Heliyon 9 (2023) e14219

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Clinical symptoms and immune injury reflected by low CD4/CD8 ratio should increase the suspicion of HIV coinfection with tuberculosis

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ARTICLE INFO

Keywords:
CD4/CD8 ratio
$CD4^+$ T cells
HIV-TB coinfection

ABSTRACT

Background: Patients who are coinfected with human immunodeficiency virus 1 (HIV) and *Mycobacterium tuberculosis* (TB) benefit from timely diagnosis and treatment. In the present study frequencies of $CD3^+$, $CD4^+$, and $CD8^+$ T cells among peripheral blood mononuclear cells (PBMCs) of patients in the Kashi region of China infected with HIV, TB, and both HIV and TB (HIV-TB) were investigated to provide a basis for rapid identification of coinfected patients.

Methods: A total of 62 patients with HIV, TB, or HIV-TB who were first hospitalized at our institution were included in the study, as were 30 controls. PBMCs were isolated, and the frequencies of $CD3^+$, $CD4^+$, and $CD8^+$ T cells were determined via flow cytometry.

Results: The frequency of $CD4^+$ T cells and the CD4/CD8 ratio were significantly lower in the HIV-TB group than in the other three groups. In fever patients the frequency of $CD4^+$ T cells and the CD4/CD8 ratio were significantly lower in the HIV-TB group than in the HIV group and the TB group. In patients who exhibited rapid weight loss there were no significant differences in the frequency of $CD4^+$ T cells or the CD4/CD8 ratio between the groups. The results of treatment were compared in the HIV, TB, and HIV-TB groups after 7 days, and there were obvious improvements in the frequency of $CD4^+$ T cells and the CD4/CD8 ratio.

Conclusion: Clinical symptoms and the degree of immune injury can heighten suspicion for HIV-TB coinfection.

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https://doi.org/10.1016/j.heliyon.2023.e14219
Received 28 April 2022; Received in revised form 29 January 2023; Accepted 24 February 2023
Available online 4 March 2023

1. Introduction

Human immunodeficiency virus 1 (HIV) infection is increasingly considered a chronic inflammatory disease that leads to immunodeficiency. Inflammatory markers are elevated throughout the asymptomatic phase of infection [1], and are correlated with the rate of progression to acquired immune deficiency syndrome (AIDS) [2]. *Mycobacterium tuberculosis* (TB) infection is caused by a facultative intracellular pathogen of macrophages [3]. HIV patients are evidently more prone to developing TB, which has been associated with an increased incidence of additional opportunistic infections and may be an independent risk factor for progression to AIDS [4,5]. HIV-TB coinfection has a lower cure rate and a higher mortality rate than infection with HIV or TB alone. Interaction between these pathogens contributes substantially to the high incidence of mortality associated with coinfection.

The Kashi region is located to the west of XinJiang Province in China, close to the Taklamakan Desert, which is the second largest desert in the world. The desert climate and geographic location of Kashi are favorable for the development of chronic obstructive pulmonary disease, lung cancer, and TB because people living in this area are frequently exposed to dust storms that can adversely affect normal lung function [6]. The incidence of HIV is increasing in Kashi due to low medical accessibility and poor health awareness among residents, but HIV-TB patients are rarely diagnosed. We conducted a survey and found that 3/19 (15.8%) of AIDS patients undergoing treatment were coinfected with TB, but their diagnostic certificates listed only "HIV+". These data suggest that among AIDS patients in the Kashi region, many with HIV-TB coinfection have likely gone undiagnosed in the past.

Testing for HIV is relatively rapid, and more convenient than testing for TB, which requires at least three positive sputum smear tests, chest CT scanning, and T-SPOT testing. The development of molecular methods over the last decade has substantially advanced the fight against TB. Many HIV-TB coinfected patients do not have access to molecular diagnostic tests however; therefore, the diagnosis of TB remains challenging in HIV-TB coinfected individuals due to the high frequency of negative sputum smear tests [7]. Due to the low average annual income in the Kashi region, most HIV patients would not be able to afford the extra cost of the TB testing that would be necessary if clinicians were directed to examine all HIV^+ patients for TB coinfection. In addition, a proportion of HIV^+ patients are discharged from hospital before symptoms of TB may be observed by their physician, because it can take some days to detect TB. Thus HIV-TB patients are rarely diagnosed in the Kashi region, and therefore they do not receive comprehensive treatment.

$CD4^+$ T cells are considered the primary targets of HIV [8], whereas $CD4^+$ T cells and macrophages are thought to be crucial for host defenses against TB [9]. Several studies have shown that numbers of $CD4^+$ T cells are lower in HIV-TB coinfected patients than in patients infected with HIV alone [10–12]. Therefore, we investigated the frequencies of $CD3^+$, $CD4^+$, and $CD8^+$ cells in the peripheral blood of TB, HIV, and HIV-TB patients to provide a basis for more rapid identification of HIV-TB patients, thereby enabling physicians to determine in advance whether to test an HIV^+ patient for TB. This would facilitate the earlier comprehensive treatment of HIV-TB patients in the Kashi region.

2. Materials and methods

2.1. Patients, selection criteria, and analytical procedures

From January 2021 to August 2021 patients with a first diagnosis of HIV, TB, and HIV-TB at the First People's Hospital of Kashi were recruited for the study along with healthy controls. Patients and controls were matched for age, sex, and body mass index (BMI). All subjects were required to provide basic information via a questionnaire, and a 5-mL sample of peripheral blood for testing. Written informed consent was obtained from all participants. All experiments were conducted in accordance with established ethical guidelines. The project was approved by the Ethics Committee of the First People's Hospital of Kashi (project number 2020-No.74). The inclusion criteria were (1) the diagnosis matched the criteria of the AIDS Diagnosis and Treatment Guidelines formulated by the Chinese Medical Association, and the patient had undergone clinical diagnostic and laboratory tests to confirm HIV antibody positivity; (2) the diagnosis matched the criteria for TB as revised and issued in 2001, and was accompanied by significant signs of TB poisoning, TB culture was positive, and imaging analyses confirmed pulmonary TB; (3) the patient was not pregnant; and (4) the patient was aged >15 years. The exclusion criteria were (1) use of glucocorticoids or immunosuppressants; and (2) immune system diseases other than HIV or other chronic diseases.

