Hematological toxicity of amphotericin B deoxycholate-based induction therapy in patients with HIV-associated talaromycosis

Yihong Zhou

1 Department of Infectious Diseases, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou, China; 2 Division of Infectious Diseases, Chongqing Public Health Medical Center, Chongqing, China

Tao LU

Department of Infectious Disease, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou Province, China

Yan Li

School of Public Health, Zunyi Medical University, Zunyi, PR China

Yuanyuan Qin

Division of Infectious Diseases, Chongqing Public Health Medical Center, Chongqing, China

Yanqiu Lu

Division of Infectious diseases, Chongqing Public Health Medical Center, Chongqing, China

Qun Tian

Department of Infectious Diseases, the Third People's Hospital of Guilin, Guangxi, China

Ke Lan

Department of Infectious Disease, Longtan Hospital of Guangxi Zhuang Autonomous Region, Guangxi, China

Guoqiang Zhou

Department of Infectious Diseases, the First Hospital of Changsha, Hunan, China

Yingmei Qin

Department of Infectious Disease, the Fourth People's Hospital of Nanning, Guangxi, China

Shunmei Li

Department of Cardiology, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou Province

Vijay Harypursat

Division of Infectious Disease, Chongqing Public Health Medical Center, Chongqing, China

shide lin (✉ 18300923071@163.com)

Department of Infectious Disease, Affiliated Hospital of Zunyi Medical University, Zunyi 5563003, Guizhou province, China

Yaokai Chen

Research Article

Keywords: HIV, talaromycosis, *Talaromyces marneffe*, anemia, leukopenia, thrombocytopenia, risk factors

Posted Date: October 3rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1269438/v2>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Abstract

Background: This study's objective was to investigate the predictors for severe anemia, severe leukopenia, and severe thrombocytopenia when amphotericin B deoxycholate-based induction therapy is used in HIV patients with talaromycosis.

Methods: A total of 170 HIV patients with talaromycosis were enrolled from January 1st, 2019 to September 30th, 2020.

Results: Approximately 42.9%, 20.6%, and 10.6% of the enrolled patients developed severe anemia, severe leukopenia and severe thrombocytopenia, respectively. Baseline lower hemoglobin levels, higher serum creatinine levels, higher AST/ALT ratios, lower sodium levels, and higher administered doses of amphotericin B were observed to be independent risk factors associated with the development of severe anemia. Co-infection with tuberculosis and lower platelet levels at baseline were shown to be independent risk factors associated with the development of severe leukopenia. A lower platelet level at baseline was the independent risk factor found to be associated with the development of severe thrombocytopenia.

Conclusion: The preceding findings reveal risk factors for severe anemia, severe leukopenia, and severe thrombocytopenia, which will favor prevention and timely treatment of hematological toxicity, improvement of patient's outcomes, shorter hospital stays, and a reduction of the requirement for blood transfusion.

Introduction

Talaromyces marneffei is a dimorphic fungus, which can cause life-threatening infection (talaromycosis) in people living with HIV/AIDS (PLWHA) in endemic areas, such as South-East Asia, Southern China, and India(1). In China, the prevalence of talaromycosis in PLWHA is approximately

3.3%, while the prevalence in South China is approximately 15.0%(2). Even in affected patients having appropriate anti-fungal treatment, the mortality rate for talaromycosis remains up to 30% (3). Talaromycosis is an independent predictor of death in PLWHA in hospital, especially in older patients (4).

Anemia is commonly present in HIV patients with talaromycosis, and its prevalence is between between 80–95.6%(5, 6). This could lead to negative outcomes, such as prolonged hospitalization, a requirement for close monitoring, and eventually the requirement for blood or platelet transfusion (7). Anemia is also associated with poor prognosis and reduced survival(8–10). The prevalence of leukopenia in HIV patients with talaromycosis approaches 40%(5). The occurrence of leukopenia in these patients may compromise the host immune system, and thus increase their susceptibility to opportunistic infections(8), which could affect progression of HIV-associated talaromycosis. Thrombocytopenia is an independent risk factor for poor prognosis in HIV patients comorbid with talaromycosis and the prevalence of thrombocytopenia approaches 50%(5). Platelets have also been shown to have antimicrobial activity against bacteria, viruses and fungus(11, 12). Therefore, leukopenia and thrombocytopenia further impair immune function

in patients with HIV-associated talaromycosis. However, the risk factors which predict the development of severe anemia, severe leukopenia and severe thrombocytopenia in HIV patients with talaromycosis are currently unknown, especially in patients receiving amphotericin B.

