was prepared. The bacterial cells were then added to the autoclaved molten agar solution at 41°C, and the contents were immediately transferred to a sterile glass Petri dish (440 × 340 mm) to cover a thin layer of agar and the bottom bacterium. The liposomal clarithromycin sample was centrifuged at 12,000 g for 20 min at 4°C in the presence of Triton X-100 at a concentration of 0.2% v/v in PBS to form a pellet from which the liposomal drug was released. At this concentration, Triton does not affect bacterial growth. Walls were made with a diameter of 5 mm and filled with 25 µL of a sample of standard clarithromycin or its liposomal solutions. The *Petri* dishes were then incubated at 37° for 18 h. Then, the diameter of the growth inhibition halo was measured and repeated three times. An average of three replications was used to quantify the efficiency of clarithromycin encapsulation in liposomes. The sensitivity of the aforementioned microbial value was 0.002 mg/L, and the minimum measurable value was 0.002 mg/L, with a correlation coefficient higher than 0.99. The standard drug curve was linear in the 0.002–0.0125 mg/L range.

2.4 Statistical analysis

Data were analyzed using SPSS software version 24 (IBM Corp., Released 2016, IBM SPSS Statistics for Windows, Version 24.0., Armonk, NY: IBM Corp.). Continuous and categorical variables were reported as mean and frequency (percentage), respectively. Basic demographic and clinical characteristics of study participants were compared between groups using an independent sample *t*-test and a chi-squared test for continuous and categorical variables, respectively. Repeated measures analysis of variance was used as the main statistical method for comparing primary outcomes over time between the two studied groups. Through repeated measures ANOVA, changes over time for primary outcomes in each group were evaluated separately (time effect), mean changes over time between groups were compared (intervention effect), and the difference in changing over time between the two groups (interaction of time and intervention) was also evaluated. $p < 0.05$ was considered statistically significant.

3 Results

The present study was performed on 60 patients with CL, who met the inclusion criteria, aged 2–62 years with a mean of 27.75 years (17.05) at the Isfahan Leishmania Center. The patients were split into two groups, as shown in Figure 1. Analysis of demographic data showed that the age of the patients treated with clarithromycin was 20.60 years (12.29), and compared to the parallel group with a mean of 34.90 years (18.29), it was significantly lower ($p < 0.001$). Due to its low sensitivity and specificity, skin biopsy should be used only in atypical cases and based on the clinical course of the disease.

The location of the lesions was categorized into four groups: head and neck, trunk, upper limbs, and lower limbs. Further analysis of the frequency of distribution of the lesion site and other baseline variables, including gender and the number of lesions, was statistically the same within the groups (Table 1).

To account for the influential age difference within the groups, adjustments were made for the variable. P1, P2, and P3 resulted from repeated measures analysis of variances, and P4 resulted from an independent sample *t*-test. The mean lesion size decreased over time in both groups, but the decrease was significantly greater in the clarithromycin group than the placebo group ($p = 0.02$). Eventually, the lesion size was significantly reduced in the clarithromycin group ($p = 0.043$) (Table 2).

Moreover, mean measurements of induration decreased over time in both groups and were statistically and clinically significant in the clarithromycin group, although there was no considerable difference between the two groups. The *p*-value for intervention was 0.38, indicating a lack of significant difference between the two groups; however, the *p*-value for intervention and time was significant at 0.007, indicating that induration decreases faster in the clarithromycin group (Table 2).

Recovery time in the clarithromycin group was 26.65 days (5.12), while in the placebo group, it was 32.84 days (24.43), which was statistically insignificant ($p = 0.18$). Additionally, patients in both groups did not report itching, dryness, swelling, redness, burning, or any other sign of allergy after treatment initiation.

4 Discussion

In the present study, we investigated the therapeutic effects of liposomal clarithromycin in combination with Glucantime on CL lesions. Although there were no significant differences between the two groups regarding the recovery time, the lesion size in the clarithromycin group was significantly reduced and was significantly smaller than that in the only-Glucantime group.

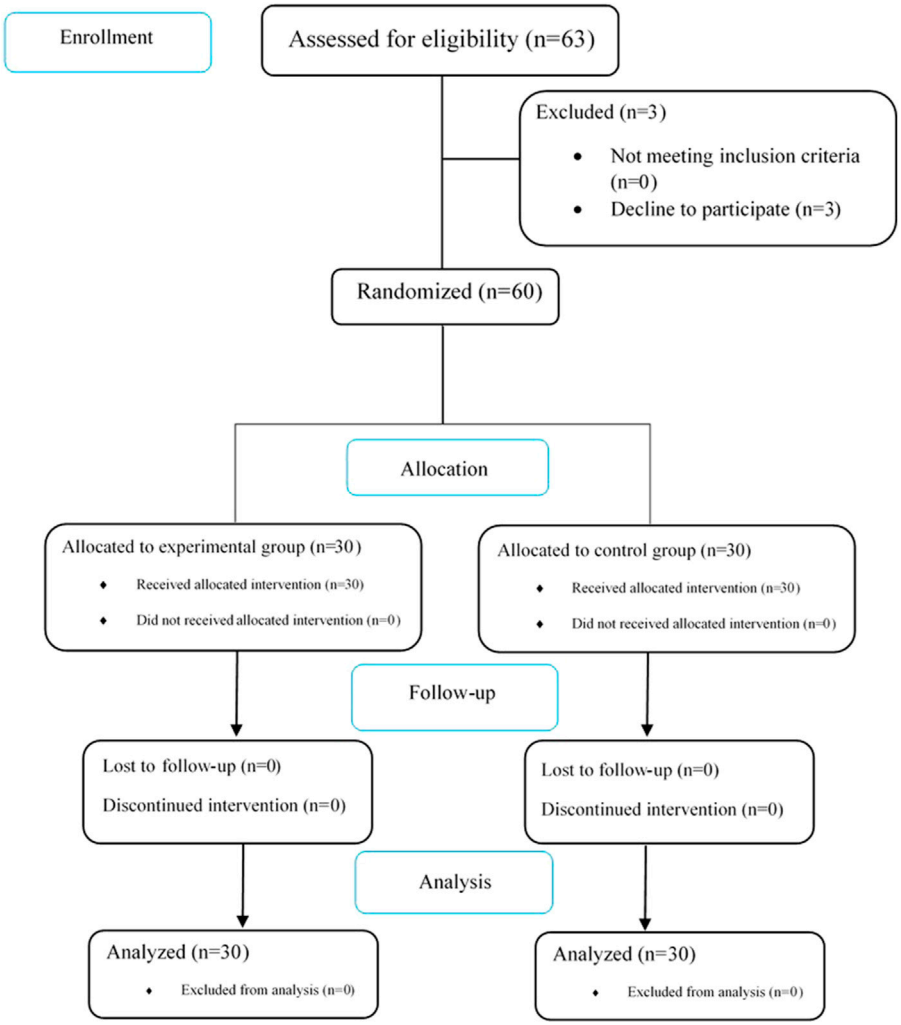

FIGURE 1
CONSORT flowchart of the study.

TABLE 1 Characteristics of the patients and investigation of the lesions in the two study groups.

Variable		Clarithromycin group (*n* = 30)	Placebo group (*n* = 30)	*p*-value
Gender	Female	3 (10%)	7 (23.33%)	0.299
	Male	27 (90%)	23 (76.67%)	
Age		20.60 (12.29)	34.90 (18.29)	0.001
Location of lesions	Head and neck	5 (16.7%)	2 (6.7%)	0.424
	Trunk	5 (16.7%)	0 (0.0%)	0.052
	Upper limb	25 (83.3%)	22 (73.3%)	0.532
	Lower limb	5 (16.7%)	12 (40.0%)	0.084
Number of lesions	1	8 (26.7%)	9 (30.0%)	0.095
	2	22 (73.3%)	17 (56.7%)	
	3	0 (0.0%)	4 (13.3%)	

Values are reported as mean (SD) and frequency (percentage) for continuous and categorical variables, respectively, resulted from independent samples. The *t*-test and chi-squared test were performed for continuous and categorical variables.

TABLE 2 Comparison of lesion size and induration.

Variable	Group	First visit	Second visit	Third visit	P1 (time)	P2 (time*intervention)	P3 (intervention)
Lesion size	1 Clarithromycin	7.73 (4.31)	3.29 (2.52)	0.48 (0.50)	0.006	0.02	0.043
	2 Placebo	5.47 (5.83)	2.35 (2.37)	0.76 (0.88)	0.03		
	P4	0.04	0.12	0.72			
Induration intensity	1 Clarithromycin	2.60 (0.77)	1.67 (0.47)	1.0 (0.00)	0.03	0.007	0.38
	2 Placebo	2.63 (0.49)	1.73 (0.94)	-	0.43		
	P4	0.87	0.80	-			

Values are reported as mean (SD). Lesion size is recorded in centimeters, and induration is recorded as intensity grading, i.e., +1, +2, and +3. According to the repeated measures analysis of variances, P1 shows the result of each approach outcome over time. According to the repeated measures analysis of variances, P2 and P3 show the comparison of within-group outcomes, and P2 considers the variable time. P4 from the independent samples *t*-test shows the comparison of within groups at each point evaluation.

The anti-microorganism property of clarithromycin is carried out through protein synthesis inhibition by reversibly connecting to 50S ribosomal subunits. It is highly concentrated in phagocytes, effectively transported to the site of infection, and has performance against intracellular microorganisms (Balcioğlu et al., 2012; Sazgarnia et al., 2012). Based on substantial evidence, clarithromycin acts against cutaneous infections, including *Mycobacterium chelonae*, *Corynebacterium minutissimum*, and *Mycobacterium intracellulare*, and has also been used in various skin conditions like rosacea, leprosy, and erythrasma (Sazgarnia et al., 2012; Abokwidir and Feldman, 2016). It was suggested to be effective not only against bacteria but also against protozoa such as *Toxoplasma gondii*, *Cryptosporidium spp*., and *Plasmodium spp*. (Hardman et al., 1996).

Balcioğlu et al. (2012) recommended azithromycin and clarithromycin as possible, effective, and safe therapeutic agents for Leishmania tropica throughout the *in vitro* study with more efficacy in clarithromycin administration. They also suggested that *in vivo* studies should be planned to detect intracellular concentrations of these drugs and determine the effective route and dosage.

Azithromycin has been used in a few research studies for CL with promising results. Possibly, due to its immunomodulatory activity, it can accelerate clinical improvement (Krolewiecki et al., 2002; Sinagra et al., 2007; Amer et al., 2016; Zabolinejad et al., 2020; Abtahi-Naeini et al., 2021). Given the similarity of clarithromycin and azithromycin, a possible therapeutic role in CL for clarithromycin has been proposed (Zabolinejad et al., 2020). Sazgarnia et al. (2012) conducted an *in vitro* study and reported that clarithromycin administration in both liposomal and non-liposomal forms had significant activity against leishmaniasis and suggested that this therapeutic technique should be used in human subjects.

Recently, Zabolinejad et al. (2020) evaluated the effects of oral clarithromycin in combination with systemic Glucantime on 20 patients and reported that this technique significantly reduced lesion size and was considered a safe and effective treatment option. Our results also showed significant effects of liposomal clarithromycin on CL, which is consistent with the previous studies that have reported the efficacy of clarithromycin on CL. The liposomal formulation of clarithromycin has been proposed to be highly effective against sensitive bacteria compared to a free drug formulation (Alhajlan et al., 2013). Additionally, liposomes have advantages like greater skin and intestinal penetration, controlled drug release, localized and limited adverse effects, targeted treatment for skin lesions, and low systemic absorption (Khamesipour et al., 2022; Banerjee et al., 2023).

Considering an increase in the rate of Leishmania resistance, a major treatment policy involves combination therapy, utilizing available drugs through new drug delivery methods such as liposomes (Tiuman et al., 2011; Ponte-Sucre et al., 2017). Other available topical drug formulations, including paromomycin and liposomal amphotericin B, have been investigated and suggested to be effective against CL, but further evaluation is needed (Wolf Nassif et al., 2017; Khamesipour et al., 2022). Macrolides, such as azithromycin and clarithromycin, are among the few suggested drugs with advantages of low risk of toxicity, diverse administration options, and safe usage during pregnancy and childhood (Balcioğlu et al., 2012).

To the best of our knowledge, the present study is the first *in vivo* clinical trial investigating topical liposomal clarithromycin's effects. Our results align with the previous studies' results, demonstrating the effectiveness of clarithromycin in reducing the size of lesions in leishmaniasis. In addition, the clarithromycin trial was associated with a faster response in reducing induration. We believe the daily administration of liposomal clarithromycin in combination with systemic Glucantime is a beneficial therapeutic strategy.

Nevertheless, our study had limitations. These include a small sample size, a short follow-up period, and a lack of comparison with other therapeutic protocols, even systemic clarithromycin. A short treatment period with clarithromycin is another limitation to our study, and we could not analyze its efficacy on complete scar formation. As a result, we recommend a large-scale, randomized, controlled trial to evaluate the effectiveness of liposomal and non-liposomal clarithromycin for CL and to compare it with other available treatments with a more prolonged treatment period.

5 Conclusion

The present study is the pilot clinical trial that investigates the effectiveness of liposomal clarithromycin. We showed that the administration of liposomal clarithromycin along with systemic Glucantime had a significant beneficial effect on lesion size in CL. These results were in line with the previous studies, but we also suggest that more studies on larger populations should be performed.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Isfahan University of Medical Science. The studies were conducted in accordance with local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The trial was approved by the Ethics Committee of the Isfahan University of Medical Science (Grant No. IR.MUI.MED.REC.1398.396). The study was conducted in accordance with the Declaration of Helsinki and subsequent revisions and was registered at the Iranian clinical trials (www.irct.ir; IRCTID: IRCT20171230038142N17).

Author contributions

AmH: Conceptualization, synthesizing and proving the medications, Investigation, Methodology, Writing–original draft, Writing–review and editing. JV: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Software, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abokwidir, M., and Feldman, S. R. (2016). Rosacea management. *Skin. Appendage Disord.* 2 (1-2), 26–34. doi:10.1159/000446215

Abtahi-Naeini, B., Hadian, S., Sokhanvari, F., Hariri, A., Varshosaz, J., Shahmoradi, Z., et al. (2021). Effect of adjunctive topical liposomal azithromycin on systemic azithromycin on Old World cutaneous leishmaniasis: a pilot clinical study. *Iran. J. Pharm. Res.* 20 (2), 383–389. doi:10.22037/ijpr.2020.113710.14445

Alhajlan, M., Alhariri, M., and Omri, A. (2013). Efficacy and safety of liposomal clarithromycin and its effect on *Pseudomonas aeruginosa* virulence factors. *Antimicrob. agents Chemother.* 57 (6), 2694–2704. doi:10.1128/AAC.00235-13

Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J., et al. (2012). Leishmaniasis worldwide and global estimates of its incidence. *PloS one* 7 (5), e35671. doi:10.1371/journal.pone.0035671

Amer, E. I., Eissa, M. M., and Mossallam, S. F. (2016). Oral azithromycin versus its combination with miltefosine for the treatment of experimental Old World cutaneous leishmaniasis. *J. Parasit. Dis.* 40 (2), 475–484. doi:10.1007/s12639-014-0529-0

Balcioğlu, İ. C., Ülgen, Z., and Özbel, Y. (2012). The *in vitro* effects of azithromycin and clarithromycin on promastigotes and amastigotes of leishmania tropica. *Kafkas Univ. Vet. Fak. Derg.* 18, A115–A20.

Banerjee, M., Sangeetha, S., Rajalakshmi, M., and Damodharan, N. (2023). Application of self nano emulsifying drug delivery system for clarithromycin. *J. Pharm. Negat. Results* 14, 821–828. doi:10.47750/pnr.2023.14.S01.114

Hailu, A., Dagne, D. A., and Boelaert, M. (2016). *Leishmaniasis. Neglected tropical diseases-sub-saharan africa.* Springer, 87–112.

Hardman, J. G., Gilman, A. G., and Limbird, L. E. (1996). *Goodman & Gilman's the pharmacological basis of therapeutics*.

