Am J Cancer Res 2025;15(4):1790-1805
www.ajcr.us /ISSN:2156-6976/ajcr0163470

Original Article

Impact of doxycycline on mycoplasma pneumonia treatment and cancer prognosis in pediatric leukemia patients post-chemotherapy: a target trial emulation

Jun Wen[1], Xiaoyan Xu[1], Yufei Su[1], Xuehao Qi[1], Qiang Fu[2], Mingjiang Chen[3], Xue Wang[1]

[1]Emergency Department, Xi'an Children's Hospital, Xi'an 710000, Shaanxi, China; [2]Hematology Department, Xi'an Children's Hospital, Xi'an 710000, Shaanxi, China; [3]Information Department, Xi'an Children's Hospital, Xi'an 710000, Shaanxi, China

Received January 21, 2025; Accepted March 19, 2025; Epub April 15, 2025; Published April 30, 2025

Abstract: Background: Acute leukemia is the most common malignancy in children. While chemotherapy is effective, it significantly compromises immune function, leading to a high incidence of infectious complications, such as secondary pneumonia, particularly Mycoplasma pneumonia (MP). The treatment of infections in pediatric leukemia patients faces challenges such as antibiotic resistance and drug interactions during chemotherapy. Objective: This study aims to evaluate the therapeutic efficacy and safety of doxycycline in treating MP in pediatric leukemia patients post-chemotherapy, as well as it impact on chemotherapy continuity. Methods: This study employed a target trial emulation design using retrospective data from pediatric leukemia patients diagnosed with MP. Patients aged 12-17 years with confirmed leukemia and clinical evidence of pneumonia following chemotherapy were included. Doxycycline was compared to azithromycin and other empirical treatments. Follow-up assessments at 3 days, 5 days, 30 days, and 180 days evaluated fever resolution, radiological improvement, additional interventions, and adverse events. Statistical analyses included Kaplan-Meier survival analysis and Cox proportional hazards models. Results: In Trial 2, doxycycline demonstrated a significantly higher treatment success rate than other empirical treatments (87.72% vs. 73.13%, P = 0.013) and was associated with faster fever resolution (P = 0.048) and shorter time to chest X-ray improvement (P = 0.048). The 30-day survival rate was significantly higher in the Doxycycline group compared to other empirical treatments (100% vs. 91.04%, P = 0.019). Fewer patients require additional interventions such as ICU admission (P = 0.019). Furthermore, patients in the Doxycycline group had a significantly higher likelihood of completing chemotherapy without delays (84.21% vs. 59.70%, P = 0.01). In Trial 1, no significant differences were observed in treatment success rate, fever resolution time, hospitalization duration, or chemotherapy tolerance between Doxycycline and Azithromycin (P > 0.05). Conclusion: Doxycycline, as a broad-spectrum antibiotic, demonstrates efficacy comparable to azithromycin in treating MP with advantages in reducing chemotherapy-related delays, hospitalization duration, and the need for additional interventions. It enhances chemotherapy tolerance and continuity. Doxycycline may serve as an economical and effective alternative for managing post-chemotherapy infections in pediatric leukemia patients, especially in cases of antibiotic resistance or intolerance.

Keywords: Doxycycline, mycoplasma pneumonia, chemotherapy delay, pediatric leukemia, target trial emulation

Introduction

Leukemia, a malignant disease of the blood and bone marrow, represents a substantial global health challenge, particularly among pediatric populations. In 2019, it accounted for 0.64 million new cases, 0.33 million deaths, and 11.66 million disability-adjusted life years (DALYs) worldwide [1]. According to the World Health Organization, leukemia accounts for approximately 30% of all childhood cancers, making it the most common cancer among children and adolescents worldwide. The annual incidence of childhood leukemia varies by region, with an estimated 3 to 4 cases per 100,000 children [2]. To address this significant health burden, standard chemotherapy regimens for leukemia include intensive treatments with drugs such as anthracyclines, vincristine, corticosteroids, and cytarabine [3]. While these regimens are essential for inducing remission and preventing relapse, they are

associated with significant immunosuppression. As a result, leukemia patients undergoing chemotherapy often experience compromised immune function, which increases their susceptibility to infections. Consequently, the incidence of pneumonia in this population is markedly higher than in immunocompetent individuals [4].

Pneumonia in leukemia patients is a major concern for clinicians, as it significantly increases the risk of mortality. Studies indicate that up to 25-30% of pediatric leukemia patients may develop pneumonia during treatment, with mortality rates in immunocompromised children reaching 15-20%, underscoring the urgent need for effective treatment strategies [5, 6]. Among patients who develop pneumonia with fever following chemotherapy, Mycoplasma pneumonia (MP) is one of the most common types, with an incidence significantly higher than in the general population [7]. Clinically, Mycoplasma infection manifests as cough, fever, and dyspnea, and in severe cases, it may progress to impaired lung function or even acute respiratory distress syndrome [8]. Standard treatment for MP typically relies on macrolide antibiotics such as azithromycin. However, the increasing prevalence of Mycoplasma resistance has led to a gradual decline in the efficacy of these traditional therapies [9]. Studies indicate that in leukemia patients, chemotherapy-induced dysbiosis and immunosuppression further complicate treatment, necessitating antibiotic selection that provides broader antimicrobial coverage while minimizing adverse effects [10]. Doxycycline, a broad-spectrum tetracycline antibiotic, offers a promising alternative. It not only exhibits strong antibacterial effects against Mycoplasma but also demonstrates potential advantages in modulating excessive immune responses and improving pulmonary pathology due to its anti-inflammatory properties [11]. Furthermore, doxycycline has good drug tolerance and a low risk of interactions with chemotherapy agents, making it a promising empirical treatment option for leukemia patients [12].

The choice of antimicrobial therapy not only affects the effectiveness of pneumonia treatment but also influences the patient's tolerance and adherence to chemotherapy, ultimately impacting the overall efficacy of the chemotherapy regimen [13]. Therefore, there is an urgent need to evaluate alternative antibiotic therapies that are effective against pneumonia pathogens while remaining compatible with chemotherapy protocols. To address this, we conducted a target trial emulation to assess the efficacy and safety of doxycycline in treating post-chemotherapy MP in pediatric leukemia patients. The goal was to determine whether doxycycline can improve pneumonia outcomes without compromising tumor control, thereby providing valuable insights for standard empirical treatment strategies.

Materials and methods

Patient selection

This retrospective cohort study was designed to emulate a target randomized controlled trial (RCT) assessing antimicrobial therapy in pediatric leukemia patients diagnosed with MP within six months after chemotherapy. Patient data were obtained from the electronic medical record system of Xi'an Children's Hospital. Based on predefined inclusion and exclusion criteria, a total of 216 patients, all of whom were first diagnosed with mycoplasma pneumonia between January 1, 2015 and March 31, 2024, were included in this study (**Figure 1**).

Inclusion criteria: a) Pediatric patients aged 12-17 years. b) Diagnosed with leukemia (e.g., acute lymphoblastic leukemia, acute myeloid leukemia, etc.) [14-16]. c) Treated according to standard pediatric leukemia chemotherapy regimens, with no change in chemotherapy protocol within 30 days prior to pneumonia diagnosis. d) Clinical diagnosis of MP during chemotherapy or within 6 months post-chemotherapy. e) Diagnosed and treated with antimicrobial therapy at the study hospital. f) Available medical records with follow-up data at 2 weeks, 1 month, and 6 months post-treatment. g) Informed consent from parents/guardians (and assent from the child, if appropriate for age) to use data in this study.

Exclusion criteria: a) Patients diagnosed with other types of cancer (e.g., solid tumors, lymphoma) or those not receiving leukemia treatment. b) Patients with a history of chronic respiratory diseases (e.g., cystic fibrosis, asthma, congenital lung disease). c) Patients receiving "high-dose chemotherapy" regimens (e.g.,

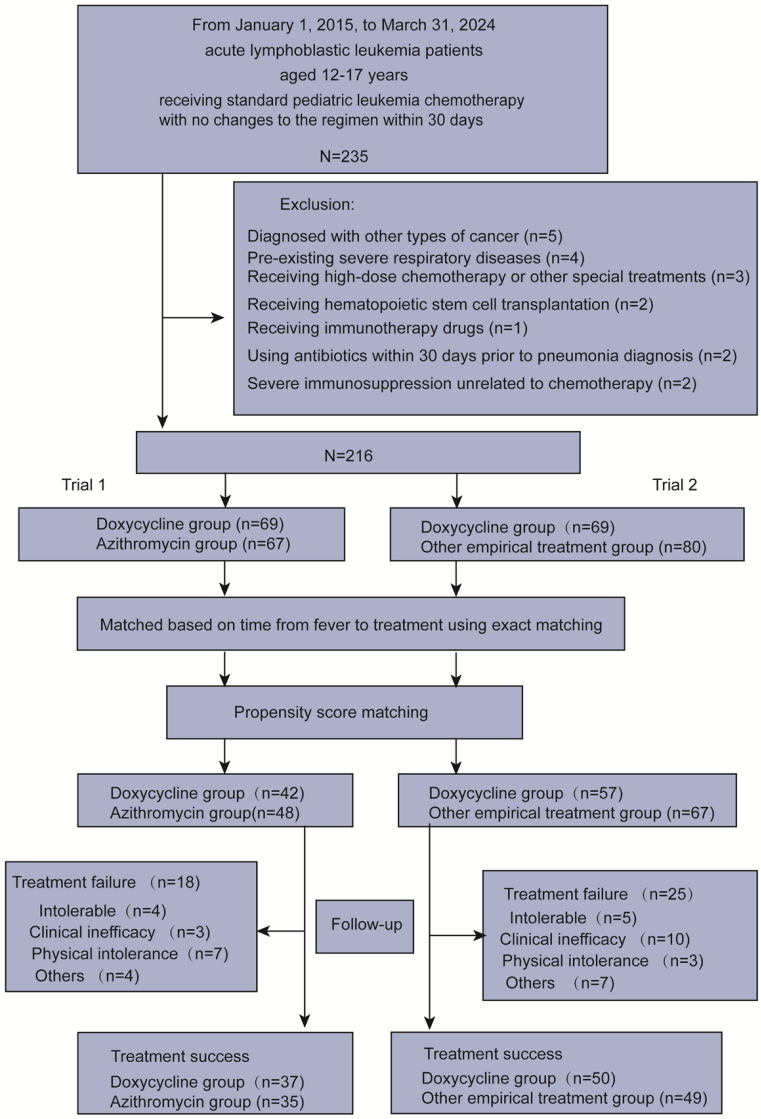


Figure 1. Flowchart of patient inclusion for two target trial emulations.

high-dose methotrexate or cyclophosphamide) or "salvage chemotherapy". d) Patients who had already undergone or scheduled for "hematopoietic stem cell transplantation (HSCT)". e) Patients treated with immunotherapy agents such as blinatumomab, CAR T-cell therapy, or immune checkpoint inhibitors. f) Patients who had received other antibiotics within 30 days prior to pneumonia onset. g) Patients with severe immunosuppression unrelated to chemotherapy (e.g., HIV/AIDS, congenital immunodeficiencies). h) Patients with other major infections at the time of pneumonia diagnosis (e.g., sepsis, fungal infections). i) Patients lacking sufficient data or follow-up information at any of the specified time points (2 weeks, 1 month, or 6 months).

Diagnostic criteria for mycoplasma pneumonia [17]: 1) Newly onset cough, sputum production, or exacerbation of pre-existing respiratory symptoms with purulent sputum, with or without chest pain. 2) Fever. 3) Pulmonary signs and/or the presence of crackles on auscultation. 4) White blood cell count (WBC) > 10 × 10^9/L or < 4 × 10^9/L, with or without neutrophil left shift. 5) Chest X-ray showing patchy or infiltrative opacities or interstitial changes, with or without pleural effusion. 6) Microbiological testing showing positive Mycoplasma DNA (PCR) from respiratory secretions, or positive Mycoplasma IgM antibodies, or a 4-fold or greater increase in IgG antibody titers compared to the acute phase.

Diagnosis can be made if any one of items 1-4 is present in conjunction with items 5 and 6, excluding non-infectious diseases.