Current guidelines recommend amphotericin B deoxycholate as the preferred induction therapy. In Vietnam, an open-label, non-inferiority trial observed that amphotericin B deoxycholate as initial treatment was superior to itraconazole(3); however, the toxic effects of amphotericin B deoxycholate, such as anemia, leukopenia, and thrombocytopenia, cannot be ignored(13, 14). Up to 40% of HIV patients with talaromycosis, whether with anemia or not at baseline, develop severe anemia after they have initiated amphotericin B deoxycholate (3). The incidence of patients who started amphotericin B deoxycholate and who subsequently developed severe leukopenia and severe thrombocytopenia are approximately 10% and 20%, respectively(3). However, the risk factors which predict severe anemia, severe leukopenia and severe thrombocytopenia in HIV patients comorbid with talaromycosis being treated with amphotericin B deoxycholate are unknown. We, therefore, conducted a multi-center prospective observational study to assess risk factors for the occurrence of severe anemia, leukopenia, and thrombocytopenia in HIV patients being treated with amphotericin B deoxycholate for talaromycosis.

Material And Methods

Study Design and Setting

This was a prospective, multi-center, observational study of HIV patients with talaromycosis admitted to hospital from January 1st, 2019 to September 30th, 2020. Patients at 11 hospitals located in 9 cities were enrolled, viz., Chongqing Public Health Medical Center, Guangzhou Eighth People's Hospital, Guangxi Longtan Hospital of Guangxi Zhuang Autonomous Region, Liuzhou General Hospital, the Third People's Hospital of Guilin, the First Hospital of Changsha, the Fourth People's Hospital of Nanning, Kunming Third People's Hospital, Guiyang Public Health Clinical Center, Beijing Youan Hospital of Capital Medical University, and Yunnan Provincial Infectious Disease Hospital. Eligible patients were confirmed HIV-infected patients 18 years of age or older and had talaromycosis confirmed by either microscopy or culture. Exclusion criteria included The patients who had hematologic diseases causing anemia (including aplastic anemia, hemolytic anemia, bleeding, and so on), severe active other infection, bacterial or other microbia infections and tumors, a hemoglobin level of less than 80g/L, a leukocyte count of less than $1.0 \times 10^9/L$, a neutrophil count of less than $0.5 \times 10^9/L$, a platelet count of less than $30 \times 10^9/L$, a blood amylase level of more than 2 times the reference level upper limit, a serum creatinine level of more than 1.5 times the reference level upper limit, an aspartate aminotransferase level (AST), alanine aminotransferase level (ALT), or alkaline phosphatase level of more than 5 times the reference level upper limit, a total bilirubin level of more than 2 times the reference level upper limit, and a serum creatine phosphokinase (CK) level of more than 2 times the reference level upper limit. Patients received treatment for 14 days with amphotericin B deoxycholate at a dose of 0.5 to 0.7 mg per kilogram per day.

Written informed consent was obtained from all patients or their representatives. The independent ethics committees of each participating hospital approved the trial protocol.

Assessments

We explored demographic factors, symptoms and signs, diagnosis of opportunistic infections, laboratory test results at admission, and types of highly active antiretroviral therapy (HAART) before diagnosis of talaromycosis. Each individual was invited to participate in 2-weeks of follow-up. At the follow-up visits at weeks 1, 2, hemoglobin, platelets, and leukocytes were evaluated.

Outcomes and definitions

Patients were evaluated for hematological toxicity, including anemia, leukopenia, and thrombocytopenia at weeks 1 and 2 after the initiation of amphotericin B deoxycholate. The primary outcome was the occurrence of severe hematological toxicity due to any cause, defined as the appearance of at least one of three laboratory abnormalities during amphotericin B deoxycholate treatment(14). Severe anemia was defined as hemoglobin level drop to 80 g/L. Severe leukopenia was defined as leukocyte count drop to 2×10^9 /L or, if baseline count was under 2×10^9 /L, a decrease of 25% in leukocyte count. Severe thrombocytopenia was defined as platelet count decrease to 50×10^9 /L, or, if the baseline count was under 50×10^9 /L, a decrease of 25% in platelet count.

Weight loss was defined as loss of more than 10% of body weight within 6 months. All patients were tested for tuberculosis, cytomegalovirus infection, syphilis, hepatitis B, and hepatitis C. These diagnoses were confirmed according to laboratory testing results. A large fraction of the diagnoses for pneumocystis pneumonia, oral candidiasis, and toxoplasma encephalopathy were presumptive, due to easier establishment of a clinical diagnosis and the limited availability of definitive pathogenic testing for these specific diseases.

Statistical Analysis

Statistical Package for the Social Sciences software, Version 25.0 (IBM-SPSS Statistics, Armonk, New York, USA) was used to analyze all study data. Standard descriptive statistics analyzed the clinical characteristics, diagnosis, and laboratory variables of patients. Continuous variables and categorical variables were compared using the Mann-Whitney U test and Chi-squared tests, respectively. In identifying independent factors associated with severe anemia, severe leukopenia, or severe thrombocytopenia, variables were initially analyzed using a bivariate model, and subsequently independent risk factors were identified by means of a logistic regression model using a forward, stepwise approach, which began with inclusion of all variables associated with severe anemia, severe leukopenia, or severe thrombocytopenia on bivariate analysis ($p \leq 0.1$), and subsequently included only those variables with $p \leq 0.05$ in the final model. We used the variance inflation factor (VIF) to make the Multicollinearity diagnosis. If the VIF is higher than 5, the multicollinearity is present.