Khamesipour, A., Mohammadi, A., Jaafari, M., Eskandari, S., Tasbihi, M., Javadi, A., et al. (2022). Pilot study of safety and efficacy of topical liposomal amphotericin B for cutaneous leishmaniasis caused by Leishmania major in Islamic Republic of Iran. *East Mediterr. Health J.* 28 (9), 658–663. doi:10.26719/emhj.22.070

Krolewiecki, A., Leon, S., Scott, P., and Abraham, D. (2002). Activity of azithromycin against Leishmania major *in vitro* and *in vivo*. *Am. J. Trop. Med. Hyg.* 67 (3), 273–277. doi:10.4269/ajtmh.2002.67.273

Ponte-Sucre, A., Gamarro, F., Dujardin, J.-C., Barrett, M. P., López-Vélez, R., García-Hernández, R., et al. (2017). Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. *PLoS neglected Trop. Dis.* 11 (12), e0006052. doi:10.1371/journal.pntd.0006052

Saghaei, M. (2004). Random allocation software for parallel group randomized trials. *BMC Med. Res. Methodol.* 4, 26. doi:10.1186/1471-2288-4-26

Sazgarnia, A., Zabolinejad, N., Layegh, P., Rajabi, O., Berenji, F., Javidi, Z., et al. (2012). Antileishmanial activity of liposomal clarithromycin against Leishmania major promastigotes. *Iran. J. basic Med. Sci.* 15 (6), 1210–1214. doi:10.22038/ijbms.2012.4942

Scott, P., and Novais, F. O. (2016). Cutaneous leishmaniasis: immune responses in protection and pathogenesis. *Nat. Rev. Immunol.* 16 (9), 581–592. doi:10.1038/nri.2016.72

Sinagra, A., Luna, C., Abraham, D., Iannella Mdel, C., Riarte, A., and Krolewiecki, A. J. (2007). The activity of azithromycin against Leishmania (Viannia) braziliensis and Leishmania (Leishmania) amazonensis in the golden hamster model. *Rev. Soc. Bras. Med. Trop.* 40 (6), 627–630. doi:10.1590/s0037-86822007000600005

Sundar, S., and Chakravarty, J. (2013). Leishmaniasis: an update of current pharmacotherapy. *Expert Opin. Pharmacother.* 14 (1), 53–63. doi:10.1517/14656566.2013.755515

Tiuman, T. S., Santos, A. O., Ueda-Nakamura, T., Dias Filho, B. P., and Nakamura, C. V. (2011). Recent advances in leishmaniasis treatment. *Int. J. Infect. Dis.* 15 (8), e525–e532. doi:10.1016/j.ijid.2011.03.021

Wolf Nassif, P., Tatiane França Perles, D. E. M., Navasconi, T. R., Mota, C. A., Demarchi, I. G., Aristides, S. M. A., et al. (2017). Safety and efficacy of current alternatives in the topical treatment of cutaneous leishmaniasis: a systematic review. *Parasitology* 144 (8), 995–1004. doi:10.1017/S0031182017000385

Zabolinejad, N., Layegh, P., Abbasi Shaye, Z., Salehi, M., and Ghanizadeh, S. (2020). Evaluating the effect of oral clarithromycin on acute cutaneous leishmaniasis lesions compared with systemic glucantime. *J. Dermatological Treat.* 33, 1418–1423. doi:10.1080/09546634.2020.1825612

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Evaluation of the efficacy and safety of nirmatrelvir/ritonavir co-administration inpatients with rheumatic disease infected with SARS-CoV-2: a real-world study

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Background: The breakthrough development of novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccines and oral antivirals have played a critical role in curtailing the spread of the pandemic and dramatically reducing the morbidity and mortality rates among those infected. Among these oral antivirals, nirmatrelvir/ritonavir (NR) has been repurposed successfully for use against coronavirus disease-2019 (COVID-19) and is now readily available on the market with promising therapeutic effects. The availability of convenient and effective NR treatments for COVID-19 greatly mitigates the severity of the epidemic and contributes to an early end to the pandemic. Furthermore, certain patient subgroups, specifically those with rheumatic disease (RD) who are currently undergoing intensive immunodeficiency and/or immunosuppressive treatments, continue to be vulnerable and at a higher risk of experiencing severe consequences from COVID-19. Additionally, it has also been observed that NR exhibited prevalent drug-drug interactions of clinical significance, and more instances of COVID-19 rebound were being recognized with increasing frequency.

Methods: A retrospective cohort study was conducted on a real-world RD population who were infected with SARS-CoV-2 and treated with NR. The time of symptom resolution, length of hospitalization, and response rate were assessed. Results were compared among the standard regimen and non-standard regimen groups, early NR regimen and late NR regimen groups, and the NR indication regimen and NR non-indication regimen groups. During the course, all grades of adverse drug reactions (ADRs) directly associated with NR administration and associated with drug-drug interactions (DDIs) were also monitored.

Results: A total of 32 patients with RD, who were infected with SARS-CoV-2 and received NR, were retrospectively identified and divided into different groups. We found that the standard regimen group and the early NR regimen group had a shorter median time of symptom resolution compared to the control group [9.0 (interquartile range [IQR], 8.3-11.3) vs. 21.5 (IQR16.0-24.0) days, $p < 0.001$ and 9.0 (IQR 8.3-11.3) vs. 23.0 (IQR 18.0-24.0) days, $p = 0.0$]. We further found that even if the NR administration time exceeds 5 days,

patients with RD who receive the NR indication regimen can still derive certain benefits from it. The proportion of patients who showed symptom improvement was higher in the NR indication regimen compared to the NR non-indication regimen group (n = 13/17 vs. 3/6, 76.5% vs. 50.0%) at the end of follow-up, and there was a statistical difference (p = 0.0) in the response rate of patients between the two groups. We also analyzed the effect of comorbidities on patient response rates and found that the percentage of patients who showed symptom improvement was higher in the group with <4 comorbidities compared to the group with ≥ 4 comorbidities (n = 7/7 vs. 16/25, 100.0% vs. 64.0%) at the end of follow-up. During the course, all grades of ADRs and grade ≥3ADRs directly associated with NR administration were not observed in any of the 32 cases. Despite discontinuing warfarin prior to NR application (using NR immediately on the first day of warfarin withdrawal), one patient still experienced an increased international normalized ratio [INR, 5.32(0.90-1.20)] and coagulation disorders (weak positive fecal occult blood test) on the second day after using NR. The INR levels decreased to nearly normal values, and coagulation disorders returned to normal after 2 days of discontinuing NR (the seventh day after the initial administration of NR).

Conclusion: We showed NR therapy to be associated with a favorable outcome and an acceptable safety profile in an immunosuppressed population with RD during the Omicron surge. Early use of NR (within 5 days of symptom onset) could improve the prognosis of patients. NR administration for symptoms and confirmed SARS-CoV-2 infection after >5 days may also mitigate progression to severe disease and is a viable strategy. Our results highlight the importance of early utilization and/or NR indication, which may yield clinical advantages for patients with RD infected with SARS-CoV-2.

KEYWORDS

nirmatrelvir-ritonavir, rheumatic disease, coronavirus disease 2019, severe acute respiratory syndrome coronavirus 2, real-world study

1 Introduction

Since the onset of the COVID-19 pandemic, numerous therapeutic solutions have emerged that have fundamentally transformed the medical landscape of COVID-19. The global community has made concerted efforts to neutralize the replicative capabilities of its causative agent: SARS-CoV-2. Several new and repurposed natural and/or synthetic compounds are undergoing extensive investigations (preclinical studies, clinical trials, and pharmacological evaluations) worldwide as potential efficacious anti-COVID-19 drugs.

Inhibitors targeting key enzymes (e.g., RNA-dependent RNA polymerase, papain-like protease, and main protease) involved in various lifecycle stages of SARS-CoV-2 have been reported. These include CoViTris2020/ChloViD 2020, Taroxaz-26, Taroxaz-104, teriflunomide, azvudine, 2′,3′-dideoxyinosine, forodesine, riboprine, cordycepin, ensitrelvir (S-217622), SLL0197800, CoViTris 2022, and ChloViD 2022 (Rabie, 2021a; Rabie, 2021b; Rabie, 2021c; Zhang et al., 2021; Rabie, 2022; Rabie and Abdalla, 2022; Eltayb et al., 2023; Rabie and Abdalla, 2023; Rabie and Eltayb, 2023; Rabie et al., 2023).

Moreover, several pharmaceutical agents, including chloroquine, hydroxychloroquine, darunavir, arbidolfavir, remdesivir, ribavirin, ritonavir, interferons, dexamethasone, and tocilizumab, have been repurposed for COVID-19 treatment in clinical settings with varying degrees of success. The clinical benefits of monoclonal antibodies targeting the spike protein of SARS-CoV-2 have also been demonstrated in COVID-19 treatment.

The main challenge of antiviral therapies is that they require implementation as soon as possible after SARS-CoV-2 infection to act directly on viral replication—delayed administration of antiviral agents can result in a lack of efficacy (Rahmah et al., 2022). Development of novel SARS-CoV-2 vaccines and oral small-molecule antiviral drugs has played a vital part in curtailing the spread of the pandemic, and reduced the morbidity and mortality rates among those infected. COVID-19 vaccination aims to decrease the prevalence of hospitalization, admission to intensive care units, and death. The drugs stated above simplify infection management and reduce the prevalence of hospitalization in patients with COVID-19 at risk of disease progression (Rahmah et al., 2022; Focosi et al., 2023).

Among these oral small-molecule antiviral drugs, nirmatrelvir/ritonavir (NR) has been repurposed for use against COVID-19. NR is readily available on the market and has promising therapeutic effects. The availability of convenient and efficacious NR treatments for COVID-19 could mitigate the severity of the COVID-19 epidemic and contribute to its early end.

NR received conditional approval from the China National Medical Products Administration on 11 February 2022 thanks to its favorable efficacy and safety profile. However, the application of NR adheres strictly to the principles outlined in the usage instructions and guidelines (U S Food and Drug Administration,

2023; National Health Commission of the People's Republic of China, 2023; Devresse et al., 2022). Adults who are diagnosed with mild-to-moderate COVID-19, and with a high risk of disease progression, are prescribed NR within 5 days of symptom onset. Studies have consistently confirmed the efficacy of NR in reducing the severity and mortality of COVID-19 when following the aforementioned administration instructions.

In the EPIC-HR trial (Hammond et al., 2022), which evaluated protease inhibition in COVID-19 for high-risk patients, NR administration resulted in significantly fewer COVID-19-related hospitalizations or deaths by day 28 when compared with the placebo group. The relative risk reduction was 89.1% and 88.9% at the interim and final analysis time points, respectively. However, the randomized controlled trials endorsing the use of NR in phases II/III were conducted before the emergence of Omicron variants, which are currently almost 100% prevalent. Those trials involved unvaccinated patients with COVID-19 and excluded individuals with rheumatic disease (RD) (Dal-Ré et al., 2022; Gerolymatou et al., 2023). Furthermore, certain patient subgroups (specifically those with RD undergoing intensive immunodeficiency and/or immunosuppressive treatments) continue to be vulnerable and are at a higher risk of experiencing severe consequences from COVID-19. In terms of treatment and prognosis, patients with COVID-19 with RD may benefit from certain disease-modifying antirheumatic drugs (DMARDs), but solid evidence to support this postulation is lacking (Gianfrancesco et al., 2020; Alzahrani et al., 2021; Santos et al., 2021; Oztas et al., 2022; Pehlivan and Aydin, 2022; Rabie et al., 2022). Whether NR is a safe and efficacious treatment method in patients suffering from RD warrants investigation. In addition, NR has exhibited drug-drug interactions (DDIs) of clinical importance, and instances of "COVID-19 rebound" are being recognized with increasing frequency (Marzolini et al., 2022). Hence, post-marketing assessments and updated real-world data regarding the efficacy and safety of NR have become increasingly important. Herein, we detail our experience of the efficacy and safety of NR in patients suffering from RD.

2 Materials and methods

2.1 Ethical approval of the study protocol

The study protocol was approved (2021PHB047-001) by the Ethics Committee of Peking University People's Hospital (Beijing, China) and complied with the Declaration of Helsinki 1964 and its later amendments.

2.2 The inclusion/exclusion criteria of the population

This was a real-world study conducted at the Department of Rheumatology and Immunology within Peking University People's Hospital. We retrospectively selected patients aged ≥18 years with pre-existing RD who were infected with SARS-CoV-2 and received NR treatment between 8 December 2022 and 13 January 2023. We selected this start date with the aim of reducing selection bias by ensuring most cases were caused by the Omicron variant. The diagnosis of COVID-19 was based on a positive test for the nucleic acids of SARS-CoV-2 as well as clinical manifestations, laboratory tests, and imaging (Rabie, 2021b). All patients were administered antiviral therapy using NR after hospital admission. They were closely monitored from hospital admission to hospital discharge or death.

Patients receiving any other form of antiviral therapy, who previously had NR treatment, or who received NR outside the hospital setting were excluded.

2.3 Data collection

We identified patients suffering from RD with COVID-19 using an electronic medical record (EMR) system. Certain information was extracted retrospectively from the EMR of each patient: demographic characteristics (age, ethnicity/race, sex); date of hospital admission; RD-related diagnoses; comorbidities; primary immunosuppression or immunomodulation regimens for specific RD; status of vaccination against SARS-CoV-2; COVID-19-related characteristics (date of infection confirmed by polymerase chain reaction (PCR) or viral-antigen testing on nasopharyngeal swabs, symptomatology, imaging features, and COVID-19-related severity); information on oral antiviral agents (daily dose and administration time, and times of initiation and discontinuation); current immunosuppressive or immunomodulatory regimens during NR administration; sequential treatment regimens for patients who did not improve after using NR; outcome following NR administration and sequential treatments; time to symptom resolution; duration of hospital stay (DoHS); mortality; side effects; and interactions of NR with other drugs.

2.4 Definitions and grouping

We identified the "standard regimen" group as individuals who had mild-to-moderate symptoms and confirmed SARS-CoV-2 infection within 5 days and who received NR therapy in accordance with the latest guidelines and NR-prescribing information in China. These were also the typical conditions under which normative and standardization patients were administrated, thus deviation from this may be considered the non-standard regimen group. The "early NR regimen" group referred to people who received NR within 5 days of symptom onset, whereas the "late NR regimen"group received NR after 5 days. The National Medical Products Administration of China has approved NR use for the treatment of adult patients with mild-to-moderate COVID-19 and high-risk factors for progression to severe disease. If an adult patient with severe COVID-19 used NR, it was defined as an "NR non-indication regimen". To account for potential confounding effects arising from a non-indication bias with NR, we conducted subgroup analyses that stratified patients with mild-to-moderate symptoms into an "early NR regimen" group and a"late NR regimen" group. To minimize the impact of the time of NR administration on patient outcome, we further categorized patients in the "late NR regimen" group into an "NR indication regimen" group and an "NR non-indication regimen" group.

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TABLE 1 Comparison of patients' characteristics between the NR standard regimen and non-standard regimen groups.

Characteristics	Total (N = 32)	Standard regimen (n = 6)	Non-standard regimen (n = 26)	*P*
Demographics				
Age years; n (%)				
19-65	13 (40.6)	4 (66.7)	9 (34.6)	0.2
>65	19 (59.4)	2 (33.3)	17 (65.4)	
Sex, n(%)				
Male	10 (46.9)	1 (16.7)	9 (34.6)	0.6
Female	22 (53.1)	5 (83.3)	17 (65.4)	
Anti-SARS-CoV-2 vaccine status				
0 dose	29 (90.6)	5 (83.3)	24 (92.3)	0.5
≥1dose	3 (9.4)	1 (16.7)	2 (7.7)	
Primary autoimmune disease diagnosis, n (%)				
Systemic lupus erythematosus	11 (34.4)	1 (16.7)	10 (38.5)	0.6
Sjögren's syndrome	9 (28.1)	2 (33.3)	7 (26.9)	1.0
Rheumatoid Arthritis	7 (21.9)	0	7 (26.9)	0.3
Dermatomyositis	4 (12.5)	1 (16.7)	3 (11.5)	1.0
Other disease	21 (65.6)	4 (66.7)	17 (65.4)	1.0
Comorbidities, n (%)				
Cardio-cerebrovascular disease	24 (75.0)	4 (66.7)	20 (76.9)	0.6
COPD and/or other chronic respiratory disease	21 (65.6)	2 (33.3)	19 (73.1)	0.1
Chronic renal disease	14 (43.8)	1 (16.7)	13 (50.0)	0.2
Diabetes mellitus	12 (37.5)	4 (66.7)	8 (30.8)	0.2
Chronic liver disease	12 (37.5)	0	12 (46.2)	0.1
Thyroid disease	11 (34.4)	2 (33.3)	9 (34.6)	1.0
Neurodevelopmental, neurodegenerative Diseases	6 (18.8)	3 (50.0)	3 (11.5)	0.1
<4 comorbidities	7 (21.8)	2 (33.3)	5 (19.2)	0.6
≥4 comorbidities	25 (78.1)	4 (66.7)	21 (80.8)	
Previous Treatment, n(%)				
Glucocorticoids, n (%)				
Prednisolone	21 (65.6)	3 (50.0)	18 (69.2)	0.4
csDMARDS n, (%)				
Hydroxychloroquine	11 (34.4)	2 (33.3)	9 (34.6)	1.0
Mycophenolate mofetil	8 (25.0)	2 (33.3)	6 (23.1)	0.6
Leflunomide	5 (15.6)	0	5 (19.2)	0.6
Cyclosporine	4 (12.5)	0	4 (15.4)	0.6
Other csDMARDS	10 (31.3)	0	10 (38.5)	0.1

(Continued on following page)

TABLE 1 (*Continued*) **Comparison of patients' characteristics between the NR standard regimen and non-standard regimen groups.**

Characteristics	Total (N = 32)	Standard regimen (n = 6)	Non-standard regimen (n = 26)	*P*
bDMARDS, n (%)				
IL-2[3]	3 (9.4)	0	3 (11.5)	1.0
Rituximab	2 (6.3)	0	2 (7.7)	1.0
Other bDMARDS	3 (9.4)	0	3 (11.5)	1.0
Botanical drug, n (%)				
Triptergium wilfordii	2 (6.3)	0	2 (7.7)	1.0
Total glucosides of paeony	2 (6.3)	0	2 (7.7)	1.0
Current treatment, n (%)				
Dexamethasone	25 (78.1)	3 (50.0)	22 (84.6)	0.1
Human Immunoglobulin	19 (59.4)	3 (50.0)	16 (61.5)	0.7
Hydroxychloroquine	10 (31.2)	1 (16.7)	9 (34.6)	0.6
Prednisolone	5 (15.6)	1 (16.7)	4 (15.4)	1.0
Tocilizumab	5 (15.6)	1 (16.7)	4 (15.4)	1.0
Methylprednisolone	4 (12.5)	3 (50.0)	1 (3.8)	0.0*
Sequential therapy, n (%)				
Convalescent plasma infusion	5 (15.6)	0	5 (19.2)	0.6
Azvudine	3 (9.4)	0	3 (11.5)	1.0

NR: nirmatrelvir/ritonavir.
Other diseases included antiphospholipid syndrome, anti-neutrophilic cytoplasmic autoantibody (ANCA) vasculitis, gout, systemic sclerosis, immune thrombocytopenic purpura, autoimmune hemolytic anemia, polyarteritis nodosa, nodular nonsuppurative panniculitis, psoriasis1 and overlap syndrome.
Other csDMARDs, included cyclophosphamide, methotrexate, iguratimod, and tacrolimus.
Other bDMARDs, included belimumab, tumor necrosis factor inhibitor (TNFi), and tocilizumab.
*A two-sided *p*-value less than 0.05 was deemed to be a statistical difference.