The protocol for isolation of peripheral blood mononuclear cells (PBMCs) was adapted from published literature [13]. Briefly, whole blood was diluted with an equal volume of phosphate-buffered saline (PBS) before layering on Ficoll-Paque PLUS in a 15-mL centrifuge tube. The tubes were then centrifuged at 950 *g* for 25 min with the centrifuge brake disengaged. PBMCs were collected from the layer between the plasma and Ficoll liquid and washed twice with PBS. Cells were pelleted by centrifugation at 350*g* for 7 min, then resuspended in complete PBS before counting and plating. The antibodies used for flow cytometry analyses were CD3-FITC (BioLegend Inc. #317306), CD4-PE (BioLegend #980804), and CD8-APC (BioLegend #344722). CD3 cells were gated from total T lymphocytes, and CD4 and CD8 cells were gated from total CD3 cells. A BD FACS Canto machine was used to conduct the flow cytometry, and the flow cytometry results were analyzed using FlowJo™ v.10.8 software (BD Life Sciences).

2.2. Statistical analyses

SPSS 21.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Normally distributed quantitative data are expressed as means ± the standard deviation. Data exhibiting homogeneity of variance were compared via analysis of variance between multiple groups, followed by pairwise comparisons. Enumeration data were evaluated using the chi-square test. $P < 0.05$ according to the least significant difference test was considered statistically significant.

Table 1
Patient characteristics.

	TB	HIV	HIV-TB	Control	*p*
	(*n* = 24)	(*n* = 21)	(*n* = 17)	(*n* = 30)	(χ^2/F)
Age (mean ± SD)	45.3 ± 10.5	40.3 ± 9.5	41.3 ± 12.5	42.7 ± 13.2	0.24 (4.21)
Sex, *n* (%)					0.36 (3.28)
male	9 (37.50)	12 (57.15)	10 (58.82)	18 (0.60)	
female	15 (62.50)	9 (42.85)	7 (41.17)	12 (0.40)	
BMI (mean ± SD)	19.39 ± 2.13	19.31 ± 2.07	18.89 ± 1.87	20.13 ± 3.42	0.13 (4.37)
Smoking status					0.15 (5.37)
yes	9 (37.50)	11 (52.38)	7 (41.17)	20 (66.67)	
no	15 (62.50)	10 (47.61)	10 (58.82)	10 (33.33)	
Annual household income (CNY, yuan)	49,194 (35,400–55,800)	42,030 (34,900–51,500)	42,041 (33,500–52,500)	56,632 (43,700–65,500)	
Occupation, *n* (%)					0.32 (3.49)
farmer	14 (58.3)	14 (66.67)	10 (58.82)	16 (53.33)	
other	10 (41.7)	7 (33.33)	7 (41.76)	14 (46.67)	

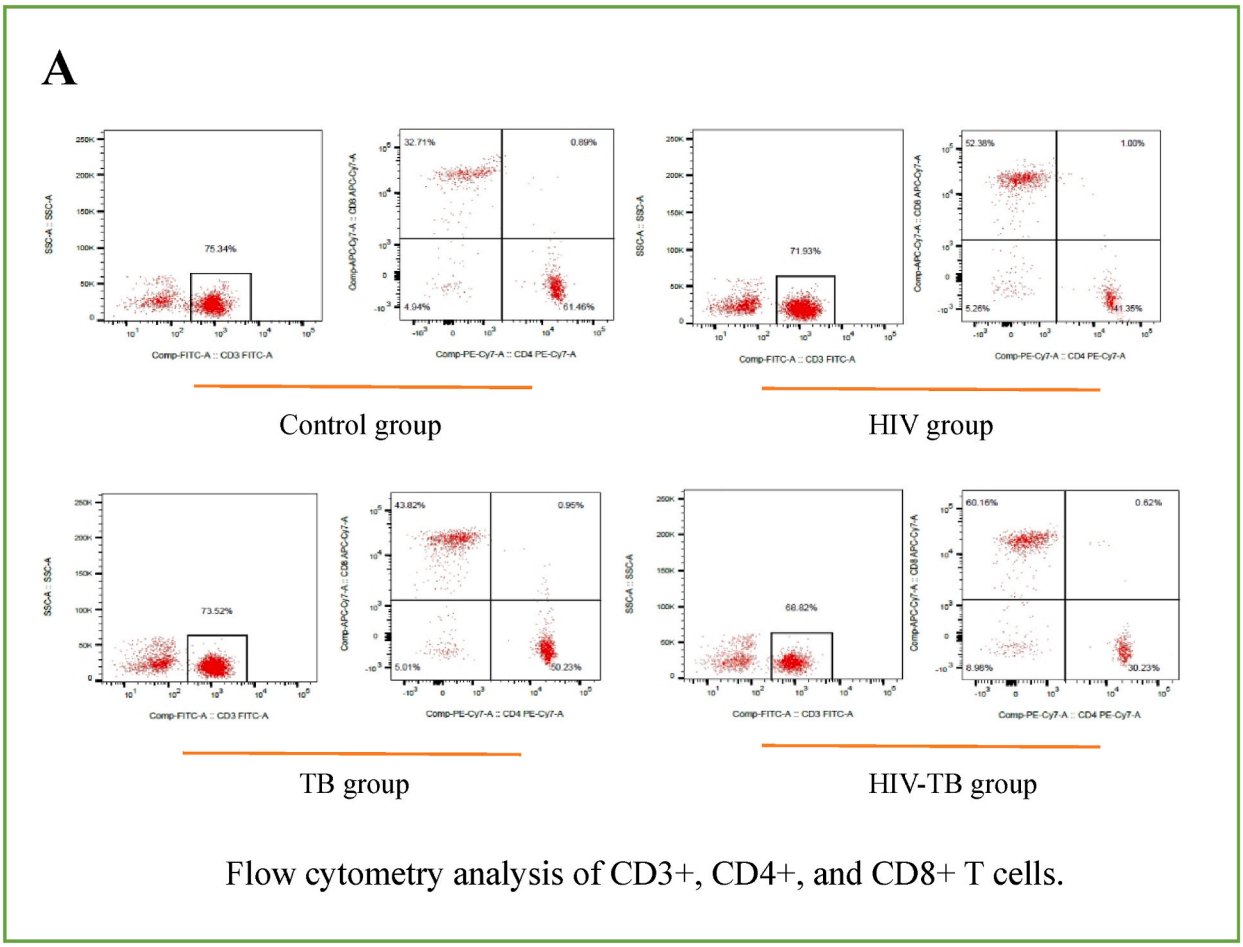


Fig. 1. a Flow cytometry analysis of $CD3^+$, $CD4^+$, and $CD8^+$ T cells. b Frequencies of $CD3^+$, $CD4^+$, and $CD8^+$ T cells in each group. Notes: Δ compared with the control group; * compared with the HIV group; ΔP < 0.05, ΔΔP < 0.01,ΔΔΔP < 0.001; *P < 0.05.