Results

Of the 255 patients who were screened, 170 patients were included after exclusion of 66 cases with a hemoglobin level of less than 80g/L, and 19 cases with partial data missing. 73 of 170 patients (42.9%) developed severe anemia, 35 of 170 patients (20.6%) developed severe leukopenia, and 18 of 170 patients (10.6%) developed severe thrombocytopenia after starting amphotericin B deoxycholate.

Table 1 shows baseline characteristics for patients. Compared with patients without severe anemia, more patients developing severe anemia had a lower BMI, weight loss, a lower hemoglobin level, a lower platelet level, a higher serum creatinine level, a higher AST/ALT ratio, a lower sodium level, and an induction therapeutic dose of amphotericin B deoxycholate of > 0.58 mg/kg/d (Table 1 and Table 2). Compared with patients who did not develop severe thrombocytopenia, more patients developing severe thrombocytopenia had lower platelet levels (Table 3).

Table 1
Clinical characteristics of the patients developing severe anemia or not at baseline

	Severe anemia (N = 73)	Without severe anemia (N = 97)	p- value
Socio-demographic			
Female sex, n (%)	14 (19.2%)	10 (10.3%)	0.100
Age, median (IQR), years	43 (35–53)	42 (32–50)	0.173
Drink, n (%)	12 (16.4%)	17 (17.5%)	0.852
Smoke, n (%)	21 (28.8%)	27 (27.8%)	0.894
BMI, median (IQR), kg/m ²	31 (29–35)	33 (30–36)	0.022
Symptoms and signs			
Fever, n (%)	59 (80.8%)	86 (88.7%)	0.153
Cough, n (%)	44 (60.3%)	52 (53.6%)	0.386
Sputum, n (%)	37 (50.7%)	38 (39.2%)	0.135
Hemoptysis, n (%)	2 (2.7%)	0 (0.0%)	0.183
Abdominal pain, n (%)	15 (20.5%)	15 (15.5%)	0.389
Diarrhea, n (%)	2 (2.7%)	8 (8.2%)	0.237
Headache, n (%)	3 (4.1%)	5 (5.2%)	1.000
Skin lesions, n (%)	21 (28.8%)	27 (27.8%)	0.894
Weight loss, n (%)	34 (46.6%)	24 (24.7%)	0.003
Diagnosis			
Oral candidiasis, n (%)	29 (39.7%)	43 (44.3%)	0.548
Cytomegalovirus infection, n (%)	8 (11.0%)	9 (9.3%)	0.718
Tuberculosis, n (%)	8 (11.0%)	7 (7.2%)	0.394
Hepatitis B, n (%)	7 (9.6%)	4 (4.1%)	0.263
Pneumocystis pneumonia, n (%)	5 (6.8%)	8 (8.2%)	0.734
Syphilis, n (%)	3 (4.1%)	1 (1.0%)	0.424
BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine.			

	Severe anemia (N = 73)	Without severe anemia (N = 97)	p- value
Hepatitis C, n (%)	0	1 (1.0%)	1.000
Toxoplasma encephalopathy, n (%)	1 (1.3%)	0	0.429
Laboratory results			
Leukocyte, median (IQR), $\times 10^9$ /L	4.04 (2.78–5.02)	3.94 (2.67–5.63)	0.611
Hemoglobin, median (IQR), g/L	89 (84–102)	107 (97–116)	< 0.001
Platelet, median (IQR), $\times 10^9$ /L	111 (61–211)	151 (85–218)	0.041
CD4 + T-cell counts, median (IQR), cells/ μ L	15 (7–39)	12 (6–35)	0.510
Serum creatinine, median (IQR), μ mol/L	68.90 (55.85–84.15)	61.00 (52.45-73.00)	0.018
Total bilirubin level, median (IQR), 10.5mg/dl	10.57 (6.94–20.69)	8.9 (6.9–12.4)	0.144
AST/ALT ratio, median (IQR)	2.03 (1.47–4.03)	1.53 (1.16–2.34)	< 0.001
Sodium, median (IQR), mmol/liter	133.1 0(128.50– 135.00)	133.7 (131.45– 138.40)	0.015
Potassium, median (IQR), mmol/liter	3.56 (3.27–3.88)	3.66 (3.39–4.08)	0.095
HAART before diagnosis			
Antiretroviral therapy, n (%)	5 (6.8%)	14 (14.4%)	0.120
3TC	5 (6.8%)	13 (13.4%)	0.169
TDF	4 (5.5%)	10 (10.3%)	0.257
EFV	5 (6.8%)	9 (9.3%)	0.568
NVP	0	2 (2.1%)	0.507
AZT	1 (1.4%)	1 (1.0%)	1.000
ABC	0 (0.0%)	1 (1.0%)	1.000
Lpv/r	0 (0.0%)	1 (1.0%)	1.000

BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine.