Patient outcomes comprised the time of symptom resolution, DoHS, and response rate. The "time of symptom resolution" was defined as the time from symptom onset to improvement based on objective assessments: continuously decreasing temperature and no fever for >3 days; improved respiratory symptoms; obvious absorption of inflammation revealed on pulmonary imaging; negative PCR results on nasopharyngeal swabs. The "response rate" was defined as the proportion of symptoms improved at the first objective assessment after 5 days of NR use. "Comorbidities" referred to any pre-existing or concurrent medical conditions that occurred during the clinical course of RD.

2.5 Statistical analyses

Data are the median (interquartile range (IQR)) for continuous variables. Results are counts and percentages for categorical variables. Differences between groups using a standard regimen and non-standard regimen, early regimen and late regimen, as well as NR indication and NR non-indication regimens were analyzed by the chi-square test for categorical variables, and the Kruskal–Wallis test (as appropriate) for continuous variables. The cumulative probability of the response rate among patients at follow-up was calculated using Kaplan–Meier methods. "Follow-up" was defined as the interval from symptom onset to the first objective assessment of patient outcome after 5 days of NR use. The log-rank test was employed using Prism 9 (GraphPad, La Jolla, CA, United States of America) to assess disparities among groups for standard and non-standard regimens, early and late regimens, and NR indication and NR non-indication regimens, as well as groups of patients with ≥4 comorbidities and 4 comorbidities. Statistical analyses were undertaken using SPSS 25.0 (IBM, Armonk, NY, United States of America). $p < 0.05$ (two-sided) was considered significant.

3 Results

3.1 Characteristics of the participants

In total, 32 patients with RD infected with SARS-CoV-2 and who received NR between 8 December 2022 and 13 January 2023 were identified retrospectively. The characteristics of the population at baseline are shown in Table 1.

The median age of all patients was 68 (IQR, 55–72) years and a higher proportion (59.4%) of patients aged >65 years were in the non-standard-regimen group. Most patients (n = 29/32, 90.6%) had not been vaccinated against SARS-CoV-2, and they were more commonly diagnosed with systemic lupus erythematosus (SLE, n = 11/32, 34.4%), Sjogren's syndrome (SS, n = 9/32, 28.1%), and RA (n = 7/32, 21.9%).

TABLE 2 Comparison of patients' outcomes between different NR regimen groups.

Patient outcome	Total (n = 32)	Standard regimen (n = 6)	Non-standard regimen (n = 26)	*P*	Early NR (n = 6)	Late NR (n = 17)	*P*	NR indication regimen YES, n (%) (n = 17)	NR non-indication regimen NO, n (%) (n = 6)	*P*
Time to symptom resolution, days, median (Q1, Q3)	18.0 (13.8, 24.0)	9.00 (8.3, 11.3)	21.5 (16.0, 24.0)	0.0*	9.00 (8.3, 11.3)	23.0 (18.0, 24.0)	0.0*	23.0 (18.0, 24.0)	17.5 (14.5, 23.5)	0.4
Length of hospitalization, days, median (Q1, Q3)	14.0 (9.8, 19.5)	15.5 (10.3, 17.8)	13.5 (10.0, 20.5)	1.0	15.5 (10.3, 17.8)	12.0 (9.0, 15.0)	0.4	12.0 (9.0, 15.0)	20.0 (12.3, 33.8)	0.1

NR: nirmatrelvir/ritonavir; *A two-sided *p*-value less than 0.05 was deemed to be a statistical difference.

The initial symptoms of COVID-19, in most cases, resembled those of other viral infections affecting the respiratory tract, typically dry cough (n = 20/32, 62.5%), fever (n = 18/32, 56.3%), and dyspnea (n = 16/32, 50.0%). The standard-regimen group had a lower prevalence of major comorbidities compared with that in the non-standard-regimen group, including cardio-cerebrovascular disease (66.7% vs. 76.9%), chronic obstructive pulmonary disease (COPD) and/or other chronic respiratory diseases (33.3% vs. 73.1%), chronic kidney disease (16.7% vs. 50.0%), and chronic liver disease (0% vs. 46.2%). Three deaths were observed (n = 3/26, 11.5%) in the non-standard-regimen group.

The immunosuppressive regimen administered previously primarily comprised glucocorticoids (n = 21/32, 65.6%), conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs, n = 22/32, 68.8%), biologic-DMARDs (n = 7/32, 21.9%), and other immunosuppressants (n = 4/32, 12.5%).

All patients diagnosed with COVID-19 received NR in addition to standard treatments according to the Clinical Guideline for COVID-19 Diagnosis and Treatment published by the National Health Commission of China (National Health Commission of the People's Republic of China, 2023). There were three types of standard treatment. The first treatment type was dexamethasone (5–7.5 mg/day) or methylprednisolone (40 mg/day) for ≤ 7 days. The second therapy type was intravenous immunoglobulin (IVIG) and the dose was dependent on COVID-19 severity: mild = 100 mg/kg; moderate = 200 mg/kg; severe and critical illness = 400 mg/kg. The dose was administered ≤5 times. The third treatment type was tocilizumab. The initial dose of tocilizumab was 4–8 mg/kg, with a single dose ≤800 mg, and given not more than twice.

Subsequently, the most efficacious treatment regimens for patients given NR were dexamethasone (n = 25/32, 78.1%), IVIG (n = 19/32, 59.4%), and tocilizumab (n = 5/32, 15.6%). The frequency of methylprednisolone use was higher in the standard-regimen group compared with that in the non-standard-regimen group (p = 0.0).

In the non-standard-regimen group, eight patients who did not respond to NR therapy received convalescent plasma or azvudine therapy subsequently, and significant improvement was observed for all of them.

3.2 Patient outcomes between standard and non-standard regimens

The standard-regimen group exhibited a shorter median time for symptom resolution in comparison with that in the non-standard-regimen group (9.0 (IQR 8.3–11.3) vs. 21.5 (IQR16.0–24.0) days) (p < 0.001). However, there was no significant difference in the median DoHS between the standard-regimen group and non-standard-regimen group (p = 1.0) (Table 2). Kaplan–Meier estimations revealed no significant differences (log-rank test, p = 0.5) in the cumulative probability of response between the standard-regimen group and non-standard-regimen group based on follow-up duration (Figure 1A). However, all symptoms improved in the standard-regimen group of patients at the end of follow-up.

3.3 Patient outcomes between early and late NR regimens

We divided 23 patients with mild-to-moderate COVID-19 into groups of early NR regimen and late NR regimen. The early-NR-regimen group had a shorter median time of resolution compared with that of the late-NR-regimen group (9.0 (IQR 8.3–11.3) vs. 23.0 (IQR 18.0–24.0) days) (p = 0.0]. There was no significant difference in the median DoHS between the two groups (p = 0.4) (Table 2). NR administration within or beyond 5 days did not yield a significant impact on the response in those individuals (log rank test, p = 0.8). Nevertheless, a substantial proportion of patients (n = 13/17, 76.5%) continued to exhibit improved symptoms during the first objective assessment at the end of follow-up in the late-NR-regimen group (Figure 1B). All patients in the early-NR-regimen group showed symptom improvement at the end of follow-up.

3.4 Patient outcomes between NR-indication-regimen and NR-non-indication-regimen groups

We categorized the 23 patients receiving the late NR regimen into two subgroups based on whether they had indications for NR treatment or not. The time of symptom resolution was longer (23.0 (IQR 18.0–24.0) vs. 17.5 (IQR 14.5–23.5) days) (p = 0.4] in the NR-indication-regimen group compared with that in the NR-non-indication-regimen group. DoHS was shorter in the NR-indication-regimen group than that in the NR-non-indication-regimen group (12.0 (IQR 9.0–15.0) vs. 20.0 (IQR 12.3–33.8)

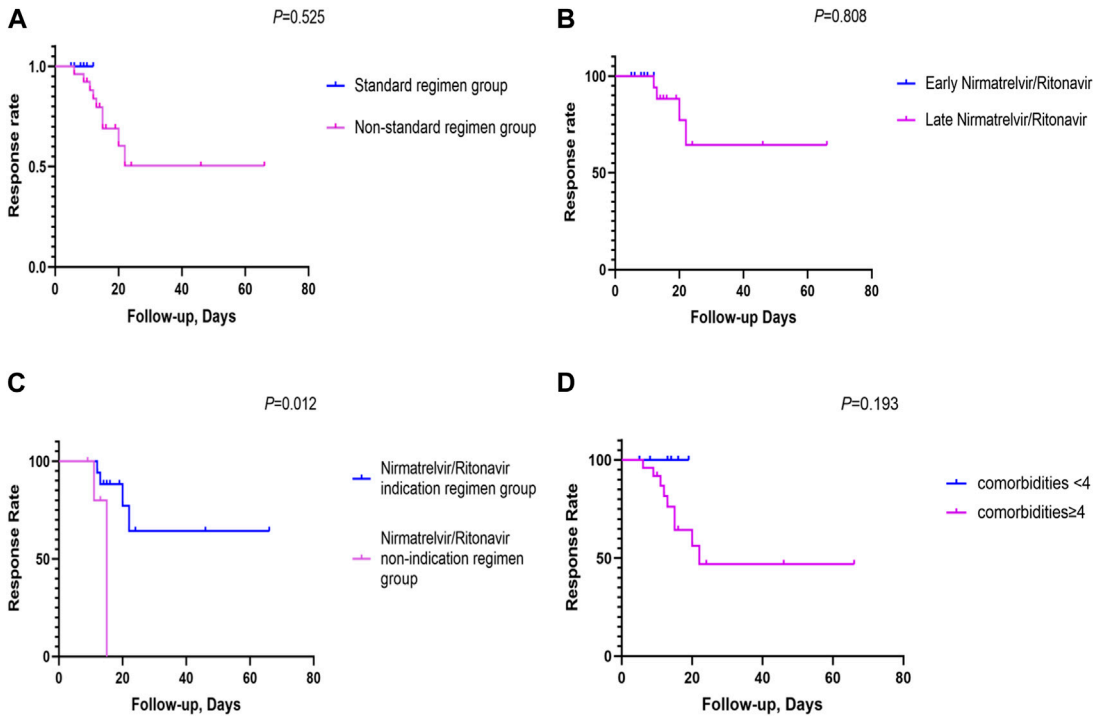

FIGURE 1
The cumulative probability of response rates between the different NR regimen groups **(A)** The cumulative probability of response rates between the NR standard regimen and the non-standard regimen groups (n = 32); **(B)** The cumulative probability of response rates between the early NR regimen group and late NR regimen group (n = 23); **(C)** The cumulative probability of response rates between the NR indication regimen group and the NR non-indication regimen group (n = 23); **(D)** The cumulative probability of response rates between comorbidities <4 and comorbidities≥4 (n = 32). (Kaplan-Meier survival curves were utilized to generate cumulative incidence curves for comparing patient response rates between different arms. The vertical axis denoted the overall response rate, and the horizontal axis represented the duration of follow-up. The follow-up period was defined as the interval from symptom onset to the first objective assessment of patient outcomes after 5 days of NR usage).

days (p = 0.1) (Table 2). The proportion of patients who showed symptom improvement was higher in the NR-indication-regimen group compared with that in the NR-non-indication-regimen group (n = 13/17 vs. 3/6, 76.5% vs. 50.0%) at the end of follow-up. A significant difference (p = 0.0) was observed in the response of patients between the two groups (Figure 1C).

3.5 ADRs induced by DDIs

During the therapy course, all grades of ADRs and ADRs of grade ≥3 directly associated with NR administration were not observed in any of the 32 cases. Ten patients received hydroxychloroquine while one patient was administered sacubitril–valsartan and clopidogrel, both of which might interact with NR. However, interactions were not observed between NR and these three drugs. Despite discontinuing warfarin before NR application (NR was used on the first day of warfarin withdrawal), one patient experienced an increased international normalized ratio (INR; 5.32 (0.90–1.20)), prothrombin time (PT; 62.8 (9.4–12.5) s), and activated partial thromboplastin time (aPTT; 66.1 (25.1–36.5) s), as well as an increased fibrinogen level (425 (200–400) mg/dL) and coagulation disorders (weak positive fecal occult blood test) on the second day after using NR. The INR (1.25), PT (14.3 s), and aPTT (37.9 s) decreased to nearly normal values, and the fibrinogen level (345 mg/dL) and coagulation disorders returned to normal after 2 days of discontinuing NR (the seventh day after initial administration of NR).

3.6 Impact of comorbidities on patient outcome

Three deaths were observed in our study, one of whom had moderate COVID-19 and the other two had severe COVID-19. The treatment regimen for these three deceased individuals was NR, dexamethasone, and IVIG. The characteristics of the three deceased individuals were identified: age >65 years and additional comorbidities such as COPD and/or other chronic respiratory diseases (including interstitial lung disease (ILD) and pulmonary infection), cardio-cerebrovascular diseases (including arrhythmia, atrial fibrillation, coronary atherosclerosis, hypertension, and pulmonary hypertension), and type-2 diabetes mellitus. All of these ailments were risk factors for COVID-19 progression. We also analyzed the effect of comorbidities on the response to therapy. The percentage of patients who showed symptom improvement was higher in the group with <4 comorbidities compared with that in the group with ≥4 comorbidities (n = 7/7 vs. 16/25, 100.0% vs. 64.0%) at the end of follow-up. However, the number of comorbidities did not have a

significant effect ($p = 0.2$) on the response rate between the two groups (comorbidities ≥4 or <4) (Figure 1D).