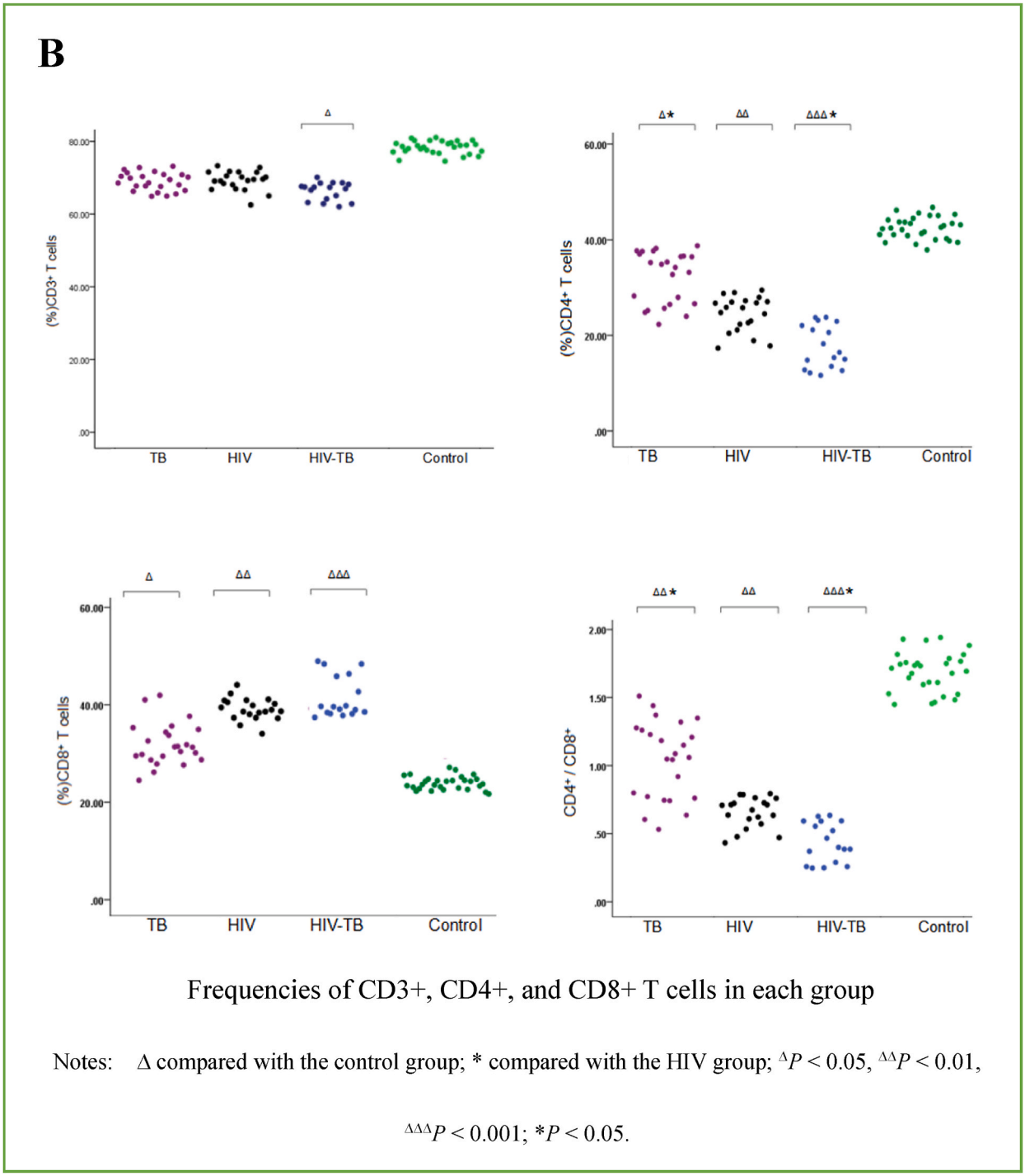


Fig. 1. (*continued*).

3. Results

3.1. Characteristics of the study population

A total of 92 subjects were invited to participate, and there were no significant differences between the patient groups and the control group in terms of mean age, sex, or BMI, or smoking history or occupation (Table 1). HIV and HIV-TB patients had lower mean

Table 2
Patients with fever and rapid weight loss.

Group	Fever	Rapid weight
	(*n*)	loss (*n*)
HIV ($n = 21$)	6	3
TB ($n = 24$)	9	0
HIV-TB ($n = 17$)	10	4

annual household incomes (CNY, yuan) than subjects in the TB group and the control group.

3.2. T lymphocyte testing for identifying the degree of immune injury in coinfection patients

Results of flow cytometry analyses of CD3, CD4, and CD8 expression in T cells in PBMCs are shown in Fig. 1A. Various parameters were compared in the different groups, and the frequency of $CD4^+$ T cells ($p < 0.001$) and the CD4/CD8 ratio ($p < 0.001$) were significantly lower in the HIV-TB group than the control group (Fig. 1B). The frequencies of $CD4^+$ T cells ($p < 0.01$) and $CD8^+$ T cells ($p < 0.01$) and the CD4/CD8 ratio ($p < 0.01$) were significantly lower in HIV patients than in the control group (Fig. 1B). The frequency of $CD4^+$ T cells ($p < 0.05$) and the CD4/CD8 ratio ($p < 0.05$) were significantly lower in TB patients than in the control group (Fig. 1B). These data suggest that the immune status of these patients in the Kashi region is related to the frequency of $CD4^+$ T cells and the CD4/CD8 ratio among PBMCs.

3.3. Effects of fever and rapid weight loss on $CD4^+$ T cells and the CD4/CD8 ratio

Some patients exhibited fever (body temperature >37.3 °C) and/or rapid weight loss (>8 kg within 20 days) (Table 2), and associations between these symptoms and the frequency of $CD4^+$ T cells and the CD4/CD8 ratio were assessed. In patients with fever the frequency of $CD4^+$ T cells and the CD4/CD8 ratio were significantly lower in HIV-TB patients than in HIV patients and TB patients [Fig. 2A and (B)]. In patients with rapid weight loss the CD4/CD8 ratio in HIV and HIV-TB patients did not differ significantly ($p = 0.59$) [Fig. 3A and (B)]. In patients who did not exhibit fever or rapid weight loss the frequency of $CD4^+$ T cells and the CD4/CD8 ratio were significantly lower in HIV-TB patients than in HIV patients and TB patients [Fig. 4A and (B)].