Criteria for early dropout or withdrawal: This study is a retrospective emulation of a target trial, with data sourced from historical prescribing practices in patient medical records. The criteria for dropout or withdrawal differ from those in prospective randomized controlled trials. Patients were excluded from analysis upon these conditions: a) Missing key follow-up data, such as data for primary outcome measures (e.g., symptom improvement, imaging results) at any key follow-up time point (3 days, 2 weeks, 1 month, or 6 months). b) Lost to follow-up: Failure to contact the patient or guardian after three consecutive attempts at follow-up.

Target trial protocol and emulation: overall study design

The target trials compared doxycycline with azithromycin or other antimicrobial treatment groups. Follow-up was extended until Septem-

ber 30, 2024, with outcomes assessed at 3 days, 5 days, 30 days, and 180 days. Key features of the target trial, as specified and emulated, are summarized in **Table 1**; only the study design methods are outlined below. We employed a matched cohort design to simulate the balance achieved through randomization. Supplementary Table 1 provides a detailed comparison of key study design features between the specified trial and the simulated target trial approach, including baseline characteristics, interventions, follow-up time points, and primary outcome measures. Treatment allocation was based on historical prescribing practices from patient medical records and propensity scores calculated from baseline characteristics, matching patients in the doxycycline group with those in the control groups (Two target trial emulations were conducted: in Trial 1, the control group received azithromycin, while in Trial 2, the control group received alternative empirical therapies). All data were extracted from the hospital's electronic medical record system, including pre-existing anonymized information. Ethical approval was granted by the Ethics Committee of Xi'an Children's Hospital (approval number: 20240036). Due to the anonymous nature of the data analysis and the protection of patient privacy, the requirement for informed consent was waived. The study adhered to Good Clinical Practice (GCP) guidelines, the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) reporting guidelines, and the Declaration of Helsinki.

Grouping and intervention methods

(1) Doxycycline Group: Patients in this group received oral doxycycline treatment. For children weighing ≥ 45 kg, the initial loading dose was 200 mg (single dose), followed by 100 mg every 12 hours. For children weighing < 45 kg, the dose was 2 mg/kg every 12 hours, with a maximum single dose of 100 mg. The treatment duration was 7-14 days, adjusted according to the severity of infection and clinical response.

(2) Azithromycin Group: Patients in this group received oral or intravenous azithromycin therapy. The oral dose followed the recommended pediatric dosage: 10 mg/kg on Day 1 (single dose), not exceeding 500 mg, followed by 5 mg/kg daily from Day 2 to Day 5, with a maximum dose of 250 mg per day. For patients with severe infections, intravenous dripping was administered at a dose of 10 mg/kg daily, not exceeding 500 mg, for 3-5 days, after which oral therapy was initiated. The treatment duration was 7-14 days, adjusted according to the severity of infection and clinical response.

(3) Other Empirical Treatment Group: Patients in this group received other antimicrobial treatments as recorded in their medical records, including erythromycin (40%), clarithromycin (30%), levofloxacin (20%), and moxifloxacin (10%). The erythromycin dosage was 20-40 mg/kg/day, divided into 3-4 doses, with a maximum daily dose of 2 g. The clarithromycin dosage was 15 mg/kg/day, divided into 2 doses, with a maximum daily dose of 1 g. The levofloxacin dosage was 8-10 mg/kg every 12 hours, with a maximum daily dose of 750 mg. Moxifloxacin, due to limited indications in children, was prescribed on a case-by-case basis, with a dose of 4 mg/kg once daily, not exceeding 400 mg per day. The treatment duration was 7-14 days, adjusted according to the severity of infection and clinical response. This group was designed to simulate other empirical treatments, as the sample size for individual antimicrobial therapies was insufficient. To avoid sample wastage, different drugs were grouped together, and the overall results of this group were used for comparison with other empirical treatments. The drug manufacturers and batch numbers are listed in Supplementary Table 2.

Sample size estimation

Based on previous literature, we estimated the efficacy rates for the azithromycin group (85%) [18], the doxycycline group (85%) [19], and the empirical treatment group (70%) [20]. Sample size calculations were performed using PASS software, with the study design incorporating a significance level ($\alpha = 0.05$) and statistical power ($1-\beta = 0.80$) for comparing efficacy between two groups.

For Trial 1 (Doxycycline vs. Azithromycin), assuming an efficacy rate of 85% in both groups, a non-inferiority design was used, with the non-inferiority margin set at 10%. The "Two Proportions - Non-Inferiority" module in PASS software was selected, with the expected efficacy rate of 85% for both the doxycycline and

Table 1. Baseline characteristics of patients in two target trial emulations

Baseline	Trial 1: Doxycycline vs. Azithromycin					Trial 2: Doxycycline vs. Other Empirical Treatment				
	Total (n = 90)	Azithromycin group (n = 48)	Doxycycline group (n = 42)	Statistic	*P*	Total (n = 124)	Doxycycline group (n = 57)	Other empirical treatment group (n = 67)	Statistic	*P*
Age (years)	14.62 ± 1.60	14.62 ± 1.70	14.62 ± 1.50	t = 0.02	0.986	14.61 ± 1.63	14.86 ± 1.49	14.40 ± 1.72	t = 1.56	0.121
Sex, n (%)				χ^2 = 0.24	0.621				χ^2 = 0.76	0.383
Female	56 (62.22)	31 (64.58)	25 (59.52)			60 (48.39)	30 (52.63)	30 (44.78)		
Male	34 (37.78)	17 (35.42)	17 (40.48)			64 (51.61)	27 (47.37)	37 (55.22)		
Presence of neutropenia, n (%)				χ^2 = 0.02	0.885				χ^2 = 2.78	0.095
No	35 (38.89)	19 (39.58)	16 (38.10)			49 (39.52)	18 (31.58)	31 (46.27)		
Yes	55 (61.11)	29 (60.42)	26 (61.90)			75 (60.48)	39 (68.42)	36 (53.73)		
Type of leukemia, n (%)				χ^2 = 0.03	0.985				χ^2 = 1.72	0.424
Acute lymphoblastic leukemia	55 (61.11)	29 (60.42)	26 (61.90)			59 (47.58)	24 (42.11)	35 (52.24)		
Acute myeloid leukemia	20 (22.22)	11 (22.92)	9 (21.43)			51 (41.13)	27 (47.37)	24 (35.82)		
Chronic myeloid leukemia	15 (16.67)	8 (16.67)	7 (16.67)			14 (11.29)	6 (10.53)	8 (11.94)		
Chemotherapy duration (months) Mean ± SD	2.99 ± 1.34	3.10 ± 1.36	2.86 ± 1.32	t = 0.87	0.385	2.90 ± 1.32	2.84 ± 1.33	2.96 ± 1.32	t = -0.47	0.637
Time from fever to admission (days), n (%)				χ^2 = 4.56	0.102				χ^2 = 1.55	0.462
1	38 (42.22)	19 (39.58)	19 (45.24)			70 (56.45)	34 (59.65)	36 (53.73)		
2	37 (41.11)	24 (50.00)	13 (30.95)			26 (20.97)	13 (22.81)	13 (19.40)		
3	15 (16.67)	5 (10.42)	10 (23.81)			28 (22.58)	10 (17.54)	18 (26.87)		

azithromycin groups, non-inferiority margin at -10%, significance level at $\alpha = 0.05$ (two-sided), and statistical power at ($1-\beta = 0.80$). The software estimated that approximately 200 patients per group would be required to achieve sufficient statistical power to detect non-inferiority. For Trial 2 (Doxycycline vs. Empirical Treatment), assuming an efficacy rate of 85% for the doxycycline group and 70% for the empirical treatment group, a superiority design was adopted, with an expected difference of 15% between the two groups. The "Two Proportions - Superiority" module in PASS software was selected, with the expected efficacy rates of 85% and 70% for the doxycycline and other empirical treatment groups respectively, significance level at $\alpha = 0.05$ (two-sided), and statistical power at ($1-\beta = 0.80$). The software estimated that approximately 118 patients per group would be required to detect the superior efficacy of doxycycline over empirical treatment.

Exact matching and propensity score matching

We first performed exact matching for each eligible participant receiving doxycycline, azithromycin, or other antimicrobial therapies based on the time from the onset of fever to hospital admission (1 day, 2 days, 3 days). After exact matching, we further performed propensity score matching within each stratum based on five factors: "age", "sex", "presence of neutropenia", "type of leukemia" and "chemotherapy duration". In Trial 1, the reference group was the "azithromycin group" and the matched group was the "doxycycline group" (Supplementary Tables 3, 4, 5, 6, 7 and Supplementary Figures 1, 2). In Trial 2, the reference group was the "other empirical treatment group" and the matched group was the "doxycycline group" (Supplementary Tables 8, 9, 10, 11 and Supplementary Figure 3). The matching ratio was either 1:1 or 1:2, with a caliper value of 0.2. Groups that showed no significant baseline differences before matching were not further subjected to propensity score matching. Finally, Trial 1 included 42 patients in Doxycycline group and 48 in Azithromycin group, and Trial 2 included 57 patients in Doxycycline group and 67 in other empirical treatment group.

Outcome measures

Infection-related outcome measures: (1) Treatment success rate: This outcome measures the proportion of patients who, during their treatment, were able to achieve resolution of fever and radiographic improvement without requiring additional interventions (e.g., changes in antibiotic therapy, ICU admission, or respiratory support), as well as those can be successfully discharged.

(2) Hospitalization time at fever resolution: A sustained normal body temperature (< 37.5°C) for at least 24 hours without the use of antipyretic medications is considered as fever resolution. During data compilation, we calculated the proportion of patients who achieved fever resolution on Day 3 and Day 5 to observe the overall efficacy in different treatment groups.

(3) Hospitalization time at chest X-ray resolution: This refers to the duration from the patient's admission to the time when the pulmonary lesions, as seen on chest X-ray, show significant improvement (Lesion reduction in lung infiltrates, nodules, cavities, etc.) or resolution (Lesion disappearance, with restoration of normal lung tissue appearance).

(4) Need for additional interventions: This assesses whether the patient requires further medical interventions, such as a change or escalation in antibiotic therapy, ICU admission, or mechanical ventilation.

(5) Adverse events: Adverse reactions, such as dizziness, nausea, rash, and other related issues were recorded.

(6) Reasons for treatment failure: Any factors that lead to the interruption or cessation of treatment, including intolerance, clinical deterioration, physical intolerance, or other causes, were documented.

Tumor-related outcome measures: (1) 30-day survival rate: The percentage of patients surviving without significant adverse events or complications within 30 days of initiating treatment was calculated.

(2) Tumor response rate: The proportion of patients achieving complete remission [21] or partial remission after treatment were calculated.

(3) Impact on chemotherapy tolerance: The impact of PM and its treatment on the patient's ability to continue chemotherapy, including delays, dose adjustments, and treatment inter-

ruptions, were evaluated. a) Chemotherapy delay: A delay in the chemotherapy cycle of more than the planned time (e.g., > 7 days). b) Dose adjustment: The actual dose administered is less than 90% of the planned dose. c) Chemotherapy discontinuation: Premature termination of chemotherapy due to adverse events or complications.

Statistical methods

Propensity score matching was performed using R version 4.2.2 (2022-10-31). The propensity scores were calculated using logistic regression. All statistical tests for balance assessment were two-tailed, with a significance level set at $P < 0.05$. Normally distributed continuous variables were expressed as mean ± standard deviation (Mean ± SD), and group comparisons were performed using one-way analysis of variance (ANOVA). For non-normally distributed continuous variables, data were presented as median and interquartile range [M (Q1, Q3)], with group comparisons performed using the Kruskal-Wallis H test. Categorical data were expressed as frequencies and proportions [n (%)], with group comparisons conducted using the Pearson χ^2 test or Fisher's exact test. The Standardized Mean Difference (SMD) was used to assess group differences. An SMD of < 0.10 indicated that the groups were balanced and acceptable, an SMD between 0.10 and 0.34 indicated small differences, an SMD between 0.35 and 0.64 indicated moderate differences, an SMD between 0.65 and 1.19 indicated large differences, and an SMD ≥ 1.20 indicated very large differences between the groups. Repeated-measures ANOVA or mixed-effects models were used to analyze the changes in serological and pulmonary function parameters over time. The Log-rank test was employed to compare survival curves. Cox proportional hazards models were used to adjust for covariates. Linear regression was used for continuous outcomes (e.g., length of hospitalization). A *P*-value of < 0.05 was considered statistically significant.