4 Discussion

We conducted this study in a real-world setting, which may differ from previous studies in terms of virus strains, study design, and settings. The EPIC-HR trial of NR was conducted during a period when the B.1.617.2 (Delta) variant was predominant in the United States (Hammond et al., 2022; Najjar-Debbiny et al., 2023). The present study was conducted at the beginning of the Omicron "wave" in China, during which the BA.5 and BF.7 variants were circulating predominantly and associated with a lower number of severe cases compared with those infected with the Delta variant. Importantly, we focused on vulnerable patient subgroups with RD undergoing therapy involving intensive immunodeficiency and/or immunosuppression. Patients with RD were typically receiving corticosteroids or other immunosuppressive drugs long-term. In our study, among 32 patients, 59.4% of individuals were aged >65 years, and 90.6% of patients had not received vaccination against COVID-19. All of these patients had received prednisolone and immunosuppressive drugs long-term, including hydroxychloroquine, mycophenolate mofetil, and biologic therapies (including rituximab) and 78.1% of patients had >4 complications (including cardio-cerebrovascular disease, COPD and/or other chronic respiratory diseases, and chronic kidney disease) (Gianfrancesco et al., 2020; Alzahrani et al., 2021; Arachchillage et al., 2022; Azizi et al., 2022; Sahebari et al., 2022). These are all risk factors for patients with RD suffering from COVID-19, which results in a potential hypofunctional immune state and puts them at high risk for severe COVID-19, hospitalization, and death. Furthermore, there may be a positive correlation between COVID-19 infection and prognosis with SLE, SSc, and RA. (Cordtz et al., 2021; Dewanjee et al., 2021; England et al., 2021; Grainger et al., 2021; Bournia et al., 2023). The expected response to SARS-CoV-2 vaccination for many patients with RD receiving systemic immunomodulatory therapies is blunted in its magnitude and duration compared with that in the general population but nonetheless emphasizes the importance of vaccination (Cordtz et al., 2022; Ammitzbøll et al., 2023; Curtis et al., 2023; Finckh et al., 2023). Moreover, other high-risk patients with RD who experience breakthrough infection (particularly among not fully vaccinated individuals infected with pre-Omicron variants) tend to have a worse prognosis (Bakasis et al., 2022; Liew et al., 2022).

The external environment, the characteristics of RD, and previous immunosuppressive therapies contributed to the particularity and complexity of our patients. Nevertheless, our data indicated that NR therapy was associated with a favorable outcome and an acceptable safety profile in a predominantly unvaccinated population with RD during the Omicron surge. The NR-standard-regimen group was associated with a lower risk of progression to severe outcomes in hospitalized patients with RD and COVID-19. Compared with the non-standard-regimen group, the standard-regimen group took less time for symptoms to resolve (Table 1) and more of them improved. All patients in the NR-standard-regimen group showed symptom improvement at the end of follow-up (Figure 1A). In this regard, the trend of our data was consistent with that in results reported in other real-world studies involving high-risk patients, RD population, and unvaccinated patients and the EPIC-HR trial (Wong et al., 2022a; Wong et al., 2022b; Hammond et al., 2022; Park et al., 2022; Aggarwal et al., 2023; Gentry et al., 2023; Kwok et al., 2023; Liu et al., 2023; Lui et al., 2023; Qian et al., 2023; Ramirez et al., 2023; Wan et al., 2023). Also, patients with RD infected with SARS-CoV-2 could benefit from early NR administration (within 5 days of symptom onset). The early-NR regimen reduced the time needed for symptom resolution to some extent, and all patients in the early-NR-standard-regimen group showed symptom improvement at the end of follow-up (Table 2; Figure 1A). The benefits to our patients align with a real-world study on early NR administration during the Omicron wave in high-risk patients (Bruno et al., 2022; Evans et al., 2023; Mutoh et al., 2023; Najjar-Debbiny et al., 2023; Yip et al., 2023).

Corticosteroids and some antirheumatic drugs may also be effective in treating COVID-19 (Raiteri et al., 2021; Rabie et al., 2022; Szekanecz et al., 2022). The number of cases taking other biologic drugs or csDMARDs was small and may have been insufficient to demonstrate other underlying effects (if present). We caution against causal inference regarding drug effects given the significant potential for residual confounding in our study. Fortunately, all patients diagnosed with COVID-19 in our study received NR in addition to standard treatments according to the Clinical Guideline for COVID-19 Diagnosis and Treatment (National Health Commission of the People's Republic of China, 2023) (National Health Commission of the People's Republic of China, 2023).

Furthermore, even if the duration of NR administration exceeded 5 days, patients with RD and mild-to-moderate COVID-19 who received NR could derive certain benefits from it (Figure 1C). NR has been approved by the US Food and Drug Administration for emergency use in adult patients with mild-to-moderate COVID-19 within 5 days of symptom onset and who are at a high risk of progression to severe disease. However, the efficacy of NR increased if it was administered in the first 24–48 h in the EPIC-HR trial (Hammond et al., 2022), which showed that treatment initiation within 5 days of symptom onset was associated with an 88% reduced risk of COVID-19-related hospitalization or death at 28 days. Our findings suggest that the NR-indication regimen enhanced improvement for patients with RD even if they experienced COVID-19 symptom onset beyond 5 days. A similar result was shown in a retrospective study from the multicenter EPICOVIDEHA registry in patients with a hematological malignancy, including those with symptom onset >5 days or patients with severe COVID-19 who continued to be administered NR (Salmanton-García et al., 2023), and an open-label, multicenter, randomized controlled trial including patients given NR within 5 days from symptom onset or a Ct value ≤ 25 of N and ORF1ab genes by real-time PCR (Liu et al., 2023). In conclusion, our results showed that early-NR or NR-indication treatment remained efficacious in patients with RD, and was a viable strategy for mitigating progression to severe disease. This awareness should prompt an immediate decision to use NR, thereby increasing the probability of disease improvement.

The percentage of patients who showed symptom improvement was higher in the group with <4 comorbidities compared with that in the group with ≥4 comorbidities. Patients in the non-standard-

TABLE 3 Comparison of sample sizes between the estimated group and the real group.

Effect size	α	Power (%)	Estimated sample size group 1	Real sample size group 1	Estimated sample size group 2	Real sample size group 2
0.2	0.05	80	394	6	394	26
0.5	0.05	80	64	6	64	26
0.8	0.05	80	26	6	26	26

regimen group had more comorbidities, were older, and had a higher prevalence of cardio-cerebrovascular disease, COPD and/or other chronic respiratory diseases, as well as chronic renal disease, compared with the standard-regimen group. These are risk factors that indicate the progression of COVID-19 into a severe and critical stage (Gianfrancesco et al., 2020; Parohan et al., 2020; Azizi et al., 2022; Hammond et al., 2022; Mena-Vázquez et al., 2022; Najjar-Debbiny et al., 2023; Tiseo et al., 2023; Zhang et al., 2023), potentially resulting in a reduced efficacy of NR. Three deaths were observed in the non-standard-regimen group. All of these deceased patients were >65 years of age and had comorbidities such as ILD, COPD, pulmonary infection, arrhythmia, atrial fibrillation, coronary atherosclerosis, hypertension, pulmonary hypertension, and type-2 diabetes mellitus. Prolonged use of immunosuppressive agents in these three patients was also associated with an increased risk of death (Mena-Vázquez et al., 2022; Najjar-Debbiny et al., 2023). These findings emphasize the importance of physicians being aware of old age, immunosuppression, and comorbidities in patients with RD infected with SARS-CoV-2.

NR is a combination of nirmatrelvir and ritonavir. Nirmatrelvir is eliminated primarily by the kidneys, with minimal liver metabolism (Rizk et al., 2023). Utilization of ritonavir to enhance the plasma concentration of nirmatrelvir by inhibiting the expression of cytochrome P450 (CYP)3A4 confers a substantial potential for clinically significant DDIs (Lemaitre et al., 2023). The most important restriction of NR use is DDIs. Ritonavir is a potent inhibitor of CYP3A4, reaching maximal inhibition at a dose of 100 mg. Therefore, ritonavir can substantially increase the plasma concentrations of concurrently administered drugs metabolized predominantly by CYP3A4 (U S Food and Drug Administration, 2023; Marzolini et al., 2022; Rizk et al., 2023; Lemaitre et al., 2023). However, ritonavir exhibits moderate inhibition of CYP2D6 if administered as a "boosting dose", and is an inducer of CYP1A2, CYP2B6, CYP2C9, and CYP2C19 enzymes (U S Food and Drug Administration, 2023; Marzolini et al., 2022; Rizk et al., 2023; Lemaitre et al., 2023). In addition, ritonavir inhibits the expression of the transporters P-glycoprotein and breast cancer resistance protein, which show high expression in the intestine, leading to the enhanced intestinal absorption of certain drugs. Ritonavir also hinders the hepatic uptake of organic anion transporting polypeptide (OATP)1B1 and OATP1B3, resulting in increased plasma concentrations of drugs such as statins (U S Food and Drug Administration, 2023; Marzolini et al., 2022).

Therefore, physicians and pharmacists should check the prescriptions given to patients. Also, the prescription should be adjusted while considering potential DDIs. For this reason (and also considering alleviation of immunosuppression), primary immunosuppressive or immunomodulatory treatments were discontinued before NR administration in our study. (Marsousi et al., 2018).

However, one patient received sacubitril–valsartan and clopidogrel, and 10 patients were administered hydroxychloroquine; these drugs could interact with NR. Sacubitril–valsartan and clopidogrel may have moderate interactions with NR (Dal-Ré et al., 2022; Eltayb et al., 2023). Sacubitril and valsartan have been reported to be substrates of human OATP1B1, OATP1B3, OAT1, and OAT3. Also, weak inhibition of the hepatic uptake transporter OATP1B1 by NR may increase the concentration of valsartan and the active metabolite of sacubitril, resulting in hypotension (U S Food and Drug Administration, 2023; Dal-Ré et al., 2022). Hence, blood pressure should be monitored upon co-administration with NR, and sacubitril–valsartan stopped if hypotension ensues (U S Food and Drug Administration, 2023; Abraham et al., 2022). Clopidogrel is a prodrug converted to its active metabolite by CYP3A4, CYP2B6, CYP2C19, and CYP1A2. Co-administration with ritonavir may reduce conversion to the active metabolite, leading to insufficient inhibition of platelet aggregation (U S Food and Drug Administration, 2023). One study assessed the combination of clopidogrel with ritonavir. The authors demonstrated that ritonavir reduced the area under the concentration–time curve by 3.2-fold (p = 0.02) and the maximum plasma concentration of a clopidogrel-active metabolite (p = 0.03), which led to diminished platelet inhibition (Ross et al., 2022). Thus, combined use of NR and clopidogrel should be avoided and, if possible, substitution with prasugrel should be attempted during NR treatment for ≥3 days (up to 5 days) after NR treatment (U S Food and Drug Administration, 2023). Significant DDIs are not expected between NR and hydroxychloroquine (U S Food and Drug Administration, 2023).

One patient was given NR on the first day after warfarin withdrawal and experienced increases in the INR, PT, aPTT, fibrinogen level, and coagulation disorders. Warfarin is a mixture of enantiomers. The S-enantiomer (more potent) is largely metabolized by CYP2C9. The R-enantiomer is metabolized by CYP3A4 and CYP1A2. Ritonavir inhibits CYP3A4 but induces CYP2C9 and CYP1A2 (U S Food and Drug Administration, 2023; Rizk et al., 2023). Reduction in warfarin exposure has been reported with chronic use of ritonavir, but an increase in warfarin exposure is anticipated with the short treatment course of NR because the onset of inhibition is more rapid than induction. The INR should be closely monitored if warfarin is administered with NR (U S Food and Drug Administration, 2023; Rizk et al., 2023; Ross et al., 2022). Despite discontinuing warfarin 1 day before NR application in one patient, an increase in the INR was observed, possibly due to the half-life of warfarin and the early, short treatment course of NR. Drug utilization for managing comorbidities may

influence COVID-19 treatment with potential DDIs. This phenomenon highlights the safety concerns regarding DDI risks in COVID-19 management among patients with comorbidities.

Clinically evident adverse events related directly to NR were not observed. This observation may have been related to our relatively small patient cohort, which could have led to certain biases. In addition, patients with RD presented with more severe clinical manifestations of COVID-19, which may have obscured some mild ADRs.

5 Limitations

Our study had two main limitations. First, this was a retrospective, single-center cohort study focusing on a population with RD in a unique background during a special time period. Hence, the sample size might have been too small and/or the study period might have been too short to observe significant differences in some patient outcomes. Nevertheless, a discernible trend of clinical importance in patient outcomes was observed. According to the G-Power Calculator on http://www.gpower.hhu.de, the achieved power (1-beta error probability) from calculations was also compared with the desired power, which was set at 80% (Serdar et al., 2021). The calculated effect size of the NR standard regimen group was 1.0, while it was 4.6 in the non-standard regimen group. The common standard deviation (sigma, σ) for the NR standard regimen group was 2.6, and 12.1 for the NR non-standard regimen group. Using a two-sided *t*-test to detect the difference between two groups and setting the probability of type I error (alpha) at 0.05, the real sample size for the non-standard group was 26, and the power was 100.0% higher than the desired power. However, the real sample size of the standard regimen group was only 6 with a power of 57.0%, which is too small to be reliable. Nevertheless, other large-scale real-world clinical studies have also demonstrated the effectiveness of the standard NR regimen in preventing COVID-19 disease progression and our results may be still persuasive. Table 3 presents a comparison between the estimated sample size and the real sample size when the effect size is 0.2, 0.5, and 0.8 and the power value is 80% respectively. Second, with regard to the observational nature of our study, patients in the non-standard-regimen group were characterized by old age and a higher probability of comorbidities. These factors may have contributed to unfavorable outcomes and increased mortality rates. To overcome a potential selection bias and make the results more robust, we conducted subgroup analyses to evaluate the impact of the duration of NR administration, NR indication, and number of comorbidities on patient outcomes. Despite these limitations, our study provided interesting data on the use of NR > 5 days after symptom onset. Several factors limited the scope of our study. Fortunately, this study is only preliminary, and a large-scale clinical research study to verify the effectiveness of our method and veracity of our results would be worthwhile.

6 Conclusion

We showed NR therapy to be associated with a favorable outcome and an acceptable safety profile in an immunosuppressed population with RD during the Omicron surge. Early use of NR (within 5 days of symptom onset) could improve the prognosis of patients. NR administration for symptoms and confirmed SARS-CoV-2 infection after >5 days may also mitigate progression to severe disease and is a viable strategy. Our results highlight the importance of early utilization and/or NR indication, which may yield clinical advantages for patients with RD infected with SARS-CoV-2. Although our study shows interesting data on the use of NR more than 5 days after the onset of symptoms, it is worth conducting a large-scale clinical research study to verify the effectiveness of this method and its results.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethic Committee of Peking University People's Hospital (2021PHB047-001). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XuZ: Conceptualization, Data interpretation and analysis, Writing of the original draft, Editing and funding acquisition. CW: Methodology, Data interpretation and analysis, Writing of the original draft. LH: Methodology, Supervision. YZ: Clinical data collection, Data curation. TL: Clinical data collection, Data curation. JH: Supervision, Writing–review and editing. XiZ: Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abraham, S., Nohria, A., Neilan, T. G., Asnani, A., Saji, A. M., Shah, J., et al. (2022). Cardiovascular drug interactions with nirmatrelvir/ritonavir in patients with COVID-19: JACC review topic of the week. *J. Am. Coll. Cardiol.* 80 (20), 1912–1924. doi:10.1016/j.jacc.2022.08.800

Aggarwal, N. R., Molina, K. C., Beaty, L. E., Bennett, T. D., Carlson, N. E., Mayer, D. A., et al. (2023). Real-world use of nirmatrelvir-ritonavir in outpatients with COVID-19 during the era of omicron variants including BA.4 and BA.5 in Colorado, USA: a retrospective cohort study. *Infect. Dis.* 23 (6), 696–705. doi:10.1016/S1473-3099(23)00011-7

Alzahrani, Z. A., Alghamdi, K. A., and Almaqati, A. S. (2021). Clinical characteristics and outcome of COVID-19 in patients with rheumatic diseases. *Rheumatol. Int.* 41 (6), 1097–1103. doi:10.1007/s00296-021-04857-9

Ammitzbøll, C., Thomsen, M. K., Andersen, J. B., Bartels, L. E., Hermansen, M. F., Johannsen, A. D., et al. (2023). COVID-19 vaccination in patients with rheumatic diseases leads to a high seroconversion rate and reduced self-imposed isolation and shielding behaviour. *Mod. Rheumatol.* 33 (4), 777–785. doi:10.1093/mr/roac069

Arachchillage, D. J., Rajakaruna, I., Pericleous, C., Nicolson, P. L. R., Makris, M., Laffan, M., et al. (2022). Autoimmune disease and COVID-19: a multicentre observational study in the United Kingdom. *Rheumatol. Oxf. Engl.* 61 (12), 4643–4655. doi:10.1093/rheumatology/keac209

Azizi, H., Karimi, H., Kazemi, M., Rezaei, S. S., Parsaei, A., Aghaali, M., et al. (2022). COVID-19 in patients with rheumatic disease using immunomodulatory drugs: imaging findings and predictors of hospitalization. *Rheumatology Ther.* 10 (1), 249–259. doi:10.1007/s40744-022-00508-y

Bakasis, A. D., Mavragani, C. P., Voulgari, P. V., Gerolymatou, N., Argyropoulou, O. D., Vlachoyiannopoulos, P. G., et al. (2022). COVID-19: clinical features and outcomes in unvaccinated 2-dose and 3-dose vaccinated against SARS-CoV-2 patients with systemic autoimmune and autoinflammatory rheumatic diseases. *J. Autoimmun.* 131, 102846. doi:10.1016/j.jaut.2022.102846