3.4. Frequencies of $CD4^+$ T cells and the CD4/CD8 ratio were tested for testify comprehensive treatment which would benefit for HIV-TB patients

The above results indicate that the $CD4^+$ T cell frequency and the CD4/CD8 ratio may provide a basis for rapid identification of patients with HIV-TB coinfection. However, whether the $CD4^+$ T cell frequency and the CD4/CD8 ratio could be improved after treatment was unclear. Therefore, PBMCs were again harvested from HIV, TB, and HIV-TB patients ($n = 6$ per group) who were hospitalized at our institution over the subsequent month, and exhibited no fever or rapid weight loss. All samples were tested for $CD4^+$ T cell frequency and CD4/CD8 ratio before and after treatment [Fig. 5A and (B), Fig. 6A and (B)]. The frequency of $CD4^+$ T cells and the CD4/CD8 ratio were obviously improved after treatment for 7 days. Interestingly the CD4/CD8 ratio did not differ significantly in HIV patients and HIV-TB patients ($p = 0.72$).

4. Discussion

HIV patients are evidently more prone to contracting TB, which is associated with an increased incidence of opportunistic infections [5]. However, HIV-TB coinfected patients are rarely diagnosed in the Kashi region. The incidences of AIDS and TB are both increasing in the Kashi region, and this is related to a number of factors such as a poor economy, comparatively low medical accessibility, and poor health awareness among residents, but it was the low rate of HIV-TB coinfection diagnoses that attracted our attention. In the current small-scale investigation, we speculated that many HIV-TB patients with AIDS have probably gone undiagnosed in the past because of economic and other factors.

Several studies have shown that the number of T lymphocytes is related to immune status [10–12]. The primary cellular targets of HIV are $CD4^+$ T cells, a subset of T lymphocytes that plays a key role in orchestrating the host immune response to pathogens. During the course of infection, the number of $CD4^+$ T cells is inversely correlated with plasma viral load. It is known that TB promotes HIV replication, which leads to a reduction in the proportion of $CD4^+$ T cells in HIV-TB coinfected patients compared with patients infected with HIV alone. $CD8^+$ T cells play an important role in the immune response against HIV infection [14]. In the initial stages of infection prior to the virus escaping the immune response, they are responsible for eliminating infected $CD4^+$ T cells. Thus, the frequency of $CD4^+$ T cells and the CD4/CD8 ratio are considered indicators of the immune response. Therefore, we investigated the frequencies of $CD3^+$, $CD4^+$, and $CD8^+$ T cells in the peripheral blood of HIV, TB, and HIV-TB patients. We compared various parameters in these patients and found that the frequency of $CD4^+$ T cells and the CD4/CD8 ratio were significantly lower in the HIV-TB group than in the other groups. In other studies, we found that CD3, CD4, and CD8 at the single cell level enable early-stage HIV diagnosis without false-negative error, and the percentage of $CD4^+$ PBMCs and the CD4/CD8 ratio decreased in early-stage HIV infection [15,16]. The initial $CD4^+$ count dropped from 855 to 472 cells/μL within 20 days from the first reactive HIV RNA test [14]. As a condition of the