Result

Baseline characteristics

The baseline characteristics of patients in the Doxycycline group and the comparator group in both trials were well-balanced, indicating the comparability between two groups (see **Table 1**).

Treatment success rate

In Trial 1, the treatment success rate was 72.92% (35/48) in the Azithromycin group and 88.10% (37/42) in the Doxycycline group. In Trial 2, the success rates were 87.72% (50/57) in the Doxycycline group and 73.13% (49/67) in the Other Empirical Treatment group (**Table 2**).

Fever resolution at days 3 and 5

In Trial 1, the proportion of patients with fever by Day 3 was lower in the Doxycycline group (71.43%) compared to the Azithromycin group (79.17%). By Day 5, the proportion of febrile patients in the doxycycline group (61.90%) was slightly higher than that in the azithromycin group (56.25%).

In Trial 2, the proportion of patients with fever by Day 3 was lower in the Doxycycline group (59.65%) compared to the Other Empirical Treatment group (76.12%). By Day 5, 47.37% of the patients in Doxycycline group remained febrile, lower than 53.73% of the Other Empirical Treatment group (**Table 2**).

In Trial 1, the mean time to fever resolution was shorter in the Doxycycline group (8.45 ± 7.68 days) compared to the Azithromycin group (11.02 ± 9.51 days), but the difference was not significant between groups (P = 0.166) (**Table 2**). Similarly, Kaplan-Meier analysis revealed no significant difference in fever resolution rate (Log-rank P = 0.402, HR 1.230, 95% CI: 0.741-2.042; **Figure 2A**).

In Trial 2, the Doxycycline group demonstrated a shorter time to fever resolution (7.11 ± 7.76 days) compared to the Other Empirical Treatment group (10.03 ± 8.36 days) also with no significant inter-group difference (P = 0.051) (**Table 2**). However, Kaplan-Meier analysis suggested a higher fever resolution rate in the Doxycycline group (Log-rank P = 0.048, HR 0.663, 95% CI: 0.430-1.023; **Figure 2C**).

Time to chest X-ray resolution

In Trial 1, the time to chest X-ray resolution was shorter in the Doxycycline group (11.12 ± 9.11 days) compared to the Azithromycin group (14.31 ± 11.03 days, P = 0.141) (**Table 2**).

Table 2. Analysis of treatment success and other outcomes in two target trial emulations

Outcome	Trial 1: Doxycycline vs Azithromycin					Trial 2: Doxycycline vs Other Empirical Treatment				
	Total (n = 90)	Azithromycin group (n = 48)	Doxycycline group (n = 42)	Statistic	*P*	Total (n = 124)	Doxycycline group (n = 57)	Other empirical treatment group (n = 67)	Statistic	*P*
Treatment success rate (%)	72 (80.00)	35 (72.92)	37 (88.10)			99 (79.84)	50 (87.72)	49 (73.13)		
Time to fever resolution (Mean ± SD)	9.82 ± 8.75	11.02 ± 9.51	8.45 ± 7.68	t = 1.40	0.166	8.69 ± 8.33	7.11 ± 7.76	10.03 ± 8.62	t = -1.97	0.051
Time to chest X-ray improvement (Mean ± SD)	12.82 ± 10.25	14.31 ± 11.03	11.12 ± 9.11	t = 1.48	0.141	11.52 ± 9.68	9.70 ± 9.12	13.06 ± 9.93	t = -1.95	0.054
Reasons for treatment failure, n (%)				-	0.077				-	0.013
Intolerance	4 (4.44)	1 (2.08)	3 (7.14)			5 (4.03)	3 (5.26)	2 (2.99)		
Clinical deterioration	3 (3.33)	3 (6.25)	0 (0.00)			10 (8.06)	0 (0.00)	10 (14.93)		
Others	4 (4.44)	3 (6.25)	1 (2.38)			7 (5.65)	3 (5.26)	4 (5.97)		
Physical intolerance	7 (7.78)	6 (12.50)	1 (2.38)			3 (2.42)	1 (1.75)	2 (2.99)		
None	72 (80.00)	35 (72.92)	37 (88.10)			99 (79.84)	50 (87.72)	49 (73.13)		
30-day survival rate (%)	89 (98.89)	47 (97.92)	42 (100.00)			118 (95.16)	57 (100.00)	61 (91.04)		0.019
Fever resolution status										
Number of patients with fever on day 3 (%)	68 (75.56)	38 (79.17)	30 (71.43)			85 (68.55)	34 (59.65)	51 (76.12)		
Number of patients with fever on day 5 (%)	53 (58.89)	27 (56.25)	26 (61.90)			63 (50.81)	27 (47.37)	36 (53.73)		
Additional interventions required, n (%)				-	0.368				-	0.017
Additional antibiotics	10 (11.11)	7 (14.58)	3 (7.14)			8 (6.45)	3 (5.26)	5 (7.46)		
Respiratory support	5 (5.56)	4 (8.33)	1 (2.38)			9 (7.26)	1 (1.75)	8 (11.94)		
None	72 (80.00)	35 (72.92)	37 (88.10)			99 (79.84)	52 (91.23)	49 (70.15)		
ICU	3 (3.33)	2 (4.17)	1 (2.38)			8 (6.45)	1 (1.75)	7 (10.45)		
Adverse reactions, n (%)				-	0.074				χ^2 = 7.21	0.065
Nausea	9 (10.00)	2 (4.17)	7 (16.67)			15 (12.10)	8 (14.04)	7 (10.45)		
Abdominal pain	10 (11.11)	8 (16.67)	2 (4.76)			19 (15.32)	4 (7.02)	15 (22.39)		
Sleep disturbances	9 (10.00)	6 (12.50)	3 (7.14)			12 (9.68)	4 (7.02)	8 (11.94)		
None	62 (68.89)	32 (66.67)	30 (71.43)			78 (62.90)	41 (71.93)	37 (55.22)		
Tumor response rate within 6 months, n (%)				χ^2 = 3.00	0.223				χ^2 = 0.43	0.807
Complete remission	27 (30.00)	14 (29.17)	13 (30.95)			47 (37.90)	22 (38.60)	25 (37.31)		
Partial remission	48 (53.33)	23 (47.92)	25 (59.52)			59 (47.58)	28 (49.12)	31 (46.27)		
No response	15 (16.67)	11 (22.92)	4 (9.52)			18 (14.52)	7 (12.28)	11 (16.42)		
Chemotherapy tolerance, n (%)				-	0.226				χ^2 = 9.12	0.01
Chemotherapy delay	7 (7.78)	6 (12.50)	1 (2.38)			10 (8.06)	3 (5.26)	7 (10.45)		
Chemotherapy adjustment	9 (10.00)	5 (10.42)	4 (9.52)			26 (20.97)	6 (10.53)	20 (29.85)		
Normal	74 (82.22)	37 (77.08)	37 (88.10)			88 (70.97)	48 (84.21)	40 (59.70)		

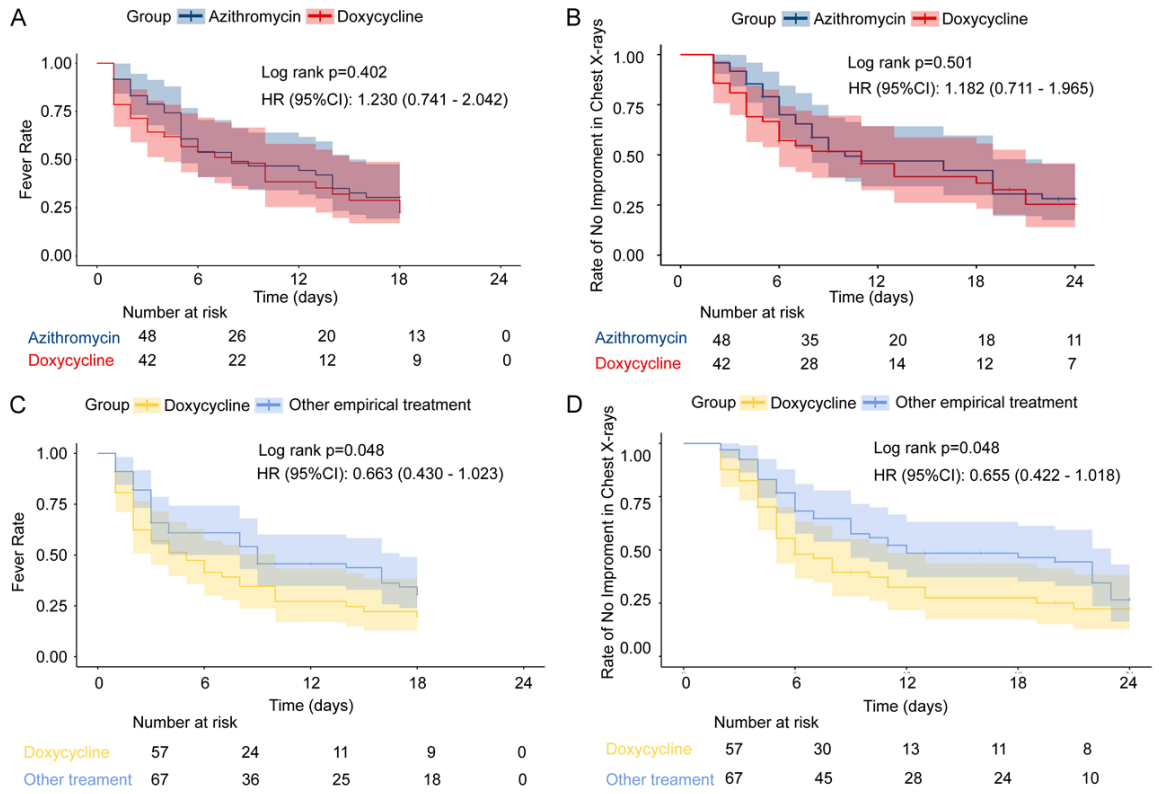


Figure 2. Kaplan-Meier curves of the 24-day cumulative rates of fever and no improvement in chest x-rays for chemotherapy patients across two target trial emulations. A: Kaplan-Meier curves of 24-day cumulative fever rate in patients from Trial 1 Emulation. B: Kaplan-Meier curves of 24-day cumulative fever rate in patients from Trial 2 Emulation. C: Kaplan-Meier curves of the 24-day cumulative rate of no improvement in chest X-rays in patients from Trial 1 Emulation. D: Kaplan-Meier curves of the 24-day cumulative rate of no improvement in chest X-rays in patients from Trial 2 Emulation.

Kaplan-Meier analysis showed no significant difference in chest X-ray improvement rate between the two groups (Log-rank P = 0.501, HR 1.182, 95% CI: 0.711-1.965; **Figure 2B**).

In Trial 2, the Doxycycline group demonstrated a shorter time to chest X-ray resolution (9.70 ± 9.12 days) compared to the Other Empirical Treatment group (13.06 ± 9.93 days, P = 0.054) (**Table 2**). Kaplan-Meier analysis also revealed a higher in chest X-ray improvement in Doxycycline group (Log-rank P = 0.048, HR 0.655, 95% CI: 0.422-1.018; **Figure 2D**).

Analysis of factors for treatment failure

In Trial 1, treatment failure was reported in 18 patients, including 7 cases of physical intolerance, 3 cases of clinical deterioration, 4 cases due to other reasons, and 4 cases of general intolerance. However, no significant disparities were observed between the two groups in trial 1 (P = 0.077).

In Trial 2, treatment failure was observed in 25 cases, comprising 10 cases of clinical deterioration, 5 cases of intolerance, 3 cases of physical intolerance, and 7 cases due to other reasons. A significant difference in the distribution of failure reasons was observed between the two groups in Trial 2 (P = 0.013; **Table 2**).