Bournia, V. K., Fragoulis, G. E., Mitrou, P., Mathioudakis, K., Tsolakidis, A., Konstantonis, G., et al. (2023). Different COVID-19 outcomes among systemic rheumatic diseases: a nation-wide cohort study. *Rheumatol. Oxf. Engl.* 62 (3), 1047–1056. doi:10.1093/rheumatology/keac422

Bruno, G., Giotta, M., Perelli, S., De Vita, G., Bartolomeo, N., and Buccoliero, G. B. (2022). Early access to oral antivirals in high-risk outpatients: good weapons to fight COVID-19. *Viruses* 14 (11), 2514. doi:10.3390/v14112514

Cordtz, R., Kristensen, S., Westermann, R., Duch, K., Pearce, F., Lindhardsen, J., et al. (2022). COVID-19 infection and hospitalization risk according to vaccination status and DMARD treatment in patients with rheumatoid arthritis. *Rheumatol. Oxf. Engl.* 62 (1), 77–88. doi:10.1093/rheumatology/keac241

Cordtz, R., Lindhardsen, J., Soussi, B. G., Vela, J., Uhrenholt, L., Westermann, R., et al. (2021). Incidence and severeness of COVID-19 hospitalization in patients with inflammatory rheumatic disease: a nationwide cohort study from Denmark. *Rheumatol. Oxf. Engl.* 60 (SI), SI59–SI67. doi:10.1093/rheumatology/keaa897

Curtis, J. R., Johnson, S. R., Anthony, D. D., Arasaratnam, R. J., Baden, L. R., Bass, A. R., et al. (2023). American college of Rheumatology guidance for COVID-19 vaccination in patients with rheumatic and musculoskeletal diseases: version 5. *Arthritis and Rheumatology (Hoboken, N.J.)* 75 (1), E1–E16. doi:10.1002/art.42372

Dal-Ré, R., Becker, S. L., Bottieau, E., and Holm, S. (2022). Availability of oral antivirals against SARS-CoV-2 infection and the requirement for an ethical prescribing approach. *Lancet. Infect. Dis.* 22 (8), e231–e238. doi:10.1016/S1473-3099(22)00119-0

Devresse, A., Briol, S., De Greef, J., Lemaitre, F., Boland, L., Haufroid, V., et al. (2022). Safety, efficacy, and relapse of nirmatrelvir-ritonavir in kidney transplant recipients infected with SARS-CoV-2. *Kidney Int. Rep.* 7 (11), 2356–2363. doi:10.1016/j.ekir.2022.08.026

Dewanjee, S., Kandimalla, R., Kalra, R. S., Valupadas, C., Vallamkondu, J., Kolli, V., et al. (2021). COVID-19 and rheumatoid arthritis crosstalk: emerging association, therapeutic options and challenges. *Cells* 10 (12), 3291. doi:10.3390/cells10123291

Eltayb, W. A., Abdalla, M., and Rabie, A. M. (2023). Novel investigational anti-SARS-CoV-2 agent ensitrelvir "S-217622": a very promising potential universal broad-spectrum antiviral at the therapeutic frontline of coronavirus species. *ACS Omega* 8 (6), 5234–5246. doi:10.1021/acsomega.2c03881

England, B. R., Roul, P., Yang, Y., Kalil, A. C., Michaud, K., Thiele, G. M., et al. (2021). Risk of COVID-19 in rheumatoid arthritis: a national veterans affairs matched cohort study in at-risk individuals. *Arthritis and Rheumatology (Hoboken, N.J.)* 73 (12), 2179–2188. doi:10.1002/art.41800

Evans, A., Qi, C., Adebayo, J. O., Underwood, J., Coulson, J., Bailey, R., et al. (2023). Real-world effectiveness of molnupiravir, nirmatrelvir-ritonavir, and sotrovimab on preventing hospital admission among higher-risk patients with COVID-19 in Wales: a retrospective cohort study. *J. Infect.* 86 (4), 352–360. doi:10.1016/j.jinf.2023.02.012

Finckh, A., Ciurea, A., Raptis, C. E., and Rubbert-Roth, A. (2023). Susceptibility to COVID-19 and immunologic response to vaccination in patients with immune-mediated inflammatory diseases. *J. Infect. Dis.* 228 (Suppl. 1), S13–S23. doi:10.1093/infdis/jiad148

Focosi, D., McConnell, S., Shoham, S., Casadevall, A., Maggi, F., and Antonelli, G. (2023). Nirmatrelvir and COVID-19: development, pharmacokinetics, clinical efficacy, resistance, relapse, and pharmacoeconomics. *Int. J. Antimicrob. agents* 61 (2), 106708. doi:10.1016/j.ijantimicag.2022.106708

Gentry, C. A., Nguyen, P. N., Thind, S. K., Kurdgelashvili, G., and Williams, R. J. (2023). Characteristics and outcomes of US veterans with immunocompromised conditions at high risk of severe acute respiratory syndrome coronavirus 2 infection with or without receipt of oral antiviral agents. *Clin. Infect. Dis.* 2023, ciad504. doi:10.1093/cid/ciad504

Gerolymatou, N., Bakasis, A. D., Voulgari, P. V., and Vlachoyiannopoulos, P. G. (2023). Oral antiviral treatment for COVID-19 in patients with systemic autoimmune rheumatic diseases. *J. Rheumatology* 50 (8), 1078–1081. doi:10.3899/jrheum.221014

Gianfrancesco, M., Hyrich, K. L., Al-Adely, S., Carmona, L., Danila, M. I., Gossec, L., et al. (2020). Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann. Rheumatic Dis.* 79 (7), 859–866. doi:10.1136/annrheumdis-2020-217871

Grainger, R., Machado, P. M., and Robinson, P. C. (2021). Novel coronavirus disease-2019 (COVID-19) in people with rheumatic disease: epidemiology and outcomes. *Clin. Rheumatol.* 35 (1), 101657. doi:10.1016/j.berh.2020.101657

Hammond, J., Leister-Tebbe, H., Gardner, A., Abreu, P., Bao, W., Wisemandle, W., et al. (2022). Oral nirmatrelvir for high-risk, nonhospitalized adults with covid-19. *N. Engl. J. Med.* 386 (15), 1397–1408. doi:10.1056/NEJMoa2118542

Kwok, W. C., Tsoi, M. F., Leung, S. H. I., Tsui, C. K., Tam, T. C. C., Ho, J. C. M., et al. (2023). Real-world study on effectiveness of molnupiravir and nirmatrelvir-ritonavir in unvaccinated patients with chronic respiratory diseases with confirmed SARS-CoV-2 infection managed in out-patient setting. *Viruses* 15 (3), 610. doi:10.3390/v15030610

Lemaitre, F., Budde, K., Van Gelder, T., Bergan, S., Lawson, R., Noceti, O., et al. (2023). Therapeutic drug monitoring and dosage adjustments of immunosuppressive drugs when combined with nirmatrelvir/ritonavir in patients with COVID-19. *Ther. Drug Monit.* 45 (2), 191–199. doi:10.1097/FTD.0000000000001014

Liew, J., Gianfrancesco, M., Harrison, C., Izadi, Z., Rush, S., Lawson-Tovey, S., et al. (2022). SARS-CoV-2 breakthrough infections among vaccinated individuals with rheumatic disease: results from the COVID-19 Global Rheumatology Alliance provider registry. *RMD Open* 8 (1), e002187. doi:10.1136/rmdopen-2021-002187

Liu, J., Pan, X., Zhang, S., Li, M., Ma, K., Fan, C., et al. (2023). Efficacy and safety of Paxlovid in severe adult patients with SARS-Cov-2 infection: a multicenter randomized controlled study. *West. Pac.* 33, 100694. doi:10.1016/j.lanwpc.2023.100694

Lui, D. T. W., Chung, M. S. H., Lau, E. H. Y., Lau, K. T. K., Au, I. C. H., Lee, C. H., et al. (2023). Analysis of all-cause hospitalization and death among nonhospitalized patients with type 2 diabetes and SARS-CoV-2 infection treated with molnupiravir or nirmatrelvir-ritonavir during the omicron wave in Hong Kong. *JAMA Netw. Open* 6 (5), e2314393. doi:10.1001/jamanetworkopen.2023.14393

Marsousi, N., Daali, Y., Fontana, P., Reny, J. L., Ancrenaz-Sirot, V., Calmy, A., et al. (2018). Impact of boosted antiretroviral therapy on the pharmacokinetics and efficacy of clopidogrel and prasugrel active metabolites. *Clin. Pharmacokinet.* 57 (10), 1347–1354. doi:10.1007/s40262-018-0637-6

Marzolini, C., Kuritzkes, D. R., Marra, F., Boyle, A., Gibbons, S., Flexner, C., et al. (2022). Recommendations for the management of drug-drug interactions between the COVID-19 antiviral nirmatrelvir/ritonavir (paxlovid) and comedications. *Clin. Pharmacol. Ther.* 112 (6), 1191–1200. doi:10.1002/cpt.2646

Mena-Vázquez, N., Manrique Arija, S., Rojas-Giménez, M., Raya-Ÿlvarez, E., Velloso-Feijoó, M. L., López-Medina, C., et al. (2022). Hospitalization and mortality from COVID-19 of patients with rheumatic inflammatory diseases in Andalusia. *Reumatol. Clin.* 18 (7), 422–428. doi:10.1016/j.reumae.2021.02.006

Mutoh, Y., Umemura, T., Nishikawa, T., Kondo, K., Nishina, Y., Soejima, K., et al. (2023). Real-world experience of the comparative effectiveness and safety of molnupiravir and nirmatrelvir/ritonavir in high-risk patients with COVID-19 in a community setting. *Viruses* 15 (3), 811. doi:10.3390/v15030811

Najjar-Debbiny, R., Gronich, N., Weber, G., Khoury, J., Amar, M., Stein, N., et al. (2023). Effectiveness of paxlovid in reducing severe coronavirus disease 2019 and mortality in high-risk patients. *Clin. Infect. Dis.* 76 (3), e342–e349. doi:10.1093/cid/ciac443

National Health Commission of the People's Republic of China (2023). Diagnosis and treatment protocol for COVID-19 patients (Trial Version 9). *Health Care Sci.* 1, 14–28. doi:10.1002/hcs2.1

Oztas, M., Bektas, M., Karacan, I., Aliyeva, N., Dag, A., Aghamuradov, S., et al. (2022). Frequency and severity of COVID-19 in patients with various rheumatic diseases treated regularly with colchicine or hydroxychloroquine. *J. Med. Virology* 94 (7), 3431–3437. doi:10.1002/jmv.27731

Park, J. J., Lee, J., Seo, Y. B., and Na, S. H. (2022). Nirmatrelvir/ritonavir prescription rate and outcomes in coronavirus disease 2019: a single center study. *Infect. Chemother.* 54 (4), 757–764. doi:10.3947/ic.2022.0123

Parohan, M., Yaghoubi, S., Seraji, A., Javanbakht, M. H., Sarraf, P., and Djalali, M. (2020). Risk factors for mortality in patients with Coronavirus disease 2019 (COVID-19) infection: a systematic review and meta-analysis of observational studies. *J. Int. Soc. Study Aging Male* 23 (5), 1416–1424. doi:10.1080/13685538.2020.1774748

Pehlivan, Ö., and Aydin, T. (2022). Clinical outcomes of patients with COVID-19 and inflammatory rheumatic diseases receiving biological/targeted therapy. *Ann. Saudi Med.* 42 (3), 155–164. doi:10.5144/0256-4947.2022.155

Qian, G., Wang, X., Patel, N. J., Kawano, Y., Fu, X., Cook, C. E., et al. (2023). Outcomes with and without outpatient SARS-CoV-2 treatment for patients with COVID-19 and systemic autoimmune rheumatic diseases: a retrospective cohort study. *Lancet. Rheumatology* 5 (3), e139–e150. doi:10.1016/S2665-9913(23)00006-1

Rabie, A. M. (2021a). Two antioxidant 2,5-disubstituted-1,3,4-oxadiazoles (CoViTris2020 and ChloViD2020): successful repurposing against COVID-19 as the first potent multitarget anti-SARS-CoV-2 drugs. *New J. Chem.* 45 (2), 761–771. doi:10.1039/D0NJ03708G

Rabie, A. M. (2021b). Potent toxic effects of Taroxaz-104 on the replication of SARS-CoV-2 particles. *Chemico-Biological Interact.* 343, 109480. doi:10.1016/j.cbi.2021.109480

Rabie, A. M. (2021c). Teriflunomide: a possible effective drug for the comprehensive treatment of COVID-19. *Curr. Res. Pharmacol. Drug Discov.* 2, 100055. doi:10.1016/j.crphar.2021.100055

Rabie, A. M. (2022). Efficacious preclinical repurposing of the nucleoside analogue didanosine against COVID-19 polymerase and exonuclease. *ACS Omega* 7 (25), 21385–21396. doi:10.1021/acsomega.1c07095

Rabie, A. M., and Abdalla, M. (2022). Forodesine and riboprine exhibit strong anti-SARS-CoV-2 repurposing potential: *in silico* and *in vitro* studies. *ACS Bio Med Chem Au* 2 (6), 565–585. doi:10.1021/acsbiomedchemau.2c00039

Rabie, A. M., and Abdalla, M. (2023). Evaluation of a series of nucleoside analogs as effective anticoronaviral-2 drugs against the Omicron-B.1.1.529/BA.2 subvariant: a repurposing research study. *Med. Chem. Res.* 32 (2), 326–341. doi:10.1007/s00044-022-02970-3

Rabie, A. M., Abdel-Dayem, M. A., and Abdalla, M. (2023). Promising experimental anti-SARS-CoV-2 agent "SLL-0197800": the prospective universal inhibitory properties against the coming versions of the coronavirus. *ACS Omega* 8 (39), 35538–35554. doi:10.1021/acsomega.2c08073

Rabie, A. M., Amer, S. A., Dawod, H. M., and Amer, Y. A. (2022). COVID-19 among rheumatoid arthritis patients on immunosuppressive therapy during the first wave of the COVID-19 pandemic: a prospective comparative study. *Afro-Egyptian J. Infect. Endemic Dis.* 12 (4), 410–419. doi:10.21608/aeji.2022.169271.1262

Rabie, A. M., and Eltayb, W. A. (2023). Potent dual polymerase/exonuclease inhibitory activities of antioxidant aminothiadiazoles against the COVID-19 omicron virus: a promising *in silico*/*in vitro* repositioning research study. *Mol. Biotechnol.* 1–20, 1–20. doi:10.1007/s12033-022-00551-8

Rahmah, L., Abarikwu, S. O., Arero, A. G., Essouma, M., Jibril, A. T., Fal, A., et al. (2022). Oral antiviral treatments for COVID-19: opportunities and challenges. *Pharmacol. Rep.* 74 (6), 1255–1278. doi:10.1007/s43440-022-00388-7

Raiteri, A., Piscaglia, F., Granito, A., and Tovoli, F. (2021). Tocilizumab: from rheumatic diseases to COVID-19. *Curr. Pharm. Des.* 27 (13), 1597–1607. doi:10.2174/1381612827666210311141512

Ramirez, G. A., Gerosa, M., Bellocchi, C., Arroyo-Sánchez, D., Asperti, C., Argolini, L. M., et al. (2023). Efficacy and safety of anti-SARS-CoV-2 antiviral agents and monoclonal antibodies in patients with SLE: a case-control study. *Biomolecules* 13 (9), 1273. doi:10.3390/biom13091273

Rizk, J. G., Lazo, J. G., Jr, Gupta, A., Lavie, C. J., and Effron, M. B. (2023). Proposal for a simple algorithmic approach to manage drug-drug interactions of oral anticoagulants with nirmatrelvir/ritonavir in COVID-19 outpatients. *Seminars Thrombosis Hemostasis* 49 (1), 85–88. doi:10.1055/s-0042-1750024

Ross, S. B., Bortolussi-Courval, É., Hanula, R., Lee, T. C., Goodwin Wilson, M., and McDonald, E. G. (2022). Drug interactions with nirmatrelvir-ritonavir in older adults using multiple medications. *JAMA Netw. Open* 5 (7), e2220184. doi:10.1001/jamanetworkopen.2022.20184

Sahebari, M., Mirfeizi, Z., Hashemzadeh, K., Salavati Nik, E., and Gholampoor Shamkani, N. (2022). Effects of biologic therapies on the chance of COVID-19 infection among rheumatoid arthritis and lupus patients during the first wave of the pandemic. *Archives Bone Jt. Surg.* 10 (11), 964–968. doi:10.22038/ABJS.2022.60064.2959

Salmanton-García, J., Marchesi, F., Gomes da Silva, M., Farina, F., Dávila-Valls, J., Bilgin, Y. M., et al. (2023). Nirmatrelvir/ritonavir in COVID-19 patients with haematological malignancies: a report from the EPICOVIDEHA registry. *EClinicalMedicine* 58, 101939. doi:10.1016/j.eclinm.2023.101939

Santos, C. S., Férnandez, X. C., Moriano Morales, C., Álvarez, E. D., Álvarez Castro, C., López Robles, A., et al. (2021). Biological agents for rheumatic diseases in the outbreak of COVID-19: friend or foe? *RMD Open* 7 (1), e001439. doi:10.1136/rmdopen-2020-001439

Serdar, C. C., Cihan, M., Yücel, D., and Serdar, M. A. (2021). Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. *Biochem. Medica* 31 (1), 010502. doi:10.11613/BM.2021.010502

Szekanecz, Z., Balog, A., Constantin, T., Czirják, L., Géher, P., Kovács, L., et al. (2022). COVID-19: autoimmunity, multisystemic inflammation and autoimmune rheumatic patients. *Expert Rev. Mol. Med.* 24, e13. doi:10.1017/erm.2022.10

Tiseo, G., Barbieri, C., Galfo, V., Occhineri, S., Matucci, T., Almerigogna, F., et al. (2023). Efficacy and safety of nirmatrelvir/ritonavir, molnupiravir, and remdesivir in a real-world cohort of outpatients with COVID-19 at high risk of progression: the PISA outpatient clinic experience. *Infect. Dis. Ther.* 12 (1), 257–271. doi:10.1007/s40121-022-00729-2

U S Food and Drug Administration (2023). Highlights of prescribing information for paxlovid. Avaliable at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/217188s000lbl.pdf.