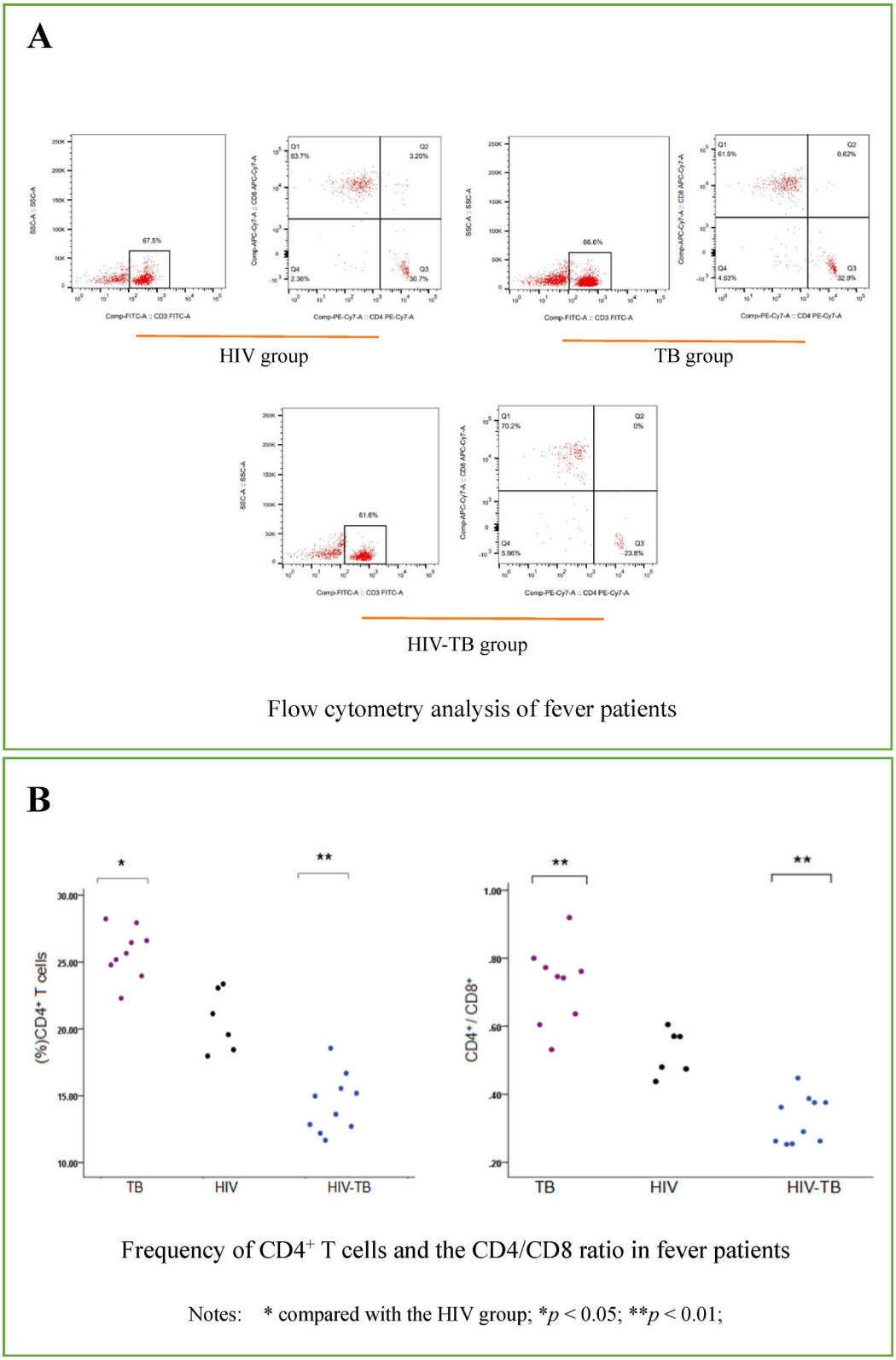


Fig. 2. a Flow cytometry analysis of fever patients. b Frequency of $CD4^+$ T cells and the CD4/CD8 ratio in fever patients. Notes: * compared with the HIV group; $*p < 0.05$; $**p < 0.01$.

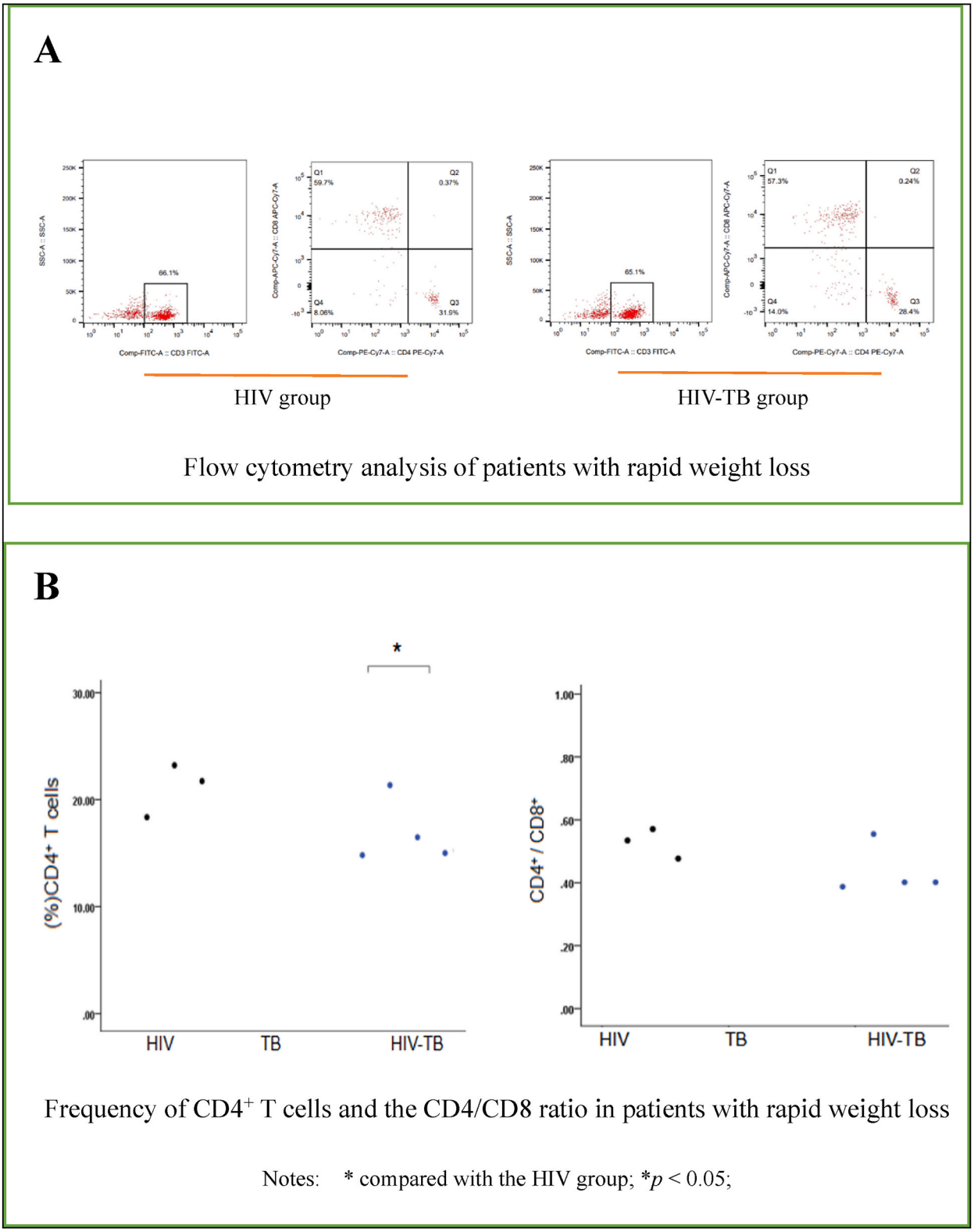
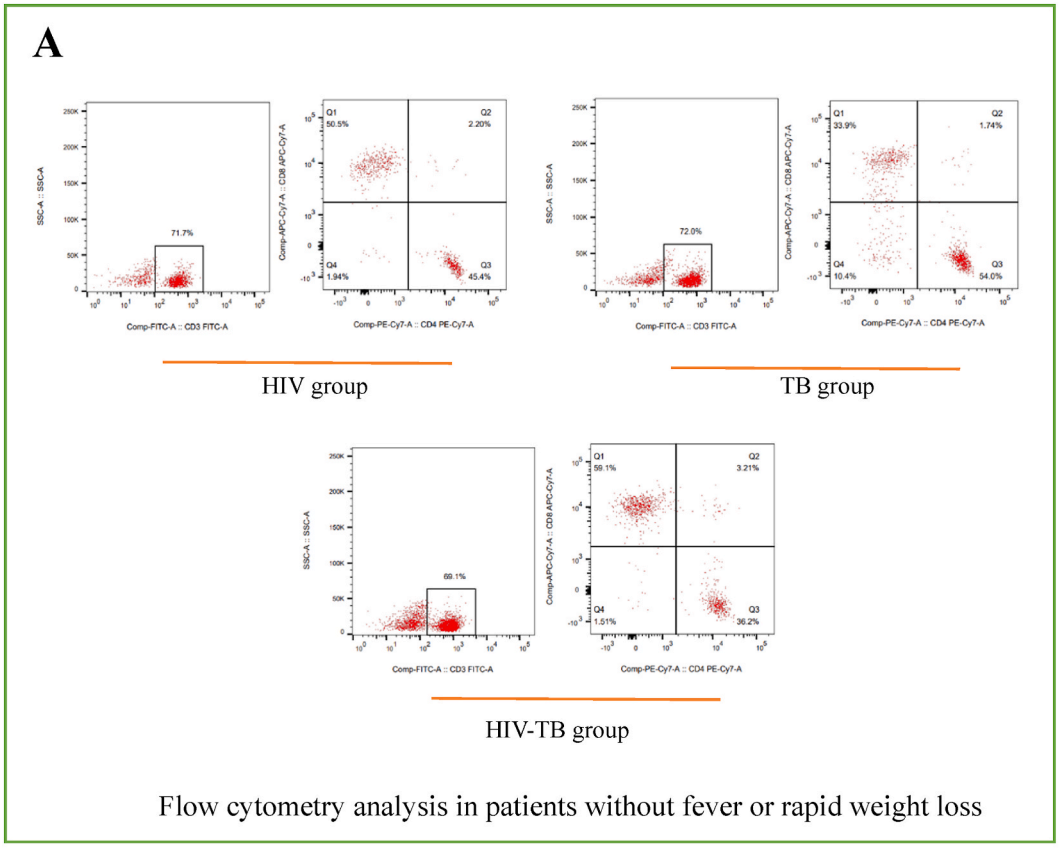