	Severe anemia (N = 73)	Without severe anemia (N = 97)	<i>p</i> -value
DTG	0	1 (1.0%)	1.000
Elvitegravir	0	1 (1.0%)	1.000
Amphotericin B			
Induction therapy > 0.58 mg/kg/d	24 (32.9%)	19 (19.6%)	0.048
BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine.			

Table 2
Clinical characteristics of the patients developing severe leukopenia or not at baseline

	Severe leukopenia (N = 35)	Without severe leukopenia (N = 135)	p-value
Socio-demographic			
Female sex, n (%)	6 (17.1%)	18 (13.3%)	0.761
Age level, median (IQR), year	44 (35–55)	32 (30–36)	0.402
Drink, n (%)	8 (22.9%)	21 (15.6%)	0.306
Smoke, n (%)	11 (31.4%)	37 (27.4%)	0.638
BMI level, median (IQR), kg/m ²	32 (30–34)	32 (30–36)	0.509
Symptoms and signs			
Fever, n (%)	30 (85.7%)	115 (85.2%)	0.937
Cough, n (%)	19 (54.3%)	77 (57.0%)	0.770
Sputum, n (%)	15 (42.9%)	60 (44.4%)	0.866
Hemoptysis, n (%)	1 (2.9%)	1 (0.7%)	0.370
Abdominal pain, n (%)	5 (14.3%)	25 (18.5%)	0.558
Diarrhea, n (%)	2 (5.7%)	8 (5.9%)	1.000
Headache, n (%)	2 (5.7%)	6 (4.4%)	1.000
Skin lesions, n (%)	8 (22.9%)	40 (29.6%)	0.428
Weight loss, n (%)	12 (34.3%)	46 (34.1%)	0.981
Diagnosis			
Oral candidiasis, n (%)	15 (42.9%)	57 (42.2%)	1.000
Cytomegalovirus infection, n (%)	3 (8.6%)	14 (10.4%)	1.000
Tuberculosis, n (%)	6 (17.1%)	9 (6.7%)	0.107
Hepatitis B, n (%)	4 (11.4%)	7 (5.2%)	0.341
Pneumocystis pneumonia, n (%)	3 (8.6%)	10 (7.4%)	1.000
Syphilis, n (%)	1 (2.9%)	3 (2.2%)	1.000
BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine,			

	Severe leukopenia (N = 35)	Without severe leukopenia (N = 135)	p-value
Hepatitis C, n (%)	0	1 (0.7%)	1.000
Toxoplasma encephalopathy, n (%)	0	1 (0.7%)	1.000
Laboratory results			
Leukocyte level, median (IQR), ×10 ⁹ /L	4.0 (2.77–4.67)	3.94 (2.60–5.60)	0.828
Hemoglobin level, median (IQR), g/L	96 (86–111)	101 (88–112)	0.275
Platelet level, median (IQR), ×10 ⁹ /L	110 (64–172)	143 (74–229)	0.068
CD4 + T-cell counts, median (IQR), cells/μL	9 (6–34)	15 (7–38)	0.233
Serum creatinine level, median (IQR), μmol/L	67.7 (52.8–77.6)	64 (53.9–77.0)	0.603
Total bilirubin level, median (IQR), mg/dl	8.9 (7.30–13.60)	9.38 (6.90-15.92)	0.673
AST/ALT ratio, median (IQR)	1.84 (1.29–2.88)	1.81 (1.27–2.65)	0.768
Sodium level, median (IQR), mmol/liter	133.7 (130.0-136.4)	133.4 (130.0-137.0)	0.804
Potassium level, median (IQR), mmol/liter	3.64 (3.3-4.0)	3.56 (3.27–3.86)	0.277
HAART before diagnosis			
Antiretroviral therapy, n (%)	3 (8.6%)	16 (11.9%)	0.804
3TC	2 (5.7%)	12 (8.9%)	0.737
TDF	1 (2.9%)	13 (9.6%)	0.340
EFV	2 (5.7%)	12 (8.9%)	0.792
NVP	0	2 (1.5%)	1.000
AZT	0	2 (1.5%)	1.000
ABC	1(2.9%)	0	0.206
Lpv/r	1 (2.9%)	0	0.206
DTG	0	1 (0.7%)	1.000

BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine,

	Severe leukopenia (N = 35)	Without severe leukopenia (N = 135)	<i>p</i> -value
Elvitegravir	0	1 (0.7%)	1.000
Amphotericin B			
Induction therapy > 0.58 mg/kg/d, n (%)	11 (31.4%)	32 (23.7%)	0.349
BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine,			

Table 3
Clinical characteristics of the patients developing severe thrombocytopenia or not at baseline