Wan, E. Y. F., Yan, V. K. C., Mok, A. H. Y., Wang, B., Xu, W., Cheng, F. W. T., et al. (2023). Effectiveness of molnupiravir and nirmatrelvir-ritonavir in hospitalized patients with COVID-19: a target trial emulation study. *Ann. Intern. Med.* 176 (4), 505–514. doi:10.7326/M22-3057

Wong, C. K. H., Au, I. C. H., Lau, K. T. K., Lau, E. H. Y., Cowling, B. J., and Leung, G. M. (2022a). Real-world effectiveness of early molnupiravir or nirmatrelvir-ritonavir in hospitalised patients with COVID-19 without supplemental oxygen requirement on admission during Hong Kong's omicron BA.2 wave: a retrospective cohort study. *Lancet. Infect. Dis.* 22 (12), 1681–1693. doi:10.1016/S1473-3099(22)00507-2

Wong, C. K. H., Au, I. C. H., Lau, K. T. K., Lau, E. H. Y., Cowling, B. J., and Leung, G. M. (2022b). Real-world effectiveness of molnupiravir and nirmatrelvir plus ritonavir against mortality, hospitalisation, and in-hospital outcomes among community-dwelling, ambulatory patients with confirmed SARS-CoV-2 infection during the omicron wave in Hong Kong: an observational study. *Lancet (London, Engl.* 400 (10359), 1213–1222. doi:10.1016/S0140-6736(22)01586-0

Yip, T. C., Lui, G. C., Lai, M. S., Wong, V. W., Tse, Y. K., Ma, B. H., et al. (2023). Impact of the use of oral antiviral agents on the risk of hospitalization in community coronavirus disease 2019 patients (COVID-19). *Clin. Infect. Dis.* 76 (3), e26–e33. doi:10.1093/cid/ciac687

Zhang, J. J., Dong, X., Liu, G. H., and Gao, Y. D. (2023). Risk and protective factors for COVID-19 morbidity, severity, and mortality. *Clin. Rev. allergy and Immunol.* 64 (1), 90–107. doi:10.1007/s12016-022-08921-5

Zhang, J. L., Li, Y. H., Wang, L. L., Liu, H. Q., Lu, S. Y., Liu, Y., et al. (2021). Azvudine is a thymus-homing anti-SARS-CoV-2 drug effective in treating COVID-19 patients. *Signal Transduct. Target. Ther.* 6 (1), 414. doi:10.1038/s41392-021-00835-6

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Individual ingredients of NP-101 (Thymoquinone formula) inhibit SARS-CoV-2 pseudovirus infection

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Thymoquinone TQ, an active ingredient of Nigella Sativa, has been shown to inhibit COVID-19 symptoms in clinical trials. Thymoquinone Formulation (TQF or NP-101) is developed as a novel enteric-coated medication derivative from Nigella Sativa. TQF consists of TQ with a favorable concentration and fatty acids, including palmitic, oleic, and linoleic acids. In this study, we aimed to investigate the roles of individual ingredients of TQF on infection of SARS-CoV-2 variants *in-vitro*, by utilizing Murine Leukemia Virus (MLV) based pseudovirus particles. We demonstrated that NP-101, TQ, and other individual ingredients, including oleic, linoleic, and palmitic acids inhibited SARS-CoV-2 infection in the MLV-based pseudovirus model. A large, randomized phase 2 study of NP-101 is planned in outpatient COVID-19 patients.

KEYWORDS

COVID-19, Thymoquinone TQ, Coronavirus, COVID-19 and anti-viral agents, TQ formula, fatty acids

1 Introduction

The novel COVID-19 virus has had a worldwide impact with more than 500 million cases and around 6 million deaths (WHO, 2022). The Food and Drug Administration (FDA) authorized the use of several medications for both inpatient and outpatient settings (Emergency Use Authorization, 2022). Many compounds have been tested for their activity against COVID-19, including herbal medicines with defined safety and tolerability profiles (Abdelrahim et al., 2022).

Nigella Sativa, also known as black seed oil, has been tested in different disease settings as a safe, complementary, and alternative medicine that demonstrated

promising anti-inflammatory and anti-tumor effects (Mostofa et al., 2017; Pop et al., 2020). Importantly, *Nigella Sativa* has been identified as a capable medicine against COVID-19. Its use was associated with a higher chance of resolution of symptoms and faster recovery in adult patients with mild COVID-19 (Koshak et al., 2021a). The active ingredient [Thymoquinone (TQ)] of *Nigella Sativa* was proposed to inhibit COVID-19 virus infection by blocking the angiotensin-converting enzyme 2 (ACE2) receptor with immunomodulatory activities (Figure 1) (Rahman, 2020).

TQ formulation (TQF or NP-101) is a novel, patent-pending, enteric-coated formulation that consists of TQ with a tight concentration and fatty acids: palmitic, oleic, and linoleic acids. In a recent randomized, double-blind, placebo-controlled, multicenter phase II trial, oral TQF has been shown to be safe and was associated with a significantly faster decline in the total symptom burden (TSB) in patients with COVID-19 than placebo (Bencheqroun et al., 2022). Notably, TQF has also significantly increased $CD4^+$ and $CD8^+$ cytotoxic and helper T lymphocytes (Bencheqroun et al., 2022).

In this study, we aim to investigate the impact of the main ingredients of TQF on SARS-CoV-2 infection. For that, we used Murine Leukemia Virus (MLV) based pseudovirus particles that were generated in Human embryonic kidney (HEK) 293T cells with a SARS-CoV-2 spike protein construct. We tested the individual components of TQF on SARS-CoV-2 pseudovirus infection by using ACE2 expressed-HEK293T. We showed that not only TQ but also other individual ingredients of NP-101, such as palmitic, oleic, and linoleic acids had inhibitory effects on SARS-CoV-2 variants with various efficacy, possibly by inhibiting viral entry via the ACE-2 receptor.

2 Materials and methods

Cell lines, constructs, and pseudoviruses. HEK293T was obtained from ATCC (American Type Culture Collection, Cat# CRL-3216) (Manassas, VA). HEK293T, stably overexpressing the hACE2 receptor, was obtained from Codex BioSolutions (Gaithersburg, Maryland). Both cell lines were maintained in Dulbecco's MEM (Cat# 25-500, Genesee Scientific) containing 10% fetal bovine serum (Cat# 35-010-CV, Corning Life Sciences). The plasmid map of the SARS-CoV-2-Spike expression vector is shown in Supplementary Figure S1.

2.1 Test compounds

TQF (NP-101) was received from Novatek Pharmaceuticals. TQ (Cat# 03416) was purchased from MilliporeSigma. Oleic acid (Cat# O1008), Linoleic acid (Cat# L1376), and Palmitic acid (Cat# P0500) were purchased from MilliporeSigma.

The preclinical work was performed by Codex BioSolutions Inc, Gaithersburg, Maryland.

2.2 Generating of SARS-CoV-2 pseudo particles

MLV particles pseudotyped with a SARS-CoV-2 spike protein construct was generated in HEK293T cells, following a protocol described previously for SARS-CoV with some modification (Mill et al., 2016; Chen et al., 2020; Xiao et al., 2021). All the plasmid DNAs were purified with ZymoPURE II Plasmid Midiprep Kit (Cat# D4201, Zymo Research). In brief, 8 million HEK293T cells were

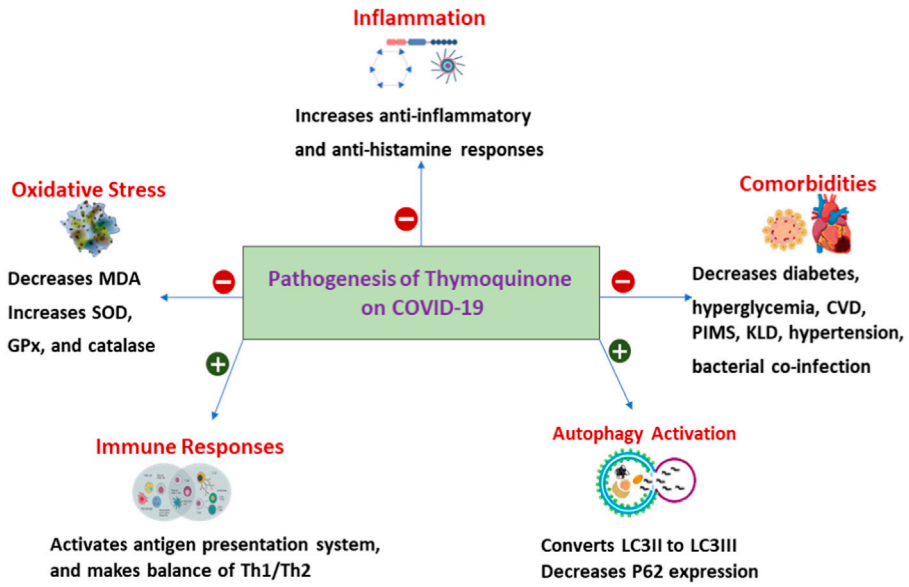


FIGURE 1
Pathogenesis *Thymoquinone* on COVID-19. SOD, Superoxide dismutase; GPx, Glutathione peroxidase; MDA, Malonaldehyde Th1, type I helper T lymphocytes; Th2, type II helper T cells; CVD, Cardiovascular disease; PIMS, Paediatric Inflammatory Multisystem Syndrome; KLD, Kawasaki-like diseases; LC3, Microtubule-associated protein 1A/1B-light chain 3; P62, protein 62.

plated into a 10-cm tissue culture dish (Cat# sc-251460, Santa Cruz) in 16 mL DMEM (Cat# 25-500, Genesee Scientific) +10% FBS (Cat# 35-010-CV, Corning Life Sciences) without any antibiotics. On the second day, the cells were transfected with 8 μg pTG-Luc, 6 μg pCMV-MLVgag-pol, and 6 μg pcDNA3.1-SARS-CoV-2-Spike-ΔC19 of different variants (Supplementary Figures S2–S5) using Lipofectamine 3,000 reagent (Cat# L3000015, Thermo Fisher). The cells were cultured for an additional 48 h. The supernatant was collected into a 50-mL Falcon tube and spun at 290 × g for 7 min. The supernatant (pseudotyped virus solution) was then passed through a 0.45 μm filter (Cat# sc 358814, Santa Cruz) using an appropriate syringe. The pseudotyped virus solution was aliquoted into cryovials and stored at −80°C. Each 10-cm cell culture dish produces about 16 mL SARS-CoV-2-PP. The SARS-CoV-2-PP was tested for quality control with the HEK293-ACE2 cell line. The quality controls were performed by two different methods: 1) RNAs in the PP were extracted with Takara's viral RNA/DNA purification kit (Cat# 740983.50). qRT-PCR was then performed with ThermoFisher's Power SYBR Green RNA-to-CT 1-Step Kit (Cat# 4389986) on QuantStudio 3 Real-Time PCR Systems. *In Vitro* transcribe luciferase RNA was used a control. The titer was calculated for each PP; 2) Each lot of PP was tested by infecting HEK293 cells. After 42-h infection, luciferase activities were measured with Codex's Luciferase assay reagent (CB-80552-010).

2.3 Testing of SARS-CoV-2 pseudovirus infection by the test compounds

In brief, 7.5 × 103 HEK293 cells, stably transfected with a full-length human ACE2 expression construct in a 15 μL culture medium, were plated into a 384-well white-clear plate coated with poly-D-Lysine to enhance the cell attachment. On Day 2, 12.5 μL of SARS-CoV-2 MLV pseudoviruses for each variant were mixed with 5 μL of each compound at different concentrations and incubated at 37°C for 30 min. After the medium in each well containing the cells was removed, 17.5 μL of each compound-virus mixture was added. The plate was centrifuged at 54 × g for 15 min at 4°C, and an additional 7·5 μL of culture medium was then added. The final total volume in each well was 25 μL. The cells were then incubated at 37°C for 42 h. Luciferase activities were measured with a Firefly Luciferase Assay Kit (CB-80552-010, Codex BioSolutions Inc). At the same time, the cell toxicities of the fatty acids on HEK293-ACE2 cells were tested using the Codex's EnerCount cell growth assay kit, which measures the ATP levels inside the cells (Cat# CB-80551-010, Codex BioSolutions). The data were normalized as the percentage of the highest reading (low concentration of each compound or no compound) of each compound (relative luciferase activity, RLU). These data were used to draw the dose-response curves against the compound concentrations. IC50 values were calculated based on curve fitting in GraphPad Prism.

3 Results

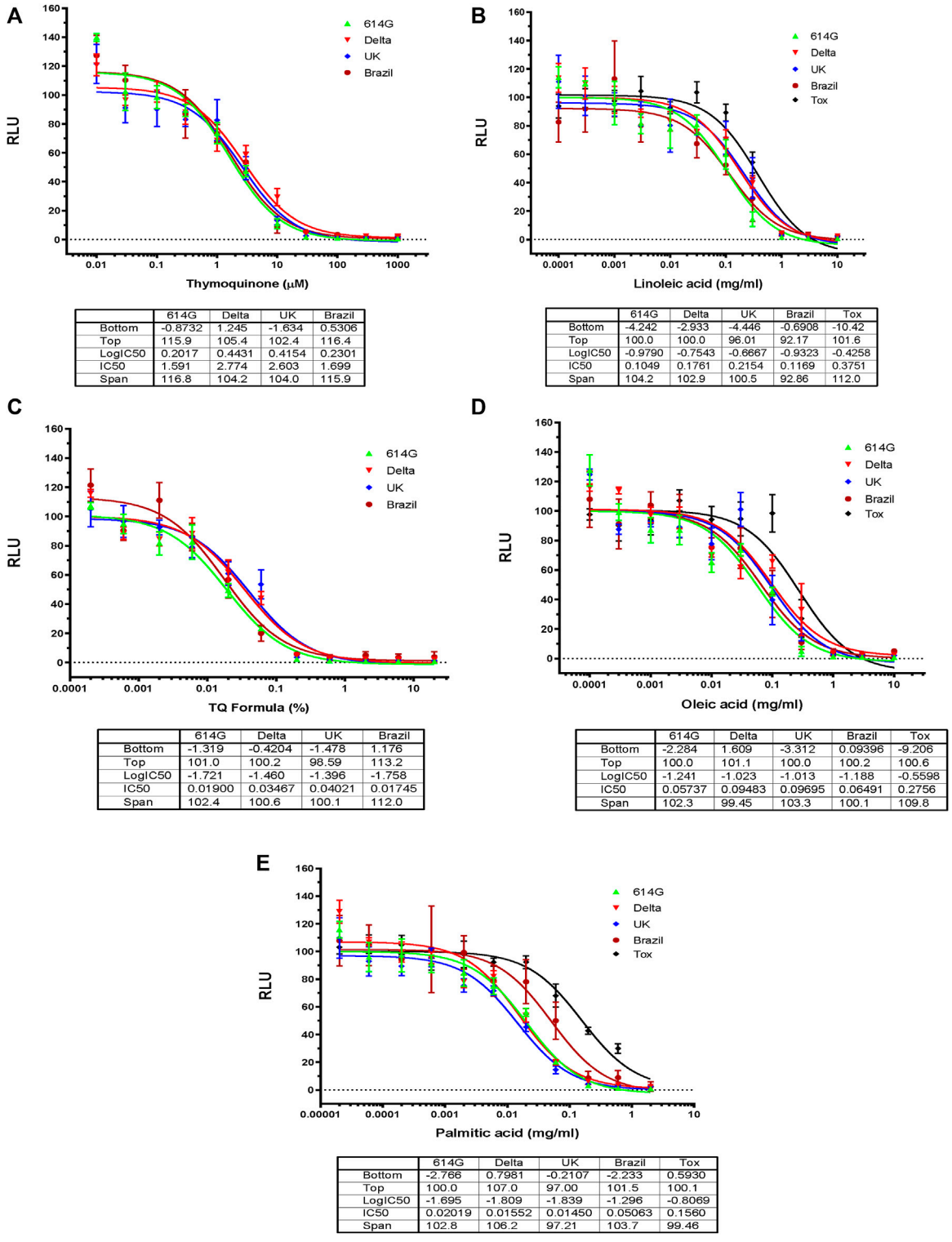
TQF is the first-ever enteric-coated capsule derived from *Nigella Sativa* oil. It is characterized and manufactured under Good Manufacturing Practices (GMP) and its patent application is currently under process (Bencheqroun et al., 2022). It consists of TQ and fatty acids, including palmitic oil, linoleic, and oleic acids. We have previously shown that both black seed oil and TQ had inhibitory effects on all four SARS-CoV-2 variants (614D, Delta, United Kingdom, Brazil) with an IC50 value range between 0.01% and 0.04% and 1.5 mM–3EmM, respectively (Figures 2A, C) (Bencheqroun et al., 2022). In this study, by using SARS-CoV-2 eGFP-luciferase spike protein pseudo-viruses, we evaluated the effect of other ingredients of the TQ formula including oleic, linoleic, and palmitic acids on viral entry into HEK293T cells stably overexpressing the hACE2 receptor (Figures 2A, C). These fatty acids also showed an inhibitory effect on infection of all four SARS-CoV-2 variants individually with an IC50 value range between 0.05 and 0.1 mg/mL for oleic acid (Figure 2D), 0.1 to 0.2 mg/mL for linoleic acid (Figure 2B), and 0.02 to 0.05 mg/mL for palmitic acid (Figure 2E). We also tested the toxicity of fatty acids on HEK293-ACE2 cells by utilizing Codex's EnerCount cell growth assay. IC50 values for cell cytotoxicity were 0.3 mg/mL for oleic acid, 0.4 mg/mL for linoleic acid, and 0.2 mg/mL for palmitic acid (Figure 2E).