Fig. 3. a Flow cytometry analysis of patients with rapid weight loss. b Frequency of $CD4^+$ T cells and the CD4/CD8 ratio in patients with rapid weight loss. Notes: * compared with the HIV group; *$p < 0.05$.



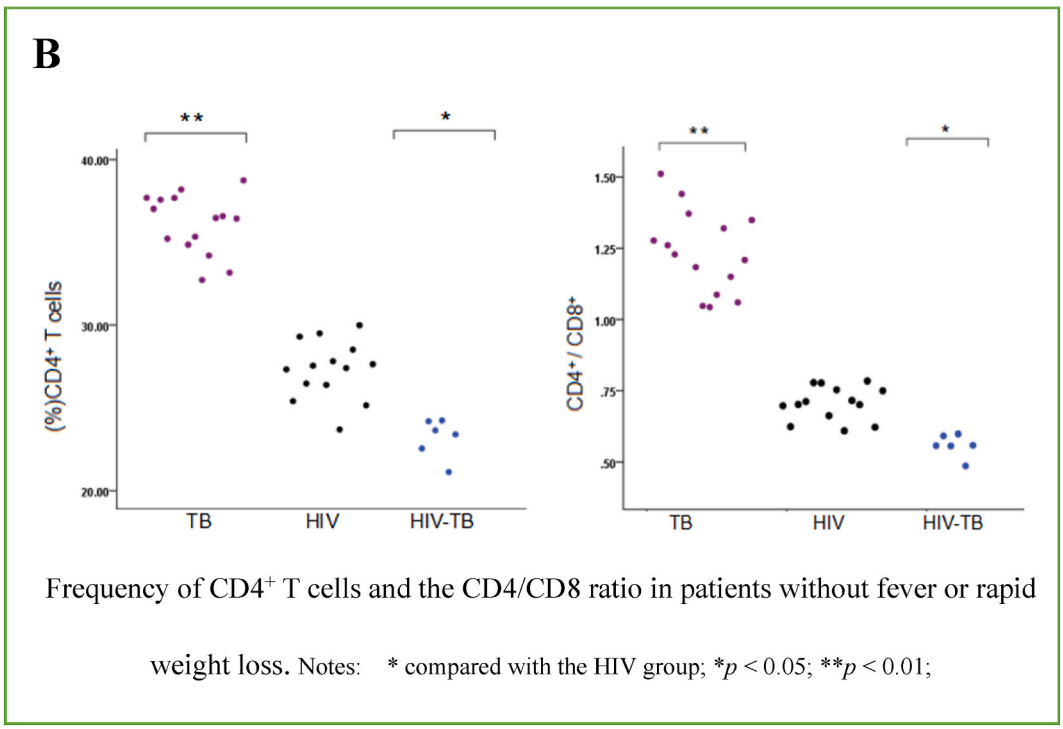
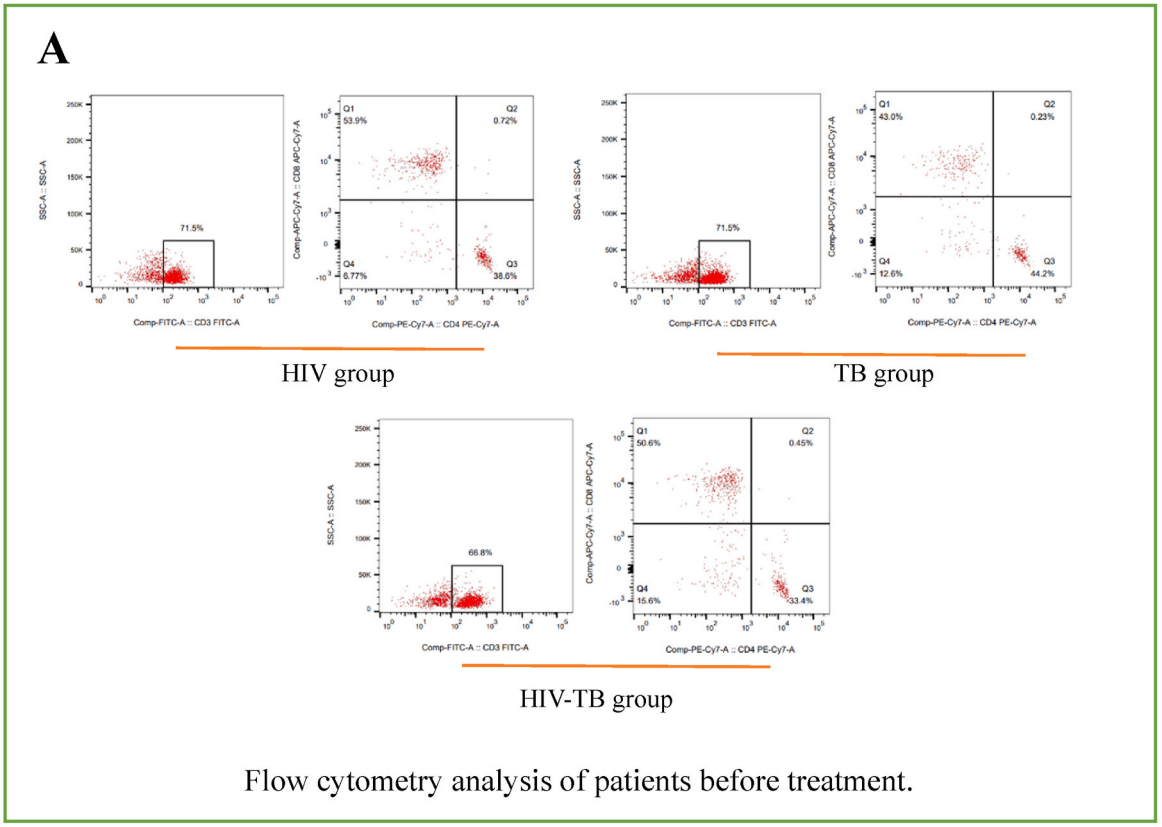