30-Day survival rate

The 30-day survival rate in Trial 1 was 100% for the Doxycycline group and 97.92% for the Azithromycin group, showing no significant difference. In Trial 2, the survival rate was 100% for the Doxycycline group, significantly higher than 91.04% for the Other Empirical Treatment group (P = 0.019; **Table 2**).

Additional interventions required

In Trial 1, fewer patients in the Doxycycline group required additional antibiotics compared to the Azithromycin group (7.14% vs. 14.58%).

Similarly, respiratory support was needed less frequently in the Doxycycline group (2.38%) compared to the Azithromycin group (8.33%). Overall, the proportion of patients requiring additional interventions was comparable between the two groups (P = 0.368; **Table 2**).

In Trial 2, the proportions of patients requiring additional antibiotics, respiratory support, or ICU transfer were lower in the Doxycycline group (5.26%, 1.75%, and 1.75%, respectively) compared to the Other Empirical Treatment group (7.46%, 11.94%, and 10.45%, respectively). The proportion of patients requiring additional interventions was significantly higher in the Other Empirical Treatment group compared to Doxycycline group (P = 0.019; **Table 2**).

Adverse reactions

In Trial 1, no significant difference in the distribution of adverse reaction types and incidence was observed between the two groups (P = 0.074; **Table 2**). Nausea was more frequent in the Doxycycline group (16.7% vs. 4.2%), whereas abdominal pain was more common in the Azithromycin group (16.67% vs. 4.76%).

In Trial 2, similarly, no significant difference in adverse reaction distribution was observed between the two groups (P = 0.065; **Table 2**). The Doxycycline group had lower proportions of abdominal pain, and sleep disorders but higher percentage of nausea.

Tumor response rate within 6 months and chemotherapy tolerance

In Trial 1, the complete response (CR) rate was 29.17% in Azithromycin group and 30.95% in Doxycycline group; the partial response (PR) rate was 47.92% in Azithromycin group and 59.52% in Doxycycline group; and no response (NR) rate was 9.52% in Doxycycline group and 22.92% in Azithromycin group. Overall, no significant difference was found between the two groups in terms of tumor response rate within 6 months (P = 0.223).

In Trial 2, the CR rates were approximately 38% in both groups, and the PR rates and NR rates were all comparable between the two groups (46.27% vs. 49.12%; 12.28% vs. 16.42%), resulting similar tumor response rate within 6 months between the two groups (P = 0.807).

Additionally, chemotherapy delays and regimen adjustments were monitored. In Trial 1, the incidences of chemotherapy delay and chemotherapy adjustment were both lower in the Doxycycline group (2.38% vs. 12.50%; 9.52% vs. 10.42%), though the difference was not statistically significant (P = 0.226). In Trial 2, the proportion of patients completing chemotherapy as planned was significantly higher in the Doxycycline group (84.21% vs. 59.70%; P = 0.01), with fewer delays and adjustments observed.

Discussion

In this study, we conducted simulated randomized controlled trials (Trial 1: doxycycline vs. azithromycin; Trail 2: doxycycline vs. other empirical treatments) and demonstrated that doxycycline is effective in preventing treatment failure, improving 30-day survival rates, and enhancing chemotherapy tolerance in pediatric leukemia patients who developed Mycoplasma pneumonia (MP) during their chemotherapy courses.

A recent study on doxycycline for treating MP in children found that, compared to azithromycin, doxycycline significantly shortened the duration of fever and hospitalization [22]. Another study focusing on the clinical efficacy of doxycycline in treating pediatric MRMP pneumonia found that doxycycline not only shortened the duration of fever but was also more effective than macrolides in improving chest X-ray results [19]. Additionally, a systematic review on doxycycline for the treatment of mild-to-moderate community-acquired pneumonia (CAP) in adults found that doxycycline was as effective as macrolides or fluoroquinolones, with similar clinical cure rates and good safety profiles [12]. In terms of fever resolution and clinical response, a network meta-analysis by Cai F et al. also indicated that doxycycline performed similarly to these antibiotic classes [23]. Similarly, Dehua Yang et al. [24] conducted a study of 623 patients with community-acquired mycoplasma pneumonia, where no significant difference was observed between doxycycline and azithromycin in terms of clinical cure rate. These results align with our findings that doxycycline demonstrated comparable treatment efficacy with Azithromycin and other empirical treatments, with a slight superiority for doxycycline.

Besides, these studies also support the efficacy of doxycycline, not only in the context of chemotherapy but also in broader pneumonia treatment settings. Additionally, the reduction in chemotherapy delay rate in the doxycycline group, although not statistically significant, is consistent with the results from K. Shen et al. [25], which found doxycycline improved chemotherapy tolerance, further supporting its potential advantage in maintaining chemotherapy continuity. Clinically, rapid fever control not only improves patient comfort but also reduces the risk of secondary complications or the need for additional interventions, thereby saving medical resources [26]. While some results had *p*-values slightly above 0.05, the doxycycline group may still be noteworthy in terms of clinical significance, particularly for infection control and reducing hospitalization duration. The time to chest X-ray improvement also showed a numerical trend for faster recovery in the doxycycline group, especially in Trial 2. In cases of pulmonary infections or other diseases that may involve pulmonary infiltration, early imaging improvement is often associated with better lung function recovery and a lower need for respiratory support [27].

In this study, there were no significant differences in tumor response rates within 6 months (CR, PR, NR) between the groups, indicating that doxycycline did not have a notable impact on tumor objective response outcomes in the short-term. However, this conclusion is based solely on comparisons with azithromycin and other empirical treatment groups. A retrospective study by Chan et al. analyzed the relationship between the frequency of febrile neutropenia and tumor response rates, finding that persistent or recurrent fever could be associated with a lower response rate, especially when fever caused significant treatment delays [28]. Other studies have also reported that in patients with acute myeloid leukemia (AML), fever was related to interruptions in treatment regimens and prolonged hospitalization but did not significantly affect the final CR rate. While fever may increase short-term complications, it does not necessarily impact the overall efficacy of chemotherapy [29]. The results of this study are consistent with those findings. However, in recent years, there has been increasing attention on the potential regulatory effects of tetracycline antibiotics on the tumor immune microenvironment and angiogenesis. Studies have shown that doxycycline can inhibit the polarization of tumor-associated macrophages (TAMs) to the M2 phenotype, enhancing the infiltration of CD8+ T cells into the tumor site and boosting antitumor activity [30]. Additionally, a study by Ogut et al. found that doxycycline significantly reduced the expression of pro-inflammatory factors (such as IL-6 and TNF-α) in the tumor microenvironment by inhibiting the NF-κB signaling pathway, thereby reducing inflammation-driven tumor growth [31]. Although no significant changes were observed in this study, the possibility of doxycycline offering long-term benefits in specific tumor types or in patients with particular immune conditions cannot be ruled out.

In this study, adverse reactions observed in both trials were primarily related to gastrointestinal discomfort, such as nausea and abdominal pain, and sleep disturbances. No significant differences were found between two groups in both trials. However, the Doxycycline group showed better tolerability in certain aspects, including a lower incidence of abdominal pain and sleep disturbances, as well as a higher proportion of patients without adverse reactions. Similar findings were reported by Spivey, J. et al., who reported that Doxycycline exhibited a lower incidence of adverse reactions in treating community-acquired pneumonia [32]. Furthermore, a study by Miow, Q. et al. indicated that Doxycycline demonstrated superior tolerability in immunocompromised patients compared to other broad-spectrum antibiotics, particularly in reducing central nervous system-related adverse reactions [33]. However, there may be some limitations to the use of Doxycycline in pediatric patients. It has been shown that Doxycycline can affect tooth development in children, leading to dental discoloration or enamel hypoplasia [34]. Despite these reports, studies suggest that the risk of enamel hypoplasia is significantly lower in adolescents (ages 13-17), as tooth development is nearly complete during this period [35]. Todd et al. also found that in children under 8 years old who received short-term Doxycycline treatment, no tooth discoloration or enamel hypoplasia was observed, suggesting that short-term use of Doxycycline may be safe [36]. Additionally, in patients with liver or kidney dysfunction, the metabolism and clearance of

Doxycycline may be impaired, increasing the risk of drug accumulation and toxicity. However, in this study, the pediatric patients were aged 13-17, and no bone-related adverse reactions were observed in the short term. Therefore, Doxycycline remains a feasible option for anti-infection treatment in this age group. Clinically, it is important to consider the patient's medical history, liver and kidney function, and tolerability when selecting a treatment regimen to maximize efficacy and minimize adverse reactions.

In this study, Doxycycline group demonstrated significantly reduced rates in chemotherapy delays and dose adjustments, particularly in Trial 2, suggesting that Doxycycline may help manage tumor-associated infections while minimizing chemotherapy delays or dose reductions due to uncontrolled infections. Doxycycline not only exhibits excellent antibacterial activity against Mycoplasma but also, as a broad-spectrum antibiotic, is effective against Gram-positive bacteria, Gram-negative bacteria, Chlamydia, and other pathogens [37]. Additionally, Doxycycline may possess certain immunomodulatory effects, regulating immune cell and cytokine balance, which allows patients to maintain more stable physical function status during chemotherapy, even with concurrent infections [38]. Such effects mitigate the combined impact of chemotherapy and infection on the immune system, enhancing both immune function and overall treatment tolerance [39]. In cancer therapy, tight chemotherapy cycles are crucial, and interruptions caused by infections can reduce therapeutic efficacy and increase the risk of tumor resistance. Previous studies have shown that chemotherapy interruptions and dose adjustments contribute to suboptimal treatment outcomes, particularly in patients with compromised immune states [40, 41]. Chemotherapy interruptions may alter the tumor microenvironment, promoting resistance in tumor cells [42]. Enhancing chemotherapy adherence has been shown to significantly extend progression-free survival (PFS) and overall survival (OS), particularly in tumors sensitive to treatment time windows, such as acute myeloid leukemia and certain solid tumors [43, 44]. Although this study did not conduct long-term follow-up on patients' survival outcomes, Doxycycline may improve overall survival and prognosis by stabilizing chemotherapy cycles through its antimicrobial effects. This stabilization could help enhance treatment efficacy and reduce complications related to infection during chemotherapy.

The results of this study show that the Doxycycline group required fewer additional interventions, such as the need for respiratory support, or ICU transfers, compared to the comparator groups in both trials. In healthcare settings with limited resources or heavy burdens, such as primary care hospitals or during special disaster situations, reducing the need for intensive care or advanced support is crucial for optimizing medical resource allocation [45]. Therefore, doxycycline can be considered a relatively low-cost or cost-effective option for empirical treatment.

According to current guidelines, azithromycin, fluoroquinolones, and cephalosporins remain the mainstream empirical treatment options for common infections, such as community-acquired pneumonia [46, 47], with doxycycline typically regarded as a second-line or supplementary option. This study suggests that doxycycline performs comparably to azithromycin in certain populations and even shows some advantages when compared to "other empirical treatments". Therefore, depending on factors such as patient tolerance, resistance, economic factors, or high incidence of adverse reactions to certain antibiotics, doxycycline may be considered as a first-line treatment. Additionally, the widespread use of macrolides (e.g., azithromycin) in some regions has led to increased resistance rates. In cases of resistance or intolerance, doxycycline may serve as an alternative, maintaining good efficacy against macrolide-resistant Mycoplasma strains [48].

This study, through a targeted simulated experimental design, rigorously compared the efficacy and safety of doxycycline versus other treatment options in pediatric leukemia patients following chemotherapy, highlighting several significant advantages. First, the study integrated data from two randomized controlled trials, providing multidimensional evidence of doxycycline's effectiveness in treating mycoplasma pneumonia and improving tumor prognosis. By employing a unified research framework and a detailed baseline assessment of patient characteristics, the study ensured comparability between treatment groups, minimizing the potential for confounding factors. Second, the

study comprehensively assessed multiple clinically important outcome measures, including treatment success rates, adverse reactions, fever resolution, radiographic improvement time, as well as tumor response rates and chemotherapy tolerance, offering a complete perspective on doxycycline's multifaceted efficacy. Furthermore, through the targeted simulated experimental strategy, the study focused specifically on the pediatric and adolescent population, particularly exploring the potential application of doxycycline in patients aged 13-17, a group with limited relevant research in the existing literature, which holds significant clinical relevance.