	Severe thrombocytopenia (N = 18)	Without severe thrombocytopenia (N = 152)	p- value
Socio-demographic			
Female sex, n (%)	5 (27.8%)	19 (12.5%)	0.161
Age level, median (IQR), year	49 (34–52)	43 (34–52)	0.512
Drink, n (%)	2 (11.1%)	27 (17.8%)	0.705
Smoke, n (%)	4 (22.2%)	44 (28.9%)	0.549
BMI level, median (IQR), kg/m ²	32 (29–37)	32 (30–35)	0.789
Symptoms and signs			
Fever, n (%)	17 (94.4%)	128 (84.2%)	0.419
Cough, n (%)	7 (38.9%)	89 (58.6%)	0.112
Sputum, n (%)	6 (33.3%)	69 (45.4%)	0.330
Hemoptysis, n (%)	0	2 (1.3%)	1.000
Abdominal pain, n (%)	4 (22.2%)	26 (17.1%)	0.832
Diarrhea, n (%)	0	10 (6.6%)	0.554
Headache, n (%)	0	8 (5.3%)	1.000
Skin lesions, n (%)	4 (22.2%)	44 (28.9%)	0.549
Weight loss, n (%)	7 (38.9%)	51 (33.6%)	0.652
Diagnosis			
Oral candidiasis, n (%)	5 (27.8%)	67 (44.1%)	0.186
Cytomegalovirus infection, n (%)	1 (5.6%)	16 (10.5%)	0.803
Tuberculosis, n (%)	1 (5.6%)	14 (9.2%)	0.938
Hepatitis B, n (%)	1 (5.6%)	10 (6.6%)	1.000
Pneumocystis pneumonia, n (%)	1 (5.6%)	12 (7.9%)	1.000
Syphilis, n (%)	0	4 (2.6%)	1.000
BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine,			

	Severe thrombocytopenia (N = 18)	Without severe thrombocytopenia (N = 152)	p- value
Hepatitis C, n (%)	1 (5.6%)	0	0.106
Toxoplasma encephalopathy, n (%)	1 (5.6%)	0	0.106
Laboratory results			
Leukocyte level, median (IQR), $\times 10^9$ /L	3.96 (2.67–4.96)	3.96 (2.71–5.53)	0.518
Hemoglobin level, median (IQR), g/L	92 (85–106)	101 (89–112)	0.199
Platelet level, median (IQR), $\times 10^9$ /L	75 (52–139)	144 (78–217)	0.007
CD4 + T-cell counts, median (IQR), cells/ μ L	13 (4–23)	15 (7–39)	0.205
Serum creatinine level, median (IQR), μ mol/L	65.95 (48.80-78.85)	64.00 (53.93-77.00)	0.871
Total bilirubin level, median (IQR), mg/dl	9.60 (8.38–12.25)	9.28 (6.82–15.98)	0.514
AST/ALT ratio, median (IQR)	1.9 (1.3–4.8)	1.8 (1.3–2.6)	0.254
Sodium level, median (IQR), mmol/liter	134.25 (129.75-136.48)	133.4 (130-137.15)	0.895
Potassium level, median (IQR), mmol/liter	3.58 (3.12–3.88)	3.63 (3.30-4.00)	0.393
HAART before diagnosis			
Antiretroviral therapy, n (%)	3 (16.7%)	16 (10.5%)	0.699
3TC	3 (16.7%)	15 (9.9%)	0.630
TDF	3 (16.7%)	11 (7.2%)	0.356
EFV	1 (5.6%)	13 (8.6%)	1.000
NVP	1 (5.6%)	1 (0.7%)	0.201
AZT	0	2 (1.3%)	1.000
ABC	0	1 (0.7%)	1.000
Lpv/r	0	1 (0.7%)	1.000

BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine,

	Severe thrombocytopenia (N = 18)	Without severe thrombocytopenia (N = 152)	<i>p</i> - value
DTG	1 (5.6%)	0	0.106
Amphotericin B			
Induction therapy > 0.58 mg/kg/d, n (%)	7 (38.9%)	36 (23.7%)	0.264
BMI: Body Mass Index. BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAART: highly active antiretroviral therapy, 3TC: lamivudine, TDF: tenofovir, EFV: efavirenz, NVP: nevirapine, AZT: zidovudine, ABC: abacavir, Lpv/r: lopinavir and ritonavir, DTG: dolutegravir, FTC: emtricitabine,			

Multivariate logistic regression analysis revealed that lower hemoglobin levels (OR = 0.938, 95% CI: 0.913 ~ 0.965), higher serum creatinine levels (OR = 1.023, 95% CI: 1.003 ~ 1.044), higher AST/ALT ratios (OR = 1.543, 95% CI: 1.170 ~ 2.036), lower sodium levels (OR = 0.922, 95% CI: 0.855 ~ 0.995), and a dose of amphotericin B deoxycholate > 0.58 mg/kg/d (OR = 2.504, 95% CI: 1.066 ~ 5.882) were independent risk factors associated with the development of severe anemia (Table 4).

Table 4

Univariate and multivariate analysis of factors associated with severe anemia, severe leukopenia and severe thrombocytopenia for the outcome of HIV patients complicated with talaromyces marneffeii infection.