Fig. 4. a Flow cytometry analysis in patients without fever or rapid weight loss. b Frequency of $CD4^+$ T cells and the CD4/CD8 ratio in patients without fever or rapid weight loss. Notes: * compared with the HIV group; *$p < 0.05$; **$p < 0.01$.



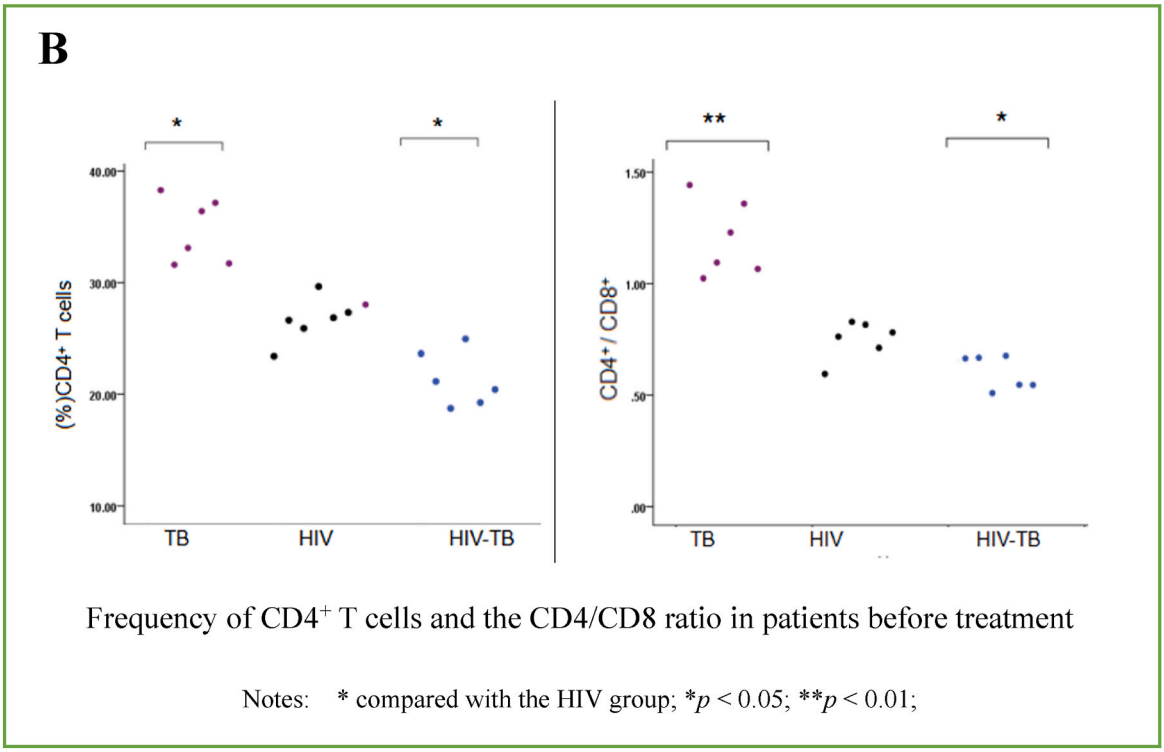
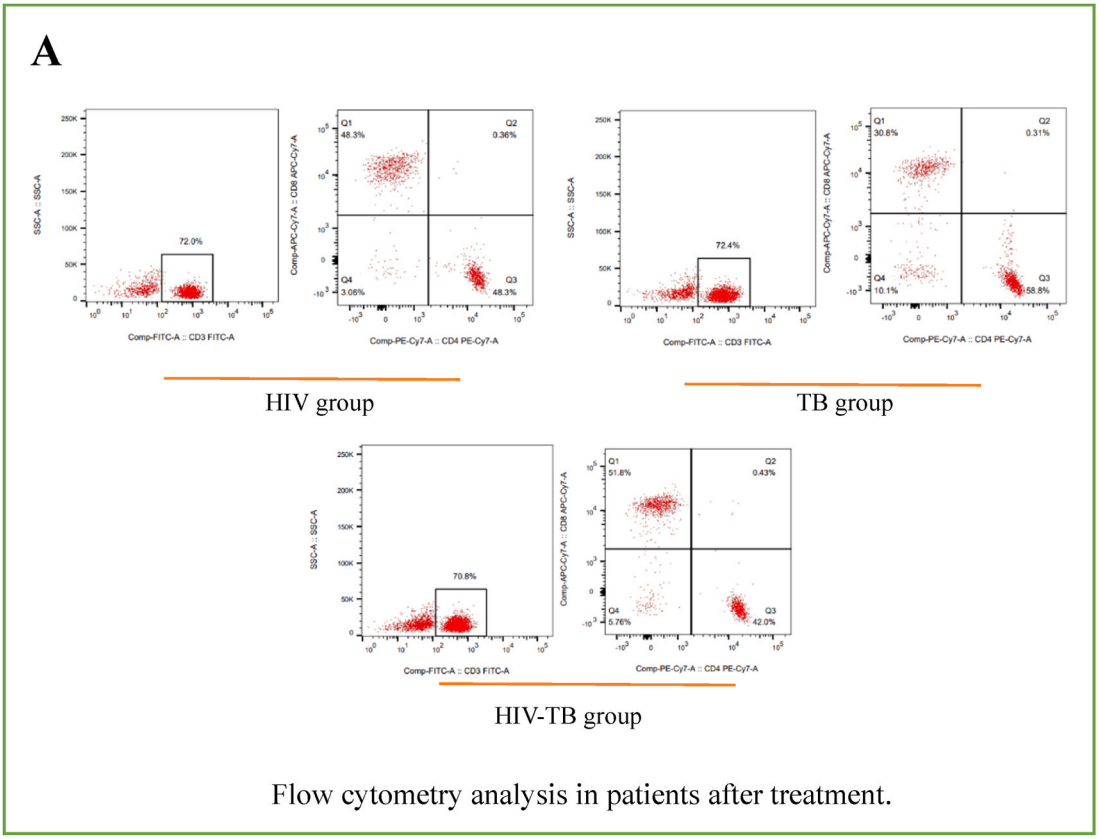


Fig. 5. a Flow cytometry analysis of patients before treatment. b Frequency of $CD4^+$ T cells and the CD4/CD8 ratio in patients before treatment. Notes: * compared with the HIV group; *$p < 0.05$; **$p < 0.01$.