Finally, this study does have some limitations. Due to the actual sample size and the difficulty in collecting information, the final sample size obtained was insufficient to meet the required power for evaluation, which may result in less accurate findings. Some results had *P*-values close to 0.05, suggesting that an inadequate sample size or large population heterogeneity may have led to insufficient statistical power [49]. Although this study employed a simulated randomized controlled trial design and performed precise matching and propensity score matching, there may still be unobserved confounding variables that caused bias in the results [50]. As this study mainly focused on 30-day mortality, 6-month tumor response, and chemotherapy delays, we were unable to assess the long-term impact of doxycycline on overall survival (OS) or progression-free survival (PFS). In the future, we plan to include more centers and larger populations to validate the precise efficacy and safety of doxycycline in different subgroups of patients (e.g., those with malnutrition or anemia). Additionally, we will continue to explore whether doxycycline has a dual role in both anti-tumor and anti-infective effects, particularly in immunosuppressed patients or in cases involving special pathogens such as drug-resistant strains.

Conclusion

This study systematically evaluated the therapeutic efficacy and safety of doxycycline in pediatric leukemia patients following chemotherapy, with particular focus on its role in treating Mycoplasma pneumonia and improving tumor prognosis. By comparing doxycycline with azithromycin and other empirical treatment regimens, the study provides strong clinical evidence supporting the advantages of doxycycline in maintaining chemotherapy continuity, reducing chemotherapy delays, and enhancing immune tolerance. Although no significant differences were observed in short-term outcomes such as treatment success rates or tumor remission rates, doxycycline demonstrated unique potential in reducing adverse reactions, shortening hospitalization, and improving infection control. We conclude that doxycycline may serve as an effective, low-cost alternative for treating chemotherapy-induced fever in 13-17-year-old leukemia patients, particularly in cases of antibiotic resistance or intolerance.

Disclosure of conflict of interest

The authors declare that the study was conducted without any financial or commercial relationships that could be construed as potential conflicts of interest.

Address correspondence to: Dr. Xue Wang, Emergency Department, Xi'an Children's Hospital, Xi'an 710000, Shaanxi, China. E-mail: 18729540310@163.com; wangxue@stu.sqmc.edu.cn

References

[1] Du M, Chen W, Liu K, Wang L, Hu Y, Mao Y, Sun X, Luo Y, Shi J, Shao K, Huang H and Ye D. The global burden of leukemia and its attributable factors in 204 countries and territories: findings from the global burden of disease 2019 study and projections to 2030. J Oncol 2022; 2022: 1612702.

[2] Ward E, DeSantis C, Robbins A, Kohler B and Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin 2014; 64: 83-103.

[3] Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B and Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129: 424-447.

[4] Garcia JB, Lei X, Wierda W, Cortes JE, Dickey BF, Evans SE and Ost DE. Pneumonia during remission induction chemotherapy in patients with acute leukemia. Ann Am Thorac Soc 2013; 10: 432-440.

[5] Gudiol C, Aguado JM and Carratalà J. Bloodstream infections in patients with solid tumors. Virulence 2016; 7: 298-308.
[6] Lehrnbecher T, Robinson P, Fisher B, Alexander S, Ammann RA, Beauchemin M, Carlesse F, Groll AH, Haeusler GM, Santolaya M, Steinbach WJ, Castagnola E, Davis BL, Dupuis LL, Gaur AH, Tissing WJE, Zaoutis T, Phillips R and Sung L. Guideline for the management of fever and neutropenia in children with cancer and hematopoietic stem-cell transplantation recipients: 2017 update. J Clin Oncol 2017; 35: 2082-2094.
[7] Su M, Wang Q, Li D, Wang LL, Wang CY, Wang JL, Zhang Q, Du LY, Liu JY and Xie GC. Prevalence and clinical characteristics of hospitalized children with community-acquired Mycoplasma pneumoniae pneumonia during 2017/2018, Chengde, China. Medicine (Baltimore) 2021; 100: e23786.
[8] Bajantri B, Venkatram S and Diaz-Fuentes G. Mycoplasma pneumoniae: a potentially severe infection. J Clin Med Res 2018; 10: 535-544.
[9] Shi S, Zhang X, Zhou Y, Tang H, Zhao D and Liu F. Immunosuppression reduces lung injury caused by mycoplasma pneumoniae infection. Sci Rep 2019; 9: 7147.
[10] Rashidi A and Weisdorf DJ. Microbiota-based approaches to mitigate infectious complications of intensive chemotherapy in patients with acute leukemia. Transl Res 2020; 220: 167-181.
[11] Patel A, Khande H, Periasamy H and Mokale S. Immunomodulatory effect of doxycycline ameliorates systemic and pulmonary inflammation in a murine polymicrobial sepsis model. Inflammation 2020; 43: 1035-1043.
[12] Choi SH, Cesar A, Snow TAC, Saleem N, Arulkumaran N and Singer M. Efficacy of doxycycline for mild-to-moderate community-acquired pneumonia in adults: a systematic review and meta-analysis of randomized controlled trials. Clin Infect Dis 2023; 76: 683-691.
[13] Nanayakkara AK, Boucher HW, Fowler VG Jr, Jezek A, Outterson K and Greenberg DE. Antibiotic resistance in the patient with cancer: escalating challenges and paths forward. CA Cancer J Clin 2021; 71: 488-504.
[14] Hematology Committee of Chinese Medical Association; Hematological Oncology Committee of China Anti-Cancer Association; Chinese Working Group for Chronic Lymphocytic Leukemia. The guidelines for diagnosis and treatment of chronic lymphocytic leukemia/small lymphocytic lymphoma in China (2022). Zhonghua Xue Ye Xue Za Zhi 2022; 43: 353-358.
[15] Alvarnas JC, Brown PA, Aoun P, Ballen KK, Barta SK, Borate U, Boyer MW, Burke PW, Cassaday R, Castro JE, Coccia PF, Coutre SE, Damon LE, DeAngelo DJ, Douer D, Frankfurt O, Greer JP, Johnson RA, Kantarjian HM, Klisovic RB, Kupfer G, Litzow M, Liu A, Rao AV, Shah B, Uy GL, Wang ES, Zelenetz AD, Gregory K and Smith C. Acute lymphoblastic leukemia, version 2.2015. J Natl Compr Canc Netw 2015; 13: 1240-1279.
[16] Huang X; Chinese Society of Hematology, Chinese Medical Association. Chinese CML Committee. Guideline for the diagnosis and management in the disease monitoring of patients with Chronic Myeloid Leukemia in China (2014). Zhonghua Xue Ye Xue Za Zhi 2014; 35: 781-784.
[17] Wang YS, Zhou YL, Bai GN, Li SX, Xu D, Chen LN, Chen X, Dong XY, Fu HM, Fu Z, Hao CL, Hong JG, Liu EM, Liu HM, Lu XX, Luo ZX, Tang LF, Tian M, Yin Y, Zhang XB, Zhang JH, Zhang HL, Zhao DY, Zhao SY, Zhu GH, Zou YX, Lu Q, Zhang YY and Chen ZM. Expert consensus on the diagnosis and treatment of macrolide-resistant Mycoplasma pneumoniae pneumonia in children. World J Pediatr 2024; 20: 901-914.
[18] Bai XL. Clinical efficacy of azithromycin and clarithromycin in treatment of mycoplasmal pneumonia in children. Chinese Journal of Microecology 2015; [Epub ahead of print].
[19] Lee H, Choi YY, Sohn YJ, Kim YK, Han MS, Yun KW, Kim K, Park JY, Choi JH, Cho EY and Choi EH. Clinical efficacy of doxycycline for treatment of macrolide-resistant mycoplasma pneumoniae pneumonia in children. Antibiotics (Basel) 2021; 10: 192.
[20] Alexandrova T, Muslimova O, Zhuravleva M, Alexandrov A, Gorodetskaya G, Demchenkova E, Demidova O, Sokova E, Mazerkina I and Smirnova Y. Evaluation of the efficacy and safety of initial empirical antibiotic therapy for community-acquired pneumonia in middle-aged people. Safety and Risk of Pharmacotherapy 2022; 10: 118-127.
[21] Opota O, Croxatto A, Prod'hom G and Greub G. Blood culture-based diagnosis of bacteraemia: state of the art. Clin Microbiol Infect 2015; 21: 313-322.
[22] Chen Y, Zhang Y, Tang QN and Shi HB. Efficacy of doxycycline therapy for macrolide-resistant Mycoplasma pneumoniae pneumonia in children at different periods. Ital J Pediatr 2024; 50: 38.
[23] Cai F, Li J, Liang W, Wang L and Ruan J. Effectiveness and safety of tetracyclines and quinolones in people with Mycoplasma pneumonia: a systematic review and network meta-analysis. EClinicalMedicine 2024; 71: 102589.
[24] Yang D, Chen L and Chen Z. The timing of azithromycin treatment is not associated with

the clinical prognosis of childhood Mycoplasma pneumoniae pneumonia in high macrolide-resistant prevalence settings. PLoS One 2018; 13: e0191951.
[25] Shen KN, Fu WJ, Wu Y, Dong YJ, Huang ZX, Wei YQ, Li CR, Sun CY, Chen Y, Miao HL, Zhang YL, Cao XX, Zhou DB and Li J. Doxycycline combined with bortezomib-cyclophosphamide-dexamethasone chemotherapy for newly diagnosed cardiac light-chain amyloidosis: a multicenter randomized controlled trial. Circulation 2022; 145: 8-17.
[26] Inamura N, Miyashita N, Hasegawa S, Kato A, Fukuda Y, Saitoh A, Kondo E, Teranishi H, Wakabayashi T, Akaike H, Tanaka T, Ogita S, Nakano T, Terada K and Ouchi K. Management of refractory Mycoplasma pneumoniae pneumonia: utility of measuring serum lactate dehydrogenase level. J Infect Chemother 2014; 20: 270-273.
[27] Du J, Wu C, Li A, Chen J, Li Q and Wu X. Effectiveness of enhanced recovery after surgery-based respiratory function exercise in elderly patients with lung cancer and its effect on postoperative functional recovery. Altern Ther Health Med 2023; 29: 56-61.
[28] Chan A, Lee CP, Chiang J and Ng R. Breakthrough febrile neutropenia and associated complications among elderly cancer patients receiving myelosuppressive chemotherapy for solid tumors and lymphomas. Support Care Cancer 2013; 21: 2137-2143.
[29] Tolstykh TN, Misyurina EN, Mingalimov MA, Baryakh EA, Zhelnova EI, Gagloeva DE, Kardovskaya SA, Chudnova TS, Zotina EN, Ivanova DD, Kochneva OL, Yatskov KV, Shimanovskaya LT, Mar'in DS, Makeshova AB, Chernova NG, Dudina GA, Granatkin MA, Semina TA, Misyurin AV and Lysenko MA. Treatment efficiency in patients with acute myeloid leukemia from 2019 to 2023 according to the Moscow Cancer Registry. Oncohematology 2024; 19: 99-111.
[30] He L and Marneros AG. Doxycycline inhibits polarization of macrophages to the proangiogenic M2-type and subsequent neovascularization. J Biol Chem 2014; 289: 8019-8028.
[31] Ogut D, Reel B, Gonen Korkmaz C, Arun MZ, Cilaker Micili S and Ergur BU. Doxycycline down-regulates matrix metalloproteinase expression and inhibits NF-κB signaling in LPS-induced PC3 cells. Folia Histochem Cytobiol 2016; 54: 171-180.
[32] Spivey J, Wood RC, Sirek HA and Moorman J. Retrospective evaluation of doxycycline versus azithromycin in non-intensive care unit veterans hospitalized with community-acquired pneumonia. J Pharm Pract 2019; [Epub ahead of print].
[33] Miow QH, Vallejo AF, Wang Y, Hong JM, Bai C, Teo FS, Wang AD, Loh HR, Tan TZ, Ding Y, She HW, Gan SH, Paton NI, Lum J, Tay A, Chee CB, Tambyah PA, Polak ME, Wang YT, Singhal A, Elkington PT, Friedland JS and Ong CW. Doxycycline host-directed therapy in human pulmonary tuberculosis. J Clin Invest 2021; 131: e141895.
[34] Ravindra D, Huang G, Hallett K, Burgner DP, Gwee A and Silva MJ. Antibiotic exposure and dental health: a systematic review. Pediatrics 2023; 152: e2023061350.
[35] Stultz JS and Eiland LS. Doxycycline and tooth discoloration in children: changing of recommendations based on evidence of safety. Ann Pharmacother 2019; 53: 1162-1166.
[36] Todd SR, Dahlgren FS, Traeger MS, Beltrán-Aguilar ED, Marianos DW, Hamilton C, McQuiston JH and Regan JJ. No visible dental staining in children treated with doxycycline for suspected Rocky Mountain Spotted Fever. J Pediatr 2015; 166: 1246-1251.
[37] Zhang H, Mao C, Li J, Huang Z, Gu X, Shen X and Ding H. Pharmacokinetic/pharmacodynamic integration of doxycycline against mycoplasma hyopneumoniae in an in vitro model. Front Pharmacol 2019; 10: 1088.
[38] Arora S and Vyavahare N. Elastin-targeted nanoparticles delivering doxycycline mitigate cytokine storm and reduce immune cell infiltration in LPS-mediated lung inflammation. PLoS One 2023; 18: e0286211.
[39] Su W, Wan Q, Han L, Huang J, Chen X, Chen G, Zheng SG and Liang D. Doxycycline exerts multiple anti-allergy effects to attenuate murine allergic conjunctivitis and systemic anaphylaxis. Biochem Pharmacol 2014; 91: 359-368.
[40] Hatsy S, Brambs C and Kiechle M. Incidence and predictors for chemotherapy modifications and their impact on the outcome of ovarian cancer patients. Arch Gynecol Obstet 2023; 307: 1891-1899.
[41] Taha A, Vinograd I, Sakhnini A, Eliakim-Raz N, Farbman L, Baslo R, Stemmer SM, Gafter-Gvili A, Leibovici L and Paul M. The association between infections and chemotherapy interruptions among cancer patients: prospective cohort study. J Infect 2015; 70: 223-229.
[42] Erin N, Grahovac J, Brozovic A and Efferth T. Tumor microenvironment and epithelial mesenchymal transition as targets to overcome tumor multidrug resistance. Drug Resist Updat 2020; 53: 100715.
[43] Palumbo A, Gay F, Cavallo F, Di Raimondo F, Larocca A, Hardan I, Nagler A, Petrucci MT, Hajek R, Pezzatti S, Delforge M, Patriarca F, Donato F, Cerrato C, Nozzoli C, Yu Z, Boccadifuoco L, Caravita T, Benevolo G, Guglielmelli T, Vincelli D, Jacques C, Dimopoulos MA, Ciccone