Variables	Univariate analysis				Multivariate analysis			
	β	OR	95% CI	<i>P</i>	β	OR	95% CI	<i>P</i>
Severe anemia								
BMI level	-0.086	0.917	0.851 ~ 0.989	0.024				
Weight loss	0.975	2.652	1.383 ~ 5.086	0.003				
Hemoglobin level	-0.068	0.934	0.910 ~ 0.959	< 0.001	-0.064	0.938	0.913 ~ 0.965	< 0.001
Platelet level	-0.003	0.997	0.994 ~ 1.000	0.083				
Serum creatinine level	0.023	1.023	1.006 ~ 1.040	0.008	0.023	1.023	1.003 ~ 1.044	0.027
AST/ALT ratio	0.463	1.588	1.241 ~ 2.032	< 0.001	0.434	1.543	1.170 ~ 2.036	0.002
Sodium level	-0.091	0.913	0.857 ~ 0.972	0.005	0.081	0.922	0.855 ~ 0.995	0.037
Potassium level	-0.498	0.608	0.350 ~ 1.054	0.076				
Amphotericin B > 0.58 mg/kg/d	0.699	2.011	0.999 ~ 4.049	0.050	0.918	2.504	1.066 ~ 5.882	0.035
Severe leukopenia								
Tuberculosis	1.064	2.897	0.955 ~ 8.781	0.060	1.198	3.313	1.052 ~ 10.439	0.041
Platelet level	-0.005	0.995	0.991 ~ 1.000	0.032	-0.005	0.995	0.990 ~ 0.999	0.024

BMI: Body Mass Index. AST: aspartate aminotransferase, ALT: alanine aminotransferase.

Variables	Univariate analysis				Multivariate analysis			
	β	OR	95% CI	<i>P</i>	β	OR	95% CI	<i>P</i>
Severe thrombocytopenia								
Male	-0.990	0.371	0.119 ~ 1.159	0.088				
Platelet level	-0.009	0.991	0.984 ~ 0.998	0.017	-0.009	0.991	0.984 ~ 0.998	0.017
BMI: Body Mass Index. AST: aspartate aminotransferase, ALT: alanine aminotransferase.								

Patients co-infected with tuberculosis and those who had a lower platelet level were shown to be at risk of progression to severe leukopenia ($p \leq 0.1$). Co-infection with tuberculosis (OR = 3.313, 95% CI: 1.052 ~ 10.439), and lower platelet levels (OR = 0.995, 95% CI: 0.990 ~ 0.999) were calculated to be independent risk factors associated with the development of severe leukopenia (Table 4).

Male gender and lower platelet level were the risk factors associated with progressing to severe thrombocytopenia ($p \leq 0.1$). Lower platelet level (OR = 0.991, 95% CI: 0.984 ~ 0.998) was identified as the independent risk factor associated with the development of severe thrombocytopenia (Table 4).

Discussion

Our results observed that the incidence of patients who developed severe anemia, severe leukopenia, and severe thrombocytopenia were 42.9%, 20.6%, and 10.6%, respectively, which approximates the observations of one previous prospective study(3).

A higher AST/ALT level, lower hemoglobin level, higher serum creatinine level, lower sodium level, and a higher administered dose of amphotericin B were found to be independent risk factors for development of severe anemia. AST and ALT are mainly produced in liver cells, and are also the main circulating hepatic enzymes in serum. Blood AST and ALT levels could increase as a consequence of hepatocellular damage or hepatic cellular death(3). Patients with talaromycosis can present with systemic infection, including that of the liver. Thus, hepatic transaminases are often elevated in patients with talaromycosis(15). One study even observed that a higher AST/ALT ratio increased the risk of death in HIV-infected patients with talaromycosis(1). However, to our knowledge, no study has investigated the connection between anemia and AST/ALT ratio in HIV-infected patients with talaromycosis. This phenomenon of higher values of AST/ALT associated with a higher prospective risk of severe anemia might be associated with abnormalities in the composition of red blood cell membranes or the limitation of effective bone marrow erythropoiesis, which are known causes of anemia associated with liver disease. Higher serum creatinine levels and lower sodium level are known to be associated with impaired renal function(16). The preceding

study observed that HIV infection and impaired renal function can result in a higher risk of anemia, which concurs with results of our study, as both HIV infection and impaired renal function have a synergistic impact on lowering hemoglobin levels(17). The adverse effects of Amphotericin B occurs in a dose-dependent manner (13). This means that higher doses of amphotericin B are associated with a higher risk of adverse effects, which is precisely what we observed in our study. It is, thus, important to establish a balance between maximizing antifungal efficacy and minimizing drug-related toxicity (18).

Patients co-infected with tuberculosis are associated with severe leukopenia. The possible reason for this is that anti-tuberculosis drugs may promote antibody generation and form antigen-antibody complexes which may be absorbed on to leukocytes, and cause leukocyte lysis and damage (19, 20).