Based on the results in Figure 2, we decided to perform dose-response assays for oleic acid, linoleic acid, and palmitic acid in the presence of 0.05 mM TQ; dose-response assays for linoleic acid and palmitic acid in the presence of 0.01 mg/mL of oleic acid; dose-response assays for palmitic acid in the presence of 0.01 mg/mL linoleic acid. Fatty acids showed an inhibitory effect on SARS-CoV-2 variants in the presence of 0.05 mM TQ with an IC50 value range between 0.4 and 0.8 mg/mL for oleic acid (Figure 3A), 0.4 to 0.7 mg/mL for linoleic acid (Figure 3 E), and 0.04 mg/mL to 0.1 mg/mL for palmitic acid (Figure 3C). Linoleic acid and palmitic acid appeared to inhibit SARS-CoV-2 variants in the presence of 0.01 mg/mL oleic acid with IC50 values ranging between 0.5 and 0.7 mg/mL and 0.04 to 5 mg/mL (Figures 3B,D,F) respectively. Linoleic acid also inhibited three SARS-CoV-2 variants (Delta, 614G, United Kingdom) in the presence of 0.01 mg/mL palmitic acid with an IC50 value range between 0.05 and 0.3 mg/mL.

Additionally, the sudden rise in prominence of subvariants such as 614G and XBB.1.5 (Omicron) made TQ formulation dose-response assays for relative effectiveness appropriate (Figure 4). The results demonstrated after a 0.01 dose of TQ formulation there was a substantial decline in the infectivity of both observed subvariants (Figure 4). Moreover, infectivity shows such a decline that both pseudo-viruses 614G and Omicron potency had touched zero by the time TQ levels reached a one percent formula (Figure 4).

4 Discussion

Oral drug therapy is urgently needed for the treatment of mild to moderate SARS-CoV-2 infections in outpatient settings for 2 main reasons: 1) to enhance disease recovery and shorten the time to resolution of symptoms, and 2) to prevent progression to severe disease and hospitalization or death. Herbal medications have been getting attention in the COVID-19 era, given their favorable safety and tolerability profiles. *Nigella Sativa*, also known as blackseed oil, has been shown to exert anti-viral effects (Barakat et al., 2013; Onifade et al., 2013; Onifade et al., 2015). *Nigella Sativa* oil was



	614G	Delta	UK	Brazil
Bottom	-0.8732	1.245	-1.634	0.5306
Top	115.9	105.4	102.4	116.4
LogIC50	0.2017	0.4431	0.4154	0.2301
IC50	1.591	2.774	2.603	1.699
Span	116.8	104.2	104.0	115.9

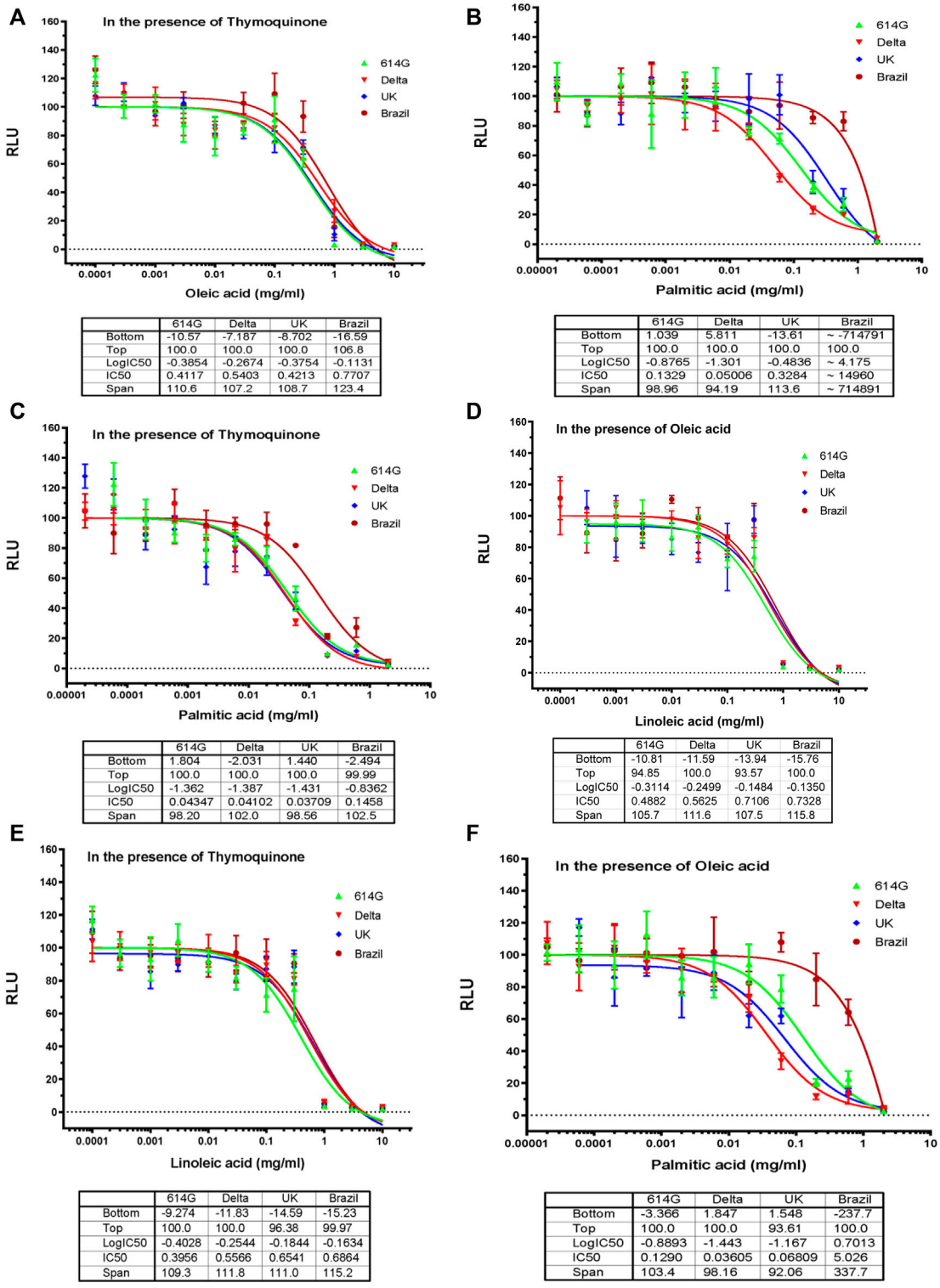
	614G	Delta	UK	Brazil	Tox
Bottom	-4.242	-2.933	-4.446	-0.6908	-10.42
Top	100.0	100.0	96.01	92.17	101.6
LogIC50	-0.9790	-0.7543	-0.6667	-0.9323	-0.4258
IC50	0.1049	0.1761	0.2154	0.1169	0.3751
Span	104.2	102.9	100.5	92.86	112.0

	614G	Delta	UK	Brazil
Bottom	-1.319	-0.4204	-1.478	1.176
Top	101.0	100.2	98.59	113.2
LogIC50	-1.721	-1.460	-1.396	-1.758
IC50	0.01900	0.03467	0.04021	0.01745
Span	102.4	100.6	100.1	112.0

	614G	Delta	UK	Brazil	Tox
Bottom	-2.284	1.609	-3.312	0.09396	-9.206
Top	100.0	101.1	100.0	100.2	100.6
LogIC50	-1.241	-1.023	-1.013	-1.188	-0.5598
IC50	0.05737	0.09483	0.09695	0.06491	0.2756
Span	102.3	99.45	103.3	100.1	109.8

	614G	Delta	UK	Brazil	Tox
Bottom	-2.766	0.7981	-0.2107	-2.233	0.5930
Top	100.0	107.0	97.00	101.5	100.1
LogIC50	-1.695	-1.809	-1.839	-1.296	-0.8069
IC50	0.02019	0.01552	0.01450	0.05063	0.1560
Span	102.8	106.2	97.21	103.7	99.46

FIGURE 2
Effects of Blackseed oil, Thymoquinone TQ and Fatty Acids on Blocking SARS-CoV-2-PP Infection of ACE2-expressing Cells. Serial dilutions of Blackseed oil, Thymoquinone TQ, Oleic acid, Linoleic acid, and Palmitic acid were tested for inhibition against an MLV-based pseudotyped virus using four different SARS-CoV-2 variant spike protein constructs (614D, Delta, United Kingdom, Brazil), in the infection of HEK293-ACE2 cells. The cell toxicities of the fatty acids on HEK293-ACE2 cells were shown as 'Tox'. *X*-axis: compound concentration. The *Y*-axis shows relative luminescence unit (RLU), reflecting the luciferase activity and the viral infectivity. The experiments were repeated three times with independent samples giving similar results. For all panels, the data points shown are mean and s.d. for n = 3 technical replicates. IC50 values derived from curve fitting are listed in the Table below each Graph.



	614G	Delta	UK	Brazil
Bottom	-10.57	-7.187	-8.702	-16.59
Top	100.0	100.0	100.0	106.8
LogIC50	-0.3854	-0.2674	-0.3754	-0.1131
IC50	0.4117	0.5403	0.4213	0.7707
Span	110.6	107.2	108.7	123.4

	614G	Delta	UK	Brazil
Bottom	1.039	5.811	-13.61	~ -714791
Top	100.0	100.0	100.0	100.0
LogIC50	-0.8765	-1.301	-0.4836	~ 4.175
IC50	0.1329	0.05006	0.3284	~ 14960
Span	98.96	94.19	113.6	~ 714891

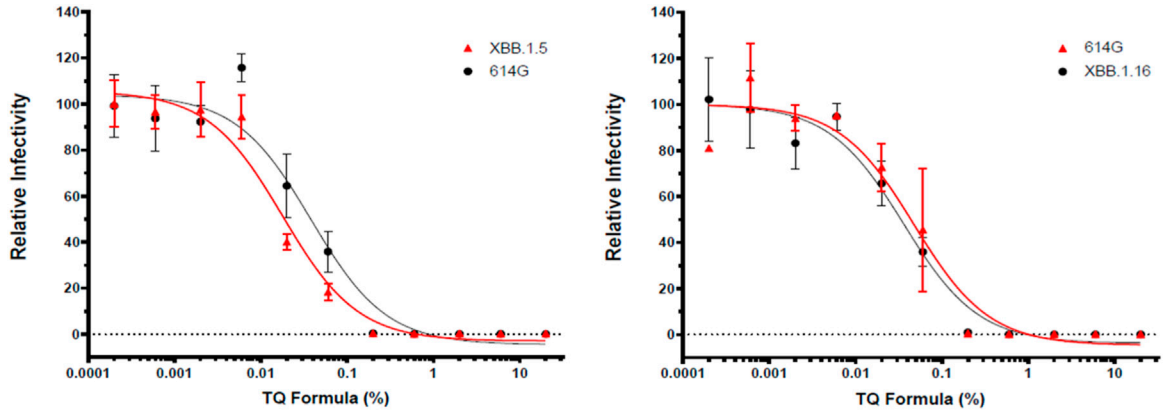
	614G	Delta	UK	Brazil
Bottom	1.804	-2.031	1.440	-2.494
Top	100.0	100.0	100.0	99.99
LogIC50	-1.362	-1.387	-1.431	-0.8362
IC50	0.04347	0.04102	0.03709	0.1458
Span	98.20	102.0	98.56	102.5

	614G	Delta	UK	Brazil
Bottom	-10.81	-11.59	-13.94	-15.76
Top	94.85	100.0	93.57	100.0
LogIC50	-0.3114	-0.2499	-0.1484	-0.1350
IC50	0.4882	0.5625	0.7106	0.7328
Span	105.7	111.6	107.5	115.8

	614G	Delta	UK	Brazil
Bottom	-9.274	-11.83	-14.59	-15.23
Top	100.0	100.0	96.38	99.97
LogIC50	-0.4028	-0.2544	-0.1844	-0.1634
IC50	0.3956	0.5566	0.6541	0.6864
Span	109.3	111.8	111.0	115.2

	614G	Delta	UK	Brazil
Bottom	-3.366	1.847	1.548	-237.7
Top	100.0	100.0	93.61	100.0
LogIC50	-0.8893	-1.443	-1.167	0.7013
IC50	0.1290	0.03605	0.06809	5.026
Span	103.4	98.16	92.06	337.7

FIGURE 3
Effects of Oleic acid, Linoleic Acid, and Palmitic Acid in Combination with TQ or with Another Fatty Acid on Blocking SARS-CoV-2-PP Infection of ACE2-expressing Cells. Serial dilutions of oleic acid, linoleic acid and palmitic acid in the presence of 0.05 μM TQ; serial dilutions of linoleic acid and palmitic acid in the presence of 0.01 mg/mL oleic acid; serial dilutions of palmitic acid in the presence of 0.01 mg/mL linoleic acid were tested for inhibition against an MLV-based pseudotyped virus using four different SARS-CoV-2 variant spike protein constructs (614D, Delta, United Kingdom, Brazil), in the infection of HEK293-ACE2 cells. *X*-axis: compound concentration. *Y*-axis: relative luminescence unit (RLU), reflecting the luciferase activity and the viral infectivity. The experiments were repeated three times with independent samples giving similar results. For all panels, data points shown are mean and s.d. for n = 3 technical replicates. IC50 values derived from curve fitting are listed in the Table below each Graph.



	XBB.1.5	614G
Bottom	-2.989	-4.560
Top	105.6	104.0
LogIC50	-1.747	-1.417
IC50	0.01790	0.03824
Span	108.6	108.6

	614G	XBB.1.16
Bottom	-4.651	-3.796
Top	100.0	100.0
LogIC50	-1.323	-1.445
IC50	0.04758	0.03589
Span	104.7	103.8

FIGURE 4
Effects of TQ Formula in percentage increments in relativity to the infectivity of Sars-CoV-19 subvariants XBB.1.5 (Omicron) and 614G. Serial dilutions of oleic acid, linoleic acid and palmitic acid in the presence of 0.05 μM TQ; serial dilutions of linoleic acid and palmitic acid in the presence of 0.01 mg/mL oleic acid; serial dilutions of palmitic acid in the presence of 0.01 mg/mL linoleic acid were tested for inhibition against an MLV-based pseudotyped virus using four different SARS-CoV-2 variant spike protein constructs (614G and Omicron) in the infection of HEK293-ACE2 cells. *X*-axis: compound concentration. *Y*-axis: relative infectivity of viral component. The experiments were repeated three times with independent samples giving similar results. For all panels, data points shown are mean and s.d. for n = 3 technical replicates. IC50 values derived from curve fitting are listed in the Table below each Graph.

associated with faster recovery in an open label randomized clinical trial (Koshak et al., 2021b). Moreover, a multicenter randomized clinical trial showed that a honey and *Nigella Sativa* combination resulted in a significant reduction in the severity of clinical symptoms, earlier viral clearance, and reduced mortality in COVID-19 patients (Ashraf et al., 2020).