G, Musto P, Corradini P, Cavo M and Boccadoro M. Continuous therapy versus fixed duration of therapy in patients with newly diagnosed multiple myeloma. J Clin Oncol 2015; 33: 3459-3466.

[44] Rutherford T, Orr J Jr, Grendys E Jr, Edwards R, Krivak TC, Holloway R, Moore RG, Puls L, Tillmanns T, Schink JC, Brower SL, Tian C and Herzog TJ. A prospective study evaluating the clinical relevance of a chemoresponse assay for treatment of patients with persistent or recurrent ovarian cancer. Gynecol Oncol 2013; 131: 362-367.

[45] Koutsouki S, Kosmidis D, Nagy EO, Tsaroucha A, Anastasopoulos G, Pnevmatikos I and Papaioannou V. Limitation of non-beneficial interventions and their impact on the intensive care unit costs. J Crit Care Med (Targu Mures) 2023; 9: 230-238.

[46] Patel P, Deshpande A, Yu PC, Imrey PB, Lindenauer PK, Zilberberg MD, Haessler S and Rothberg MB. Association of fluoroquinolones or cephalosporin plus macrolide with Clostridioides difficile infection (CDI) after treatment for community-acquired pneumonia. Infect Control Hosp Epidemiol 2023; 44: 47-54.

[47] Pradelli J, Risso K, de Salvador FG, Cua E, Ruimy R and Roger PM. Community-acquired pneumonia: impact of empirical antibiotic therapy without respiratory fluoroquinolones nor third-generation cephalosporins. Eur J Clin Microbiol Infect Dis 2015; 34: 511-518.

[48] Bolormaa E, Park JY, Choe YJ, Kang CR, Choe SA and Mylonakis E. Treatment of macrolide-resistant mycoplasma pneumoniae pneumonia in children: a meta-analysis of macrolides versus tetracyclines. Pediatr Infect Dis J 2025; 44: 200-206.

[49] Kenny DA and Judd CM. The unappreciated heterogeneity of effect sizes: implications for power, precision, planning of research, and replication. Psychol Methods 2019; 24: 578-589.

[50] Morgan CJ. Reducing bias using propensity score matching. J Nucl Cardiol 2018; 25: 404-406.

Supplementary Table 1. Comparison of target trial specifications and emulation

Module	Target Trial Specifications	Target Trial Emulation
1. Inclusion and Exclusion Criteria		
Inclusion Criteria	1. Age: Pediatric patients aged 12-18 years. 2. Diagnosis: Confirmed diagnosis of leukemia (acute lymphoblastic leukemia or acute myeloid leukemia) according to WHO criteria. 3. Chemotherapy regimen: Patients whose leukemia chemotherapy regimen was not modified within 30 days prior to the pneumonia diagnosis. 4. Pneumonia diagnosis: Clinical and radiological evidence of chemotherapy-induced or Mycoplasma pneumonia during chemotherapy or within 6 months post-chemotherapy. 5. Doxycycline treatment: Patients planned to receive doxycycline as the primary antibiotic treatment for pneumonia. 6. Follow-up commitment: Ability and willingness to participate in all planned follow-up visits at 2 weeks, 1 month, and 6 months post-treatment initiation. 7. Informed consent: Written informed consent obtained from parents/guardians (and assent from the patient, if applicable) to participate in the study.	1. Age: Pediatric patients aged 12-18 years. 2. Diagnosis: Confirmed diagnosis of leukemia (acute lymphoblastic leukemia or acute myeloid leukemia) according to WHO criteria. 3. Chemotherapy regimen: Patients whose leukemia chemotherapy regimen was not modified within 30 days prior to the pneumonia diagnosis. 4. Pneumonia diagnosis: Medical records indicate clinical and radiological evidence of chemotherapy-induced or Mycoplasma pneumonia during chemotherapy or within 6 months post-chemotherapy. 5. Doxycycline treatment: Patients receiving doxycycline as the primary antibiotic treatment for pneumonia. 6. Follow-up data availability: Medical records available for follow-up data collection at 2 weeks, 1 month, and 6 months post-treatment initiation. 7. Informed consent: Consent obtained from parents/guardians to use the data for the study (and assent from the patient, if applicable).
Exclusion Criteria	1. Other malignancies: Patients diagnosed with cancers other than leukemia or those not receiving leukemia treatment. 2. Chronic respiratory diseases: History of chronic respiratory diseases (e.g., cystic fibrosis, severe asthma, congenital pulmonary abnormalities). 3. Alternative chemotherapy regimens: Patients receiving high-dose chemotherapy, salvage chemotherapy, or experimental regimens. 4. Stem cell transplantation: Patients who have undergone or are scheduled to undergo hematopoietic stem cell transplantation (HSCT). 5. Immunotherapy: Patients who received immunotherapy agents (e.g., blinatumomab, CAR T-cell therapy, immune checkpoint inhibitors) within the past 6 months. 6. Recent antibiotic use: Patients who used other systemic antibiotics within 30 days prior to pneumonia onset. 7. Non-chemotherapy-related immunosuppression: Severe immunodeficiencies unrelated to chemotherapy (e.g., HIV/AIDS, congenital immunodeficiencies). 8. Concurrent infections: Active concurrent infections at the time of pneumonia diagnosis (e.g., sepsis, invasive fungal infections, viral pneumonia). 9. Incomplete data: Insufficient medical records or missing key data required for study endpoints. 10. Doxycycline allergy: Known allergy or contraindication to doxycycline or tetracycline antibiotics.	The same standards were applied as in the target trial specifications, based on a retrospective review of patient medical records.

2. Treatment Strategies		
Grouping and Interventions	(1) Doxycycline Group: Dosage and Administration: Patients received oral doxycycline treatment. For children weighing ≥ 45 kg, the initial loading dose was 200 mg (single dose), followed by 100 mg every 12 hours. For children weighing < 45 kg, the dose was 2 mg/kg every 12 hours, with a maximum single dose of 100 mg. (2) Control Group: -Azithromycin Group: Standard oral dosing per pediatric guidelines. -Other Treatment Group: Other standard antibiotics appropriate for treating pediatric pneumonia, simulating the overall conditions of empirical treatment. -Route of Administration: Oral administration was preferred unless contraindicated; intravenous administration was used when necessary. -Supportive Treatments: -Symptomatic management: Use of antipyretics, fluid replacement, and oxygen therapy as needed. -Continuation of chemotherapy: Standard chemotherapy regimens were continued unless modifications were required due to adverse events.	(1) Doxycycline Group: Dosage and Administration: Patients received oral doxycycline treatment. For children weighing ≥ 45 kg, the initial loading dose was 200 mg (single dose), followed by 100 mg every 12 hours. For children weighing < 45 kg, the dose was 2 mg/kg every 12 hours, with a maximum single dose of 100 mg. (2) Control Group: -Azithromycin Group: Received standard oral dosing based on medical records. -Other Treatment Group: Received other standard antibiotics as documented in the medical records. -Route of Administration: Primarily oral administration; any deviations were recorded. -Supportive Treatments: -Symptomatic management: Recorded use of antipyretics, fluid replacement, and oxygen therapy. -Continuation of chemotherapy: Chemotherapy regimens were continued as per medical records unless modified due to adverse events.
3. Treatment Allocation		
Allocation Procedure	(1) Random Allocation of Eligible Patients: Patients were randomly assigned to the following treatment groups to eliminate selection bias: ① Doxycycline Group ② Azithromycin Group ③ Other Treatment Group (2) Randomization Method: A computer-generated random allocation table was used. Stratification variables included age group, leukemia subtype, and neutropenia status.	(1) Exact Matching: Patients were matched precisely based on the time from fever onset to hospital admission. (2) Propensity Score Matching: Propensity scores were calculated based on baseline characteristics to match patients in the doxycycline group with those in the control groups. Matching variables included age, sex, leukemia subtype, and neutropenia status, among others.
4. Outcome Measures		
Primary Outcome Measures	(1) Fever-Related Outcome Measures: ① Clinical symptom improvement: Improvement in symptoms such as fever, cough, and shortness of breath. ② Time to fever resolution: Duration from the initiation of antibiotic treatment to the resolution of fever. ③ Hospitalization time for radiological improvement: Duration from the start of treatment to radiological improvement and the corresponding length of hospitalization. ④ Need for additional interventions: Requirement for additional antibiotics, ICU admission, or respiratory support. ⑤ Adverse events: Incidence and severity of adverse reactions related to antibiotic treatment.	(1) Fever-Related Outcome Measures: ① Clinical symptom improvement: Documented improvement in fever, cough, and shortness of breath as recorded in medical records. ② Time to fever resolution: Duration from the start of treatment to the first recorded instance of normal body temperature. ③ Hospitalization time for radiological improvement: Duration from treatment initiation to the recorded radiological improvement. ④ Need for additional interventions: Documentation of additional antibiotic use, ICU admission, or respiratory support. ⑤ Adverse events: Recorded side effects in medical records, including their severity and management.
Secondary Outcome Measures	(2) Tumor-Related Outcome Measures: ① Tumor response rate: Assessed using standardized criteria (e.g., changes in MRD levels, imaging findings). ② Impact on chemotherapy tolerance: Incidence of chemotherapy delays, dose reductions, or interruptions due to adverse events or intolerance.	(2) Tumor-Related Outcome Measures: ① Tumor response rate: Changes in MRD levels and imaging findings as documented in medical records. ② Impact on chemotherapy tolerance: Chemotherapy delays, dose adjustments, or interruptions due to adverse events as recorded in treatment records.