Lower platelet levels at baseline are also associated with severe leukopenia. At present, the reason of baseline lower platelet levels associated with a higher risk of severe leukopenia is unclear. It may be related to the function of platelets and leukocytes, owing to both of them participate in the defense against fungal pathogens. As the disease of talaromycosis progresses, when platelets loss happen, leukopenia also might happen. In terms of platelets, antimicrobial effector molecules and mechanisms in platelets enable a direct inhibitory effect on fungus, which might be a decrease of fungal load(21). However, since activated platelets are cleared from the blood stream, platelet loss might happen (21, 22). Leukocytes are a heterogeneous group of nucleated cells and neutrophils are the most abundant. In term of prevention and clearance of invasive fungal infections, neutrophils also play an important role (22–24).

Lower platelets levels at baseline were found to be associated with the development of severe thrombocytopenia. Talaromycosis itself, together with the toxic effects of amphotericin B deoxycholate, may both promote the development of severe thrombocytopenia. The progression of severe thrombocytopenia is associated with an increased risk of hemorrhage. Discontinuation of the causative drug should sometimes be considered, if necessary. High doses of intravenous immunoglobulin may also be used for treatment of patients with severe thrombocytopenia who are actively bleeding (25).

One of the strengths of our study is that this is a prospective study, so missing data is minimal. Study limitations include missing serum drug concentration determinations, an exclusively Chinese study cohort, and some seriously ill patients having to be excluded from the study, which limits our study's overall generalizability.

Conclusions

In conclusion, our study observed that in HIV-positive patients with talaromycosis being treated with amphotericin B deoxycholate, hemoglobin levels and leukocyte levels tend to have a downward trend, and platelet levels also have a falling trend at baseline, week 1, and week 2 after initiation of amphotericin B deoxycholate. Patients who develop severe anemia after starting amphotericin B deoxycholate are associated with HIV patients co-infected with hepatitis B, a lower hemoglobin level at baseline, a higher serum creatinine level at baseline, a higher AST/ALT level at baseline, and a lower sodium level at

baseline. Patients who develop severe leukopenia are associated with patients co-infected with tuberculosis, and lower platelet levels at baseline. Patients who develop severe thrombocytopenia are associated with lower platelet levels at baseline. These findings will assist in the prediction of possible progression of hematological toxicity, and will favor the prevention and timely treatment of hematological toxicity, improvement of patient outcomes, cause shorter hospital stays, and a may result in a reduction in the requirement for blood or platelet transfusion in HIV positive patients being treated with amphotericin B deoxycholate for talaromycosis.

Declarations

Conflicts of interest

The authors declare that they have no conflict of interest.

Funding

Funding information: The trial was funded by: the National Science and Technology Major Project of China during the 13th Five-year plan period (No. 2018ZX10302104), the medical scientific research project of Chongqing Health Commission (No.2022WSJK037), the Joint Medical Research Projects of Chongqing Municipal Health Committee and Chongqing Municipal Science and Technology Bureau (No.2020MSXM097, No.2022QNXM032, No.2020FYXX066, No.2020FYXX161, No.2018MSXM013), the Chinese National Natural Science Foundation Project (No.81860114) The funding bodies have had no role in the trial design, trial execution, collection and interpretation of data, manuscript writing and editing, or decision to submit for publication.

Acknowledgments

We express our sincere thanks and gratitude to all medical personnel, including clinical fellows, nurses, and cleaners of 11 hospitals from around China, viz., Chongqing Public Health Medical Center, Guangzhou Eighth People's Hospital, Guangxi Longtan Hospital of Guangxi Zhuang Autonomous Region, Liuzhou General Hospital, the Third People's Hospital of Guilin, the First Hospital of Changsha, the Fourth People's Hospital of Nanning, Kunming Third People's Hospital, Guiyang Public Health Clinical Center, Beijing Youan Hospital of Capital Medical University, Yunnan Provincial Infectious Disease Hospital, and Affiliated Hospital of Zunyi Medical University for their valuable support and contributions during study period. We thank all study participants and everyone who contributed to the recruitment and follow-up of participants.

Author contributions

Y. Chen, S. Lin, and Y. Zhou designed and implemented the study. T. Lu and S. Li were involved in data collection. Y. Li did the statistical analyses. Q. Tian, K. Lan, G. Zhou, and Y. Qin organized investigational procedures at the different study sites. Y. Chen, S. Lin, and Y. Zhou interpreted the data and wrote the manuscript. Y. Qin and Y. Lu were responsible for data management and quality control of baseline

investigation at the different study sites. V. Harypursat revised, copy-edited, and proofread the manuscript. All contributing authors read and approved the final version of the manuscript.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for Publication

All of the nine authors agree to submit the manuscript for possible publication in "*Mycopathologia*".