TQF (NP-101) is a novel patent-pending, enteric-coated oral formulation, derived from *Nigella Sativa*, with TQ being its main ingredient. Our group has recently conducted a randomized double-blind, placebo-controlled clinical trial with TQF. The primary endpoints of this study were safety and the median time-to-sustained-clinical-response (SCR).

SCR was 6 days in the treatment arm vs. 8 days in the control arm ($p = 0.77$). Importantly, SCR was 5 days in the treatment vs. 7.5 days in the placebo arm in the high-risk patients; HR 1.55 (95% CI: 0.70, 3.43, $p = 0.25$). High-risk features were defined based on the presence of at least one of the following risk factors: age≥60, obesity, diabetes mellitus, hypertension, chronic cardiopulmonary disease, or auto-immune disease, which were selected based on similar high-risk features reported in recent COVID-19 studies. Notably, we did not observe a significant rate of adverse event differences between the two arms ($p = 0.16$). Furthermore, in this study, TQF-treated patients were found to have a significantly faster decline in their TSB ($p < 0.001$), and our biomarkers data showed a significant increase in the treatment *versus* placebo arm in cytotoxic $CD8^+$ ($p = 0.042$) and helper $CD4^+$ ($p = 0.042$) central memory T lymphocytes. Although there was no statistically significant difference in SCR between the TQF and placebo arms, SCR was shorter in the treatment arm, especially in high-risk patients and TQF activities across multiple endpoints were significant. Therefore, a large, randomized phase 2 double-blind, placebo-controlled study in high-risk outpatient COVID-19 patients is planned accordingly. Importantly, pre-clinical studies showed that TQ and TQF inhibit the entry and infection of five SARS-CoV-2 variants by blocking the ACE2 receptor (Bencheqroun et al., 2022). This is very relevant to COVID-19 patients since ACE2 expression has been found to be closely related to morbidity and mortality of COVID-19 infection (Chaudhry et al., 2020; Ni et al., 2020; Obukhov et al., 2020; Chen et al., 2021; Kaseb et al., 2021).

Free fatty acids, such as arachidonic acid, oleic acid, and linoleic acid, have inactivated enveloped viruses, such as influenza and herpes (Kohn et al., 1980a; Kohn et al., 1980b). Polyunsaturated fatty acids, such as linoleic acid, have exerted antimicrobial effects by enhancing the generation of free radicals, augmenting the formation of cytotoxic lipid peroxides, and by increasing the formation of their bioactive metabolites (Das, 2018). Here we show that linoleic acid, oleic acid, and palmitic acid have an inhibitory effect on SARS-CoV-2 pseudovirus infection at non-toxic concentrations. The exact mechanism of the inhibition is currently unknown. However, it has been shown that polyunsaturated fatty acids including linolenic acid effectively interfered with binding to hACE2 in a dose-dependent manner (Goc et al., 2021). The same study further demonstrated that linolenic acid and eicosapentaenoic acid showed a significant direct inhibitory effects on the activity of the host proteases TMPRSS2 and cathepsin L in addition to inhibiting viral binding (Goc et al., 2021). Additionally, in observing the relative infectivity response of pseudo-viruses 614G and Omicron to incremental TQ formula doses showed a similar pattern of decline to both subvariant viruses, despite any differences in their mutation profiles. Both had a particularly

sudden dose response point, TQ, at .01%, and sharply declined the infectivity for both the subvariants. Evident to the data, the fatty acid, TQ formulation, has an effective inhibitory effect on SARS-CoV-2 viruses.

We also tested combinations of fatty acids and TQ to mimic the effect of novel NP-101. Especially palmitic acid showed a strong inhibition when combined with TQ or other fatty acids. However, this inhibitory effect differed for the Delta, 614G, United Kingdom, and Brazil COVID-19 variants, with the strongest inhibition observed for the Delta variant and the weakest inhibition observed for the Brazil variant. These findings were unexpected, and at this time we do not have a clear explanation for them. It is possible that the varied levels of inhibition could be partly due to different mutational profiles in the receptor-binding domain (RBD) that each variant carries. The Brazil variant bears three mutations (K417T, E484K, and N501Y) in the RBD that is thought to enhance the binding affinity of the spike protein for ACE2 (Gu et al., 2020; Starr et al., 2020; Villoutreix et al., 2021). In contrast, the Delta variant also carries a L452R mutation in the receptor binding site which appears to increase the interaction between RBD and the ACE-2 and infectivity (Kirola, 2021). Although different studies have shown different binding affinities between RBD and ACE, one group reported that the United Kingdom variant (alpha) had a 10-fold increase, the Brazil variant (gamma) had a 5-fold increase, and the Delta variant had a 2-fold increase in ACE binding affinity compared to the ancestral RBD (Liu et al., 2022).

Our study has some limitations, including the lack of *in-vivo* studies and the exact mechanism of inhibiting ACE2 enzymatic activity. However, in line with this literature, we also showed that oleic, palmitic, and linoleic acid had an inhibitory effect on SARS-CoV-2 pseudovirus. Importantly, polyunsaturated fatty acids, particularly linolenic acid, eicosapentaenoic acid, and linoleic acid, have also been shown to inhibit SARS-CoV-2 binding and entry by interfering with binding to the human ACE receptor (Goc et al., 2021). Finally, our promising signals from the recently completed small randomized phase 2 study acted as a significant proof of concept of the effects of NP-101 on COVID-19 in the outpatient setting (Bencheqroun et al., 2022).

5 Conclusion

This study, we showed that not only TQ but also other individual ingredients of NP-101, such as palmitic, oleic, and linoleic acids had inhibitory effects on SARS-CoV-2 variants with various efficacy, possibly by inhibiting viral entry via the ACE-2 receptor. Further *in vivo* experiments are warranted to validate the findings of this study. Accordingly, a large, randomized phase 2 study is planned in high-risk COVID-19 patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AbM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing. BG: Conceptualization, Investigation, Software, Writing–original draft, Writing–review and editing. YM: Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Writing–original draft, Writing–review and editing. AE: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing. JL: Conceptualization, Investigation, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–review and editing. AmM: Conceptualization, Investigation, Writing–original draft. AA: Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Writing–review and editing. MoK: Conceptualization, Data curation, Supervision, Writing–review and editing. OK: Conceptualization, Investigation, Methodology, Supervision, Writing–review and editing. DL: Methodology, Validation, Writing–review and editing. MG: Methodology, Validation, Writing–review and editing. MeK: Methodology, Supervision, Writing–review and editing. AS: Methodology, Supervision, Writing–review and editing. QM: Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing–review and editing. AK: Methodology, Project administration, Supervision, Validation, Writing–original draft, Writing–review and editing.

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Conflict of interest

Author JL was employed by Codex BioSolutions Inc. Authors MoK, OK, and MG were employed by Novatek Pharmaceuticals, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1291212/full#supplementary-material

References

Abdelrahim, M., Esmail, A., Al Saadi, N., Zsigmond, E., Al Najjar, E., Bugazia, D., et al. (2022). Thymoquinone's antiviral effects: it is time to be proven in the covid-19 pandemic era and its Omicron variant surge. *Front. Pharmacol.* 13, 848676. PubMed PMID: 35462919. doi:10.3389/fphar.2022.848676

Ashraf, S., Ashraf, S., Ashraf, M., Imran, M. A., Kalsoom, L., Siddiqui, U. N., et al. (2020). Therapeutic efficacy of Honey and Nigella sativa against COVID-19: a multi-center randomized controlled clinical trial (HNS-COVID-PK). medRxiv. Available at: https://doi.org/10.1101/2020.10.30.20217364.

Barakat, E. M., El Wakeel, L. M., and Hagag, R. S. (2013). Effects of Nigella sativa on outcome of hepatitis C in Egypt. *World J. Gastroenterol.* 19 (16), 2529–2536. PubMed PMID: 23674855; PMCID: PMC3646144. Epub 2013/05/16. doi:10.3748/wjg.v19.i16.2529

Bencheqroun, H., Ahmed, Y., Kocak, M., Villa, E., Barrera, C., Mohiuddin, M., et al. (2022). A randomized, double-blind, placebo-controlled, multicenter study to evaluate the safety and efficacy of ThymoQuinone formula (TQF) for treating outpatient SARS-CoV-2. *Pathogens* 11 (5), 551. doi:10.3390/pathogens11050551

Chaudhry, F., Lavandero, S., Xie, X., Sabharwal, B., Zheng, Y.-Y., Correa, A., et al. (2020). Manipulation of ACE2 expression in COVID-19. *Open Heart* 7 (2), e001424. doi:10.1136/openhrt-2020-001424

Chen, C. Z., Xu, M., Pradhan, M., Gorshkov, K., Petersen, J. D., Straus, M. R., et al. (2020). Identifying SARS-CoV-2 entry inhibitors through drug repurposing screens of SARS-S and MERS-S pseudotyped particles. *ACS Pharmacol. Transl. Sci.* 3 (6), 1165–1175. PubMed PMID: 33330839; PMCID: PMC7586456. Epub 2020/12/18. doi:10.1101/2020.07.10.197988

Chen, F., Zhang, Y., Li, X., Li, W., Liu, X., and Xue, X. (2021). The impact of ACE2 polymorphisms on COVID-19 disease: susceptibility, severity, and therapy. *Front. Cell. Infect. Microbiol.* 11, 753721. doi:10.3389/fcimb.2021.753721

Das, U. N. (2018). Arachidonic acid and other unsaturated fatty acids and some of their metabolites function as endogenous antimicrobial molecules: a review. *J. Adv. Res.* 11, 57–66. PubMed PMID: 30034876; PMCID: PMC6052656. Epub 2018/07/24. doi:10.1016/j.jare.2018.01.001

Emergency Use Authorization (2022). U.S. Food and drug administration. Available from https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs (Accessed May 13, 2022).

Goc, A., Niedzwiecki, A., and Rath, M. (2021). Polyunsaturated ω-3 fatty acids inhibit ACE2-controlled SARS-CoV-2 binding and cellular entry. *Sci. Rep.* 11 (1), 5207. doi:10.1038/s41598-021-84850-1

Gu, H., Chen, Q., Yang, G., He, L., Fan, H., Deng, Y. Q., et al. (2020). Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science* 369 (6511), 1603–1607. PubMed PMID: 32732280; PMCID: PMC7574913. Epub 2020/08/01. doi:10.1126/science.abc4730

Kaseb, A. O., Mohamed, Y. I., Malek, A. E., Raad, II, Altameemi, L., Li, D., et al. (2021). The impact of angiotensin-converting enzyme 2 (ACE2) expression on the incidence and severity of COVID-19 infection. *Pathogens* 10 (3), 379. PubMed PMID:. doi:10.3390/pathogens10030379

Kirola, L. (2021). Genetic emergence of B.1.617.2 in COVID-19. *New Microbes New Infect.* 43, 100929. PubMed PMID: 34336227; PMCID: PMC8302888. Epub 2021/08/03. doi:10.1016/j.nmni.2021.100929

Kohn, A., Gitelman, J., and Inbar, M. (1980a). Unsaturated free fatty acids inactivate animal enveloped viruses. *Arch. Virol.* 66 (4), 301–307. PubMed PMID: 7447706. Epub 1980/01/01. doi:10.1007/bf01320626

Kohn, A., Gitelman, J., and Inbar, M. (1980b). Interaction of polyunsaturated fatty acids with animal cells and enveloped viruses. *Antimicrob. Agents Chemother.* 18 (6), 962–968. PubMed PMID: 7235682. doi:10.1128/AAC.18.6.962

Koshak, A. E., Koshak, E. A., Mobeireek, A. F., Badawi, M. A., Wali, S. O., Malibary, H. M., et al. (2021a). Nigella sativa for the treatment of COVID-19: an open-label randomized controlled clinical trial. *Complementary Ther. Med.* 61, 102769. doi:10.1016/j.ctim.2021.102769

Koshak, A. E., Koshak, E. A., Mobeireek, A. F., Badawi, M. A., Wali, S. O., Malibary, H. M., et al. (2021b). Nigella sativa for the treatment of COVID-19: an open-label randomized controlled clinical trial. *Complement. Ther. Med.* 61, 102769. PubMed PMID: 34407441; PMCID: PMC8364675. Epub 2021/08/19. doi:10.1016/j.ctim.2021.102769

Liu, H., Wei, P., Kappler, J. W., Marrack, P., and Zhang, G. (2022). SARS-CoV-2 variants of concern and variants of interest receptor binding domain mutations and virus infectivity. *Front. Immunol.* 13, 825256. doi:10.3389/fimmu.2022.825256

Millet, J. K., and Whittaker, G. R. (2016). Murine leukemia virus (MLV)-based Coronavirus spike-pseudotyped particle production and infection. *Bio Protoc.* 6 (23), e2035. PubMed PMID: 28018942; PMCID: PMC5181643. Epub 2016/12/27. doi:10.21769/BioProtoc.2035

Mostofa, A. G. M., Hossain, M. K., Basak, D., and Bin Sayeed, M. S. (2017). Thymoquinone as a potential adjuvant therapy for cancer treatment: evidence from preclinical studies. *Front. Pharmacol.* 8, 295. doi:10.3389/fphar.2017.00295

Ni, W., Yang, X., Yang, D., Bao, J., Li, R., Xiao, Y., et al. (2020). Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. *Crit. Care* 24 (1), 422. PubMed PMID: 32660650; PMCID: PMC7356137. Epub 20200713. doi:10.1186/s13054-020-03120-0

Obukhov, A. G., Stevens, B. R., Prasad, R., Li Calzi, S., Boulton, M. E., Raizada, M. K., et al. (2020). SARS-CoV-2 infections and ACE2: clinical outcomes linked with increased morbidity and mortality in individuals with diabetes. *Diabetes* 69 (9), 1875–1886. PubMed PMID: 32669391; PMCID: PMC7458035. Epub 20200715. doi:10.2337/dbi20-0019

Onifade, A. A., Jewell, A. P., and Adedeji, W. A. (2013). Nigella sativa concoction induced sustained seroreversion in HIV patient. *Afr. J. traditional, complementary, Altern. Med.* 10 (5), 332–335. PubMed PMID: 24311845. doi:10.4314/ajtcam.v10i5.18

Onifade, A. A., Jewell, A. P., and Okesina, A. B. (2015). Seronegative conversion of an HIV positive subject treated with Nigella sativa and honey. *Afr. J. Infect. Dis.* 9 (2), 47–50. doi:10.4314/ajid.v9i2.6

Pop, R. M., Sabin, O., Suciu, Ş., Vesa, S. C., Socaci, S. A., Chedea, V. S., et al. (2020). Nigella sativa's anti-inflammatory and antioxidative effects in experimental inflammation. *Antioxidants* 9 (10), 921. doi:10.3390/antiox9100921

Rahman, M. T. (2020). Potential benefits of combination of Nigella sativa and Zn supplements to treat COVID-19. *J. Herb. Med.* 23, 100382. doi:10.1016/j.hermed.2020.100382

Starr, T. N., Greaney, A. J., Hilton, S. K., Ellis, D., Crawford, K. H. D., Dingens, A. S., et al. (2020). Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. *Cell* 182 (5), 1295–1310. PubMed PMID: 32841599; PMCID: PMC7418704. Epub 2020/08/26. doi:10.1016/j.cell.2020.08.012

Villoutreix, B. O., Calvez, V., Marcelin, A. G., and Khatib, A. M. (2021). *In silico* investigation of the new UK (B.1.1.7) and South African (501Y.V2) SARS-CoV-2 variants with a focus at the ACE2-spike RBD interface. *Int. J. Mol. Sci.* 22 (4), 1695. PubMed PMID: 33567580; PMCID: PMC7915722. Epub 2021/02/12. doi:10.3390/ijms22041695

WHO (2022). WHO Coronavirus (COVID-19) dashboard: world health organization. Available from: https://covid19.who.int/ (Accessed May 12, 2022).

Xiao, T., Lu, J., Zhang, J., Johnson, R. I., McKay, L. G. A., Storm, N., et al. (2021). A trimeric human angiotensin-converting enzyme 2 as an anti-SARS-CoV-2 agent. *Nat. Struct. Mol. Biol.* 28 (2), 202–209. PubMed PMID: 33432247; PMCID: PMC7895301. Epub 2021/01/13. doi:10.1038/s41594-020-00549-3

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