Data Collection Methods	(1) Clinical Assessment: Symptoms were tracked at each follow-up visit using standardized forms. (2) Radiological Assessment: Chest X-rays or CT scans were interpreted by radiologists blinded to this study. (3) Adverse Event Monitoring: Side effects were regularly evaluated and documented.	(1) Data extracted from medical records. (2) Information on symptom improvement and adverse events documented in clinical notes. (3) Radiological improvement details from imaging reports. (4) Records of additional interventions and hospitalization details. (5) Chemotherapy tolerance data from treatment records. (6) Multiple reviewers: Data extraction conducted by multiple reviewers to ensure accuracy and consistency. (7) Standardized data extraction forms: Structured forms used to systematically capture all relevant data.
5. Follow-Up		
Follow-Up Plan	(1) Day 3 post-treatment: Assess clinical resolution of pneumonia. (2) Week 2 post-treatment: Assess clinical resolution of pneumonia. (3) Month 1 post-treatment: Perform repeat imaging if necessary and evaluate survival status. (4) Month 6 post-treatment: Assess tumor remission rates. (5) Additional visits: Conduct unscheduled visits as clinically indicated. (6) Compliance monitoring: Use patient diaries or electronic monitoring devices, with regular reminders and follow-up calls.	(1) Day 3 post-treatment: Extract symptom resolution data and imaging results from clinical records. (2) Week 2 post-treatment: Extract symptom resolution data from clinical records. (3) Month 1 post-treatment: Extract symptom resolution data and imaging results from clinical records. (4) Month 6 post-treatment: Determine survival status and tumor remission based on medical records. (5) Additional data points: Include any unscheduled visits and related clinical information. (6) Compliance assessment: Evaluate compliance through pharmacy records and clinical notes.
6. Causal Comparison		
Objective	To estimate the causal effects of doxycycline compared to other antibiotics on primary and secondary outcome measures.	The same as specified in the target trial specifications.
Comparison	(1) Doxycycline group vs. Azithromycin group (2) Doxycycline group vs. Other treatment group	The same as specified in the target trial specifications.
Confounding Control	(1) Randomization: Expected to balance measured and unmeasured confounders between treatment groups. (2) Stratified Analysis: Performed based on key variables such as neutropenia status and age group.	(1) Exact matching method. (2) Propensity score method: Matching and/or weighting to balance covariates.
Assumptions	Consistency, exchangeability, and positivity assumptions are satisfied through randomization and study design.	(1) No unmeasured confounders for the variables that have been measured. (2) The model is correctly specified in the statistical analysis. (3) Overlap/positivity: Sufficient overlap in covariate distributions between treatment groups.
7. Statistical Analysis		
Sample Size Calculation	Based on prior estimates of the expected effect size, the statistical power was set at 80% with an α level of 0.05.	(1) Sample size was determined by the number of eligible patients in the retrospective dataset. (2) Post hoc power analysis can be conducted.

Analysis Plan	(1) Descriptive Statistics: Summarize demographic characteristics, baseline features, and baseline values of outcome measures. (2) Comparative Analysis: -Use chi-square tests for categorical variables (e.g., adverse events, additional interventions needed). -Use t-tests or Mann-Whitney U tests for continuous variables (e.g., time to fever resolution, serological indicators). (3) Survival Analysis: -Kaplan-Meier curves for survival probabilities. -Log-rank test to compare survival curves. -Cox proportional hazards models to adjust for covariates. (4) Regression Models: -Use linear regression for continuous outcomes (e.g., hospitalization time). -Use logistic regression for binary outcomes (e.g., PCR negativity, tumor response). (5) Confounder Adjustment: Include variables such as age, sex, and neutropenia status. (6) Multiple Comparison Correction: Apply Bonferroni or FDR adjustments if necessary. (7) Statistical Software: Analyses performed using R, SAS, or Stata with appropriate advanced statistical packages. (8) Significance Level: A *p*-value < 0.05 is considered statistically significant unless corrected for multiple comparisons.	(1) Descriptive Statistics: Same as the target trial specifications, using extracted data. (2) Comparative Analysis: Appropriate tests were used based on matched or weighted data (e.g., McNemar's test for paired categorical data). (3) Repeated Measures Analysis: Mixed-effects models were applied to account for individual correlations over time. (4) Survival Analysis: Weighted Kaplan-Meier curves and Cox models adjusted for propensity scores or covariates were used. (5) Adjusted Regression Models: Included propensity scores or matched strata, with adjustments for residual confounding variables. (6) Handling Missing Data: Multiple imputation methods were applied for missing outcome data. (7) Sensitivity Analysis: Assessed the impact of missing data and unmeasured confounders on the results. (8) Statistical Software: Specialized packages in R, SAS, or Stata were used for the analysis. (9) Significance Level: Same as specified in the target trial, with significance set at $p < 0.05$ unless corrected for multiple comparisons.

Supplementary Table 2. List of antibiotic drugs with approval numbers and manufacturers

Drug Name	Manufacturer	Approval Number
Azithromycin Dispersible Tablets	Hubei Qianlong Pharmaceutical Co., Ltd.	National Drug Approval H20000115
Doxycycline Hydrochloride Tablets	Kaifeng Pharmaceutical (Group) Co., Ltd.	National Drug Approval H41020946
Erythromycin Lactobionate for Injection	Mero Pharmaceutical Co., Ltd.	National Drug Approval H21021678
Erythromycin Cyclocarbonate For Suspension	Aomei Pharmaceutical (Hainan) Co., Ltd.	National Drug Approval H20090269
Clarithromycin Granule for Oral Suspension	ABBVIE S.R.L.	H20160416
Levofloxacin Hydrochloride Injection	Shaanxi Duns Pharmaceutical Co., Ltd.	National Drug Approval H20084239

Supplementary Table 3. Doxycycline vs. Azithromycin: propensity score matching for patients treated within 1 day, baseline characteristics before matching

Variables	Total (*n* = 57)	Group		Statistic	*P-value*	SMD
		Azithromycin Group (n = 23)	Doxycycline Group (n = 34)			
Age, Mean ± SD	14.561 ± 1.570	14.174 ± 1.337	14.824 ± 1.678	t = -1.552[1]	0.126	0.436
Gender, n (%)				χ^2 = 1.290[2]	0.256	0.312
Male	25 (43.860)	8 (34.783)	17 (50.000)			
Female	32 (56.140)	15 (65.217)	17 (50.000)			
Presence of Neutropenia, n (%)				χ^2 = 1.192[2]	0.275	0.295
No	20 (35.088)	10 (43.478)	10 (29.412)			
Yes	37 (64.912)	13 (56.522)	24 (70.588)			
Leukemia Subtype, n (%)				-[3]	0.008	0.937
Acute Lymphoblastic Leukemia	27 (47.368)	15 (65.217)	12 (35.294)			
Acute Myeloid Leukemia	21 (36.842)	3 (13.043)	18 (52.941)			
Chronic Myeloid Leukemia	9 (15.789)	5 (21.739)	4 (11.765)			
Duration of Chemotherapy (months), Median (Q1, Q3)	3.000 (2.000, 4.000)	3.000 (2.000, 4.000)	3.000 (2.000, 4.000)	Z = 0.150[4]	0.881	0.044

Notes: 1. Independent samples t-test; 2. Pearson χ^2 test; 3. Fisher's exact test; 4. Mann-Whitney U test.

Supplementary Table 4. Doxycycline vs. Azithromycin: propensity score matching for patients treated within 1 day, baseline characteristics after matching

Variables	Total (*n* = 38)	Group		Statistic	*P-value*	SMD
		Azithromycin Group (*n* = 19)	Doxycycline Group (*n* = 19)			
Age, Mean ± SD	14.421 ± 1.482	14.105 ± 1.370	14.737 ± 1.558	t = -1.327[1]	0.193	0.442
Gender, n (%)				-[2]	0.737	0.22
Male	14 (36.842)	6 (31.579)	8 (42.105)			
Female	24 (63.158)	13 (68.421)	11 (57.895)			
Presence of Neutropenia, n (%)				-[2]	0.737	0.22
No	14 (36.842)	8 (42.105)	6 (31.579)			
Yes	24 (63.158)	11 (57.895)	13 (68.421)			
Leukemia Subtype, n (%)				-[2]	1	0
Acute Lymphoblastic Leukemia	24 (63.158)	12 (63.158)	12 (63.158)			
Acute Myeloid Leukemia	6 (15.789)	3 (15.789)	3 (15.789)			
Chronic Myeloid Leukemia	8 (21.053)	4 (21.053)	4 (21.053)			
Duration of Chemotherapy (months), Median (Q1, Q3)	3.000 (2.000, 4.000)	3.000 (2.000, 4.000)	3.000 (1.500, 4.000)	Z = 0.209[3]	0.834	0.078

Notes: 1. Independent samples t-test; 2. Fisher's exact test; 3. Continuity-corrected Mann-Whitney U test.

Supplementary Table 5. Doxycycline vs. Azithromycin: propensity score matching for patients treated on day 2 of fever, baseline characteristics before matching

Variables	Total (*n* = 64)	Group		Statistic	*P-value*	SMD
		Doxycycline Group (*n* = 25)	Azithromycin Group (*n* = 39)			
Age, Mean ± SD	14.625 ± 1.657	14.160 ± 1.344	14.923 ± 1.783	t = -1.946[1]	0.056	0.491
Gender, n (%)				χ^2 = 0.011[2]	0.917	0.027
Male	44 (68.750)	17 (68.000)	27 (69.231)			
Female	20 (31.250)	8 (32.000)	12 (30.769)			
Presence of Neutropenia, n (%)				χ^2 = 3.386[2]	0.066	0.49
No	37 (57.812)	18 (72.000)	19 (48.718)			
Yes	27 (42.188)	7 (28.000)	20 (51.282)			
Leukemia Subtype, n (%)				-[3]	< 0.001	1.145
Acute Lymphoblastic Leukemia	38 (59.375)	8 (32.000)	30 (76.923)			
Acute Myeloid Leukemia	20 (31.250)	15 (60.000)	5 (12.821)			
Chronic Myeloid Leukemia	6 (9.375)	2 (8.000)	4 (10.256)			
Duration of Chemotherapy (months), Median (Q1, Q3)	3.000 (2.000, 4.000)	3.000 (2.000, 4.000)	3.000 (2.000, 4.000)	Z = -0.707[4]	0.479	0.197

Notes: 1. Variance-corrected independent samples t-test; 2. Pearson χ^2 test; 3. Fisher's exact test; 4. Mann-Whitney U test.

Supplementary Table 6. Doxycycline vs. Azithromycin: propensity score matching for patients treated on day 2 of fever, baseline characteristics after matching

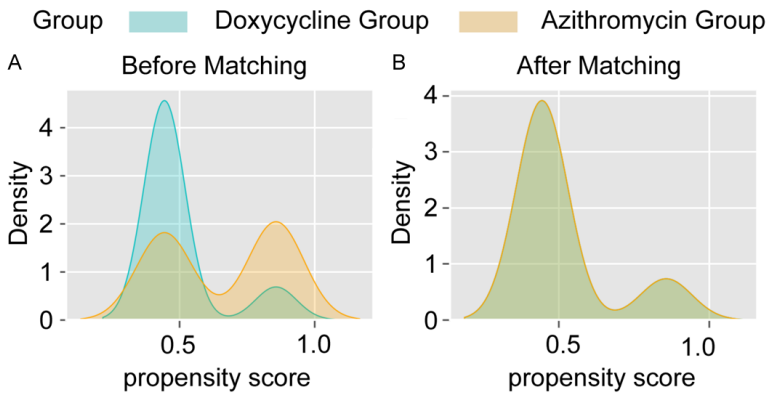
Variables	Total (*n* = 37)	Group		Statistic	*P-value*	SMD
		Doxycycline Group (*n* = 13)	Azithromycin Group (*n* = 24)			
Age, Mean ± SD	14.649 ± 1.767	14.000 ± 1.472	15.000 ± 1.842	t = -1.685[1]	0.101	0.617
Gender, n (%)				-[2]	0.476	0.318
Male	25 (67.568)	10 (76.923)	15 (62.500)			
Female	12 (32.432)	3 (23.077)	9 (37.500)			
Presence of Neutropenia, n (%)				-[2]	1	0.091
No	21 (56.757)	7 (53.846)	14 (58.333)			
Yes	16 (43.243)	6 (46.154)	10 (41.667)			
Leukemia Subtype, n (%)				-[2]	1	0.111
Acute Lymphoblastic Leukemia	24 (64.865)	8 (61.538)	16 (66.667)			
Acute Myeloid Leukemia	8 (21.622)	3 (23.077)	5 (20.833)			
Chronic Myeloid Leukemia	5 (13.514)	2 (15.385)	3 (12.500)			
Duration of Chemotherapy (months), Median (Q1, Q3)	3.000 (2.000, 4.000)	3.000 (2.000, 3.000)	3.000 (2.750, 5.000)	Z = -1.102[3]	0.27	0.401

Notes: 1. Independent samples t-test; 2. Fisher's exact test; 3. Continuity-corrected Mann-Whitney U test.