References

1. Qin Y, Zhou Y, Lu Y, Chen H, Jiang Z, He K, et al. Multicentre derivation and validation of a prognostic scoring system for mortality assessment in HIV-infected patients with talaromycosis. *Mycoses*. 2021;64(2):203–11.
2. Qin Y, Huang X, Chen H, Liu X, Li Y, Hou J, et al. Burden of *Talaromyces marneffei* infection in people living with HIV/AIDS in Asia during ART era: a systematic review and meta-analysis. *BMC Infect Dis*. 2020;20(1):551.
3. Le T, Kinh NV, Cuc NTK, Tung NLN, Lam NT, Thuy PTT, et al. A Trial of Itraconazole or Amphotericin B for HIV-Associated Talaromycosis. *N Engl J Med*. 2017;376(24):2329–40.
4. Zhou Y, Yang Z, Liu M, Lu Y, Qin Y, He X, et al. Independent Risk Factors for Deaths due to AIDS in Chongqing, China: Does Age Matter? *Front Med (Lausanne)*. 2020;7:586390.
5. Ying RS, Le T, Cai WP, Li YR, Luo CB, Cao Y, et al. Clinical epidemiology and outcome of HIV-associated talaromycosis in Guangdong, China, during 2011–2017. *HIV Med*. 2020;21(11):729–38.
6. Ranjana KH, Priyokumar K, Singh TJ, Gupta Ch C, Sharmila L, Singh PN, et al. Disseminated *Penicillium marneffei* infection among HIV-infected patients in Manipur state, India. *J Infect*. 2002;45(4):268–71.
7. Brandriss MW, Wolff SM, Moores R, Stohlman F. Jr. ANEMIA INDUCED BY AMPHOTERICIN B. *JAMA*. 1964;189:663–6.
8. Belperio PS, Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature. *Am J Med*. 2004;116(Suppl 7A):27s–43s.

9. Albrecht S, Franzeck FC, Mapesi H, Hatz C, Kalinjuma AV, Glass TR, et al. Age-related comorbidities and mortality in people living with HIV in rural Tanzania. *Aids*. 2019;33(6):1031–41.
10. Haider BA, Spiegelman D, Hertzmark E, Sando D, Duggan C, Makubi A, et al. Anemia, Iron Deficiency, and Iron Supplementation in Relation to Mortality among HIV-Infected Patients Receiving Highly Active Antiretroviral Therapy in Tanzania. *Am J Trop Med Hyg*. 2019;100(6):1512–20.
11. Yeaman MR. The role of platelets in antimicrobial host defense. *Clin Infect Dis*. 1997;25(5):951–68. quiz 69–70.
12. Madzime M, Rossouw TM, Theron AJ, Anderson R, Steel HC. Interactions of HIV and Antiretroviral Therapy With Neutrophils and Platelets. *Front Immunol*. 2021;12:634386.
13. Lemke A, Kiderlen AF, Kayser O. Amphotericin B. *Appl Microbiol Biotechnol*. 2005;68(2):151–62.
14. Falci DR, da Rosa FB, Pasqualotto AC. Hematological toxicities associated with amphotericin B formulations. *Leuk Lymphoma*. 2015;56(10):2889–94.
15. Le T, Wolbers M, Chi NH, Quang VM, Chinh NT, Lan NP, et al. Epidemiology, seasonality, and predictors of outcome of AIDS-associated *Penicillium marneffei* infection in Ho Chi Minh City, Viet Nam. *Clin Infect Dis*. 2011;52(7):945–52.
16. Kannapiran M, Nisha D, Madhusudhana Rao A. Underestimation of impaired kidney function with serum creatinine. *Indian J Clin Biochem*. 2010;25(4):380–4.
17. Abraham AG, Palella FJ, Li X, Estrella MM, Kingsley LA, Witt MD, et al. The impact of impaired kidney function and HIV infection on the risk of anemia. *AIDS Res Hum Retroviruses*. 2012;28(12):1666–71.
18. Le T, Ly VT, Thu NTM, Nguyen A, Thanh NT, Chau NVV, et al. Population Pharmacodynamics of Amphotericin B Deoxycholate for Disseminated Infection Caused by *Talaromyces marneffei*. *Antimicrob Agents Chemother*. 2019;63(2).
19. De Vriese AS, Robbrecht DL, Vanholder RC, Vogelaers DP, Lameire NH. Rifampicin-associated acute renal failure: pathophysiologic, immunologic, and clinical features. *Am J Kidney Dis*. 1998;31(1):108–15.
20. Lin FS, Wu MY, Tu WJ, Pan HQ, Zheng J, Shi JW, et al. A cross-sectional and follow-up study of leukopenia in tuberculosis patients: prevalence, risk factors and impact of anti-tuberculosis treatment. *J Thorac Dis*. 2015;7(12):2234–42.
21. Speth C, Rambach G, Lass-Flörl C. Platelet immunology in fungal infections. *Thromb Haemost*. 2014;112(4):632–9.
22. Flaujac C, Boukour S, Cramer-Bordé E. Platelets and viruses: an ambivalent relationship. *Cell Mol Life Sci*. 2010;67(4):545–56.
23. Mansour MK, Levitz SM. Interactions of fungi with phagocytes. *Curr Opin Microbiol*. 2002;5(4):359–65.
24. Lehrer RI, Cline MJ. Interaction of *Candida albicans* with human leukocytes and serum. *J Bacteriol*. 1969;98(3):996–1004.

25. Bakchoul T, Marini I. Drug-associated thrombocytopenia. Hematol Am Soc Hematol Educ Program. 2018;2018(1):576–83.