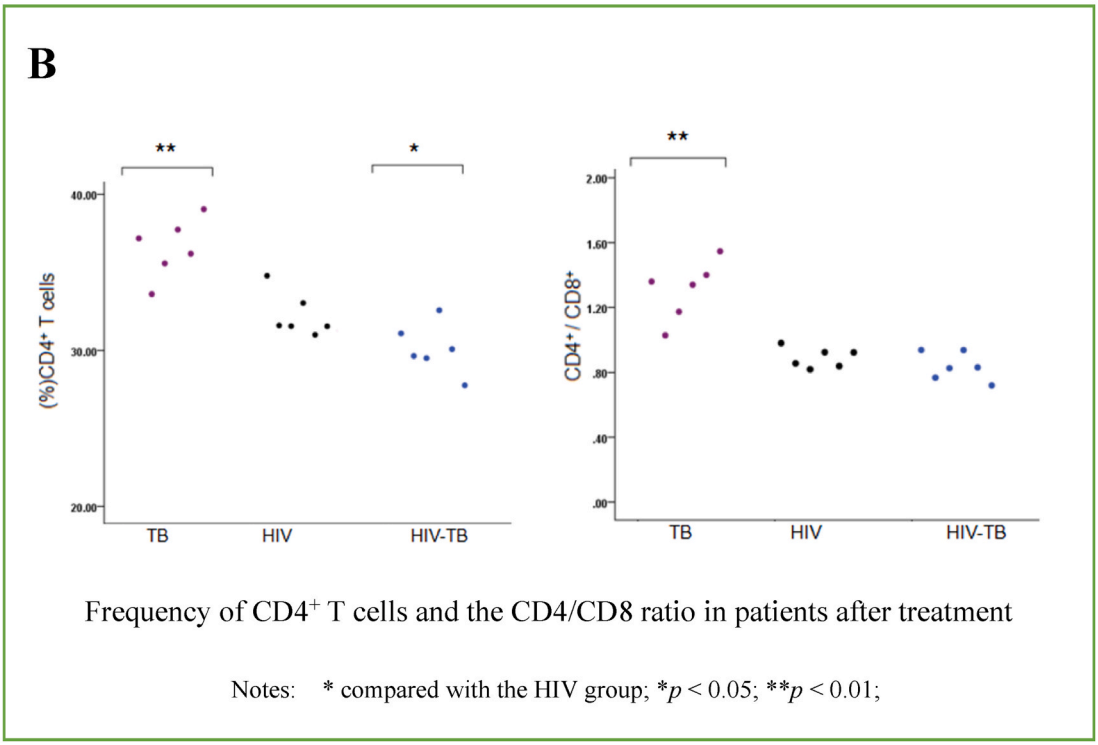


Fig. 6. a Flow cytometry analysis in patients after treatment. b Frequency of $CD4^+$ T cells and the CD4/CD8 ratio in patients after treatment. Notes: * compared with the HIV group; *$p < 0.05$; **$p < 0.01$.

selection criteria in the present study all diagnoses of HIV, TB, and HIV-TB were first diagnoses, implying early-stage infection, and the results are similar to those of the other study [14–16].

In the present study, in patients with fever the frequency of $CD4^+$ T cells and the CD4/CD8 ratio were lowest in HIV-TB patients. In patients with rapid weight loss however, there was no significant difference in CD4/CD8 ratio between HIV patients and HIV-TB patients. This may be due to the low number of patients in the study. In patients without fever or rapid weight loss the frequency of $CD4^+$ T cells and the CD4/CD8 ratio were significantly lower in HIV-TB patients than in HIV patients and TB patients. This indicates that the degree of immune injury can heighten suspicion for HIV-TB coinfection in the Kashi region, even in patients with fever. However, the data indicate that the frequency of $CD4^+$ T cells and the CD4/CD8 ratio are obviously improved after treatment for 7 days. Interestingly, the CD4/CD8 ratio did not differ significantly between HIV and HIV-TB patients after treatment. This may be due to individual differences or HIV-TB patients getting comprehensive treatment which would benefit them. There is short of a thorough clinical consideration in all HIV infected patients for TB, but our data suggests if resources are limiting focusing on patients with rapid weight loss, fever and perhaps low CD4/CD8 ratios is most fruitful.

Ethics statement

We confirm that these experiments were conducted in accordance with established ethical guidelines, and informed consent was obtained from all participants. This project was approved by the Ethics Committee of the First People's Hospital of Kashi (project number 2020-No.74).

Submission statement

All authors declare that this manuscript was submitted solely to this journal, and the work described therein is original research that has not been published previously, and is not under consideration for publication elsewhere in whole or in part.

Author contribution statement

Li Li; Fan Yang; DaYong Zheng: Conceived and designed the experiments; Wrote the paper.
Zulipikaer Abudureheman: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
XueMei Zhong; Hui Gong: Performed the experiments; Contributed reagents, materials, analysis tools or data.
Abuduweili Awuti: Performed the experiments.
Ayiguli Alimu; Subinuer Yilamujiang: Analyzed and interpreted the data.
XiaoGuang Zou: Conceived and designed the experiments.

Funding statement

Mrs li li was supported by State Key Laboratory of Pathogenesis, Prevention and Treatment of Central Asian High Incidence Diseases in Central Asia & The First People's Hospital of Kashi Fund [SKL-HIDCA-2020-KS1], State Key Laboratory of Pathogenesis, Prevention and Treatment of Central Asian High Incidence Diseases Fund [SKL-HIDCA-2020-10], First People's Hospital of Kashi Fund [KDYY