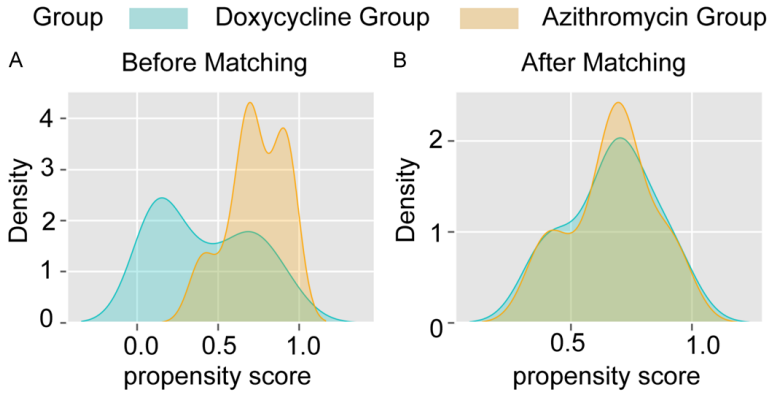
Supplementary Table 7. Doxycycline vs. Azithromycin: baseline characteristics for patients treated on day 3 of fever

Variables	Total (*n* = 15)	Group		Statistic	*P-value*	SMD
		Azithromycin Group (*n* = 5)	Doxycycline Group (*n* = 10)			
Age, Mean ± SD	15.067 ± 1.438	14.800 ± 1.924	15.200 ± 1.229	*t* = -0.494[1]	0.629	0.272
Gender, n (%)				-[2]	0.608	0.408
Male	7 (46.667)	3 (60.000)	4 (40.000)			
Female	8 (53.333)	2 (40.000)	6 (60.000)			
Presence of Neutropenia, n (%)				-[2]	0.6	0.447
No	10 (66.667)	4 (80.000)	6 (60.000)			
Yes	5 (33.333)	1 (20.000)	4 (40.000)			
Leukemia Subtype, n (%)				-[2]	0.336	0.894
Chronic Myeloid Leukemia	2 (13.333)	1 (20.000)	1 (10.000)			
Acute Myeloid Leukemia	6 (40.000)	3 (60.000)	3 (30.000)			
Acute Lymphoblastic Leukemia	7 (46.667)	1 (20.000)	6 (60.000)			
Duration of Chemotherapy (months), Median (Q1, Q3)	3.000 (2.000, 4.000)	2.000 (2.000, 3.000)	3.000 (2.250, 4.000)	*Z* = -0.630[3]	0.529	0.378

Notes: 1. Independent samples t-test; 2. Fisher's exact test; 3. Continuity-corrected Mann-Whitney U test.



Supplementary Figure 1. Doxycycline vs. Azithromycin: probability density curves before and after propensity score matching for patients treated within 1 day. A: Probability density curves before matching: The propensity score probability density curves of the two groups intersect, suggesting that propensity score matching is feasible. B: Probability density curves after matching: the greater the overlap of the probability density curves after matching, the better the matching quality.



Supplementary Figure 2. Doxycycline vs. Azithromycin: probability density curves before and after propensity score matching for patients treated on day 2 of fever. A: Probability density curves before matching: the propensity score probability density curves of the two groups intersect, suggesting that propensity score matching is feasible. B: Probability density curves after matching: the greater the overlap of the probability density curves after matching, the better the matching quality.

Supplementary Table 8. Doxycycline vs. other empirical treatments: baseline characteristics for patients treated within 1 day

Variables	Total (*n* = 70)	Group		Statistic	*P-value*	SMD
		Other Empirical Treatment (*n* = 36)	Doxycycline Group (*n* = 34)			
Age, Mean ± SD	14.800 ± 1.665	14.778 ± 1.675	14.824 ± 1.678	*t* = -0.114[1]	0.909	0.028
Gender, n (%)				χ^2 = 0.217[2]	0.642	0.111
Male	33 (47.143)	16 (44.444)	17 (50.000)			
Female	37 (52.857)	20 (55.556)	17 (50.000)			
Presence of Neutropenia, n (%)				χ^2 = 1.144[2]	0.285	0.258
No	25 (35.714)	15 (41.667)	10 (29.412)			
Yes	45 (64.286)	21 (58.333)	24 (70.588)			
Leukemia Subtype, n (%)				-[3]	0.647	0.249
Acute Myeloid Leukemia	34 (48.571)	16 (44.444)	18 (52.941)			
Chronic Myeloid Leukemia	7 (10.000)	3 (8.333)	4 (11.765)			
Acute Lymphoblastic Leukemia	29 (41.429)	17 (47.222)	12 (35.294)			
Duration of Chemotherapy (months), Median (Q1, Q3)	3.000 (2.000, 4.000)	3.000 (2.000, 4.000)	3.000 (2.000, 4.000)	*Z* = 0.972[4]	0.331	0.231

Notes: 1. Independent samples t-test; 2. Pearson χ^2 test; 3. Fisher's exact test; 4. Mann-Whitney U test.

Supplementary Table 9. Doxycycline vs. other empirical treatments: propensity score matching for patients treated on day 2 of fever, baseline characteristics before matching

Variables	Total (*n* = 51)	Group		Statistic	*P-value*	SMD
		Other Empirical Treatment (*n* = 26)	Doxycycline Group (*n* = 25)			
Age, Mean ± SD	14.314 ± 1.556	14.462 ± 1.749	14.160 ± 1.344	*t* = 0.688[1]	0.494	0.197
Gender, n (%)				χ^2 = 3.398[2]	0.065	0.535
Male	23 (45.098)	15 (57.692)	8 (32.000)			
Female	28 (54.902)	11 (42.308)	17 (68.000)			
Presence of Neutropenia, n (%)				χ^2 = 2.588[2]	0.108	0.463
No	20 (39.216)	13 (50.000)	7 (28.000)			
Yes	31 (60.784)	13 (50.000)	18 (72.000)			
Leukemia Subtype, n (%)				-[3]	0.021	0.815
Acute Lymphoblastic Leukemia	25 (49.020)	17 (65.385)	8 (32.000)			
Acute Myeloid Leukemia	21 (41.176)	6 (23.077)	15 (60.000)			
Chronic Myeloid Leukemia	5 (9.804)	3 (11.538)	2 (8.000)			
Duration of Chemotherapy (months), Median (Q1, Q3)	3.000 (1.000, 3.000)	2.000 (1.000, 3.000)	3.000 (2.000, 4.000)	*Z* = -1.528[4]	0.127	0.418

Notes: 1. Independent samples t-test; 2. Pearson χ^2 test; 3. Fisher's exact test; 4. Mann-Whitney U test.

Supplementary Table 10. Doxycycline vs. other empirical treatments: propensity score matching for patients treated on day 2 of fever, baseline characteristics after matching

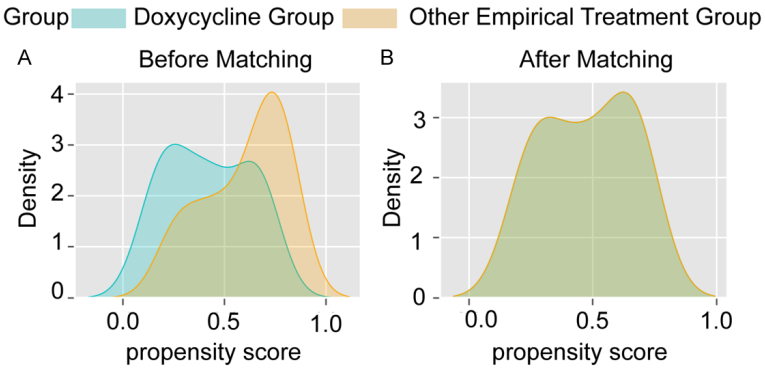
Variables	Total (*n* = 26)	Group		Statistic	*P-value*	SMD
		Other Empirical Treatment (*n* = 13)	Doxycycline Group (*n* = 13)			
Age, Mean ± SD	14.269 ± 1.614	13.846 ± 1.908	14.692 ± 1.182	*t* = -1.359[1]	0.187	0.555
Gender, n (%)				-[2]	0.428	0.48
Male	11 (42.308)	7 (53.846)	4 (30.769)			
Female	15 (57.692)	6 (46.154)	9 (69.231)			
Presence of Neutropenia, n (%)				-[2]	0.688	0.32
No	10 (38.462)	6 (46.154)	4 (30.769)			
Yes	16 (61.538)	7 (53.846)	9 (69.231)			
Leukemia Subtype, n (%)				-[2]	1	0
Acute Lymphoblastic Leukemia	12 (46.154)	6 (46.154)	6 (46.154)			
Acute Myeloid Leukemia	12 (46.154)	6 (46.154)	6 (46.154)			
Chronic Myeloid Leukemia	2 (7.692)	1 (7.692)	1 (7.692)			
Duration of Chemotherapy (months), Median (Q1, Q3)	3.000 (3.000, 3.000)	3.000 (3.000, 3.000)	3.000 (3.000, 3.000)	*Z* = 0.000[3]	1	0

Notes: 1. Independent samples t-test; 2. Fisher's exact test; 3. Continuity-corrected Mann-Whitney U test.

Supplementary Table 11. Doxycycline vs. other empirical treatments: baseline characteristics for patients treated on day 3 of fever

Variables	Total (*n* = 28)	Group		Statistic	*P-value*	SMD
		Other Empirical Treatment (*n* = 18)	Doxycycline Group (*n* = 10)			
Age, Mean ± SD	14.464 ± 1.551	14.056 ± 1.589	15.200 ± 1.229	*t* = -1.968[1]	0.06	0.836
Gender, n (%)				-[2]	1	0.09
Male	12 (42.857)	8 (44.444)	4 (40.000)			
Female	16 (57.143)	10 (55.556)	6 (60.000)			
Presence of Neutropenia, n (%)				-[2]	0.695	0.315
No	14 (50.000)	10 (55.556)	4 (40.000)			
Yes	14 (50.000)	8 (44.444)	6 (60.000)			
Leukemia Subtype, n (%)				-[2]	0.533	0.542
Chronic Myeloid Leukemia	5 (17.857)	4 (22.222)	1 (10.000)			
Acute Myeloid Leukemia	5 (17.857)	2 (11.111)	3 (30.000)			
Acute Lymphoblastic Leukemia	18 (64.286)	12 (66.667)	6 (60.000)			
Duration of Chemotherapy (months), Median (Q1, Q3)	2.500 (2.000, 4.000)	2.000 (2.000, 3.750)	3.000 (2.250, 4.000)	*Z* = -0.619[3]	0.536	0.218

Notes: 1. Independent samples t-test; 2. Fisher's exact test; 3. Continuity-corrected Mann-Whitney U test.



Supplementary Figure 3. Doxycycline vs. other empirical treatments: probability density curves before and after propensity score matching for patients treated on day 2 of fever. A: Probability density curves before matching: the propensity score probability density curves for the two groups intersect, indicating that propensity score matching is feasible. B: Probability density curves after matching: the greater the overlap of the probability density curves after matching, the better the matching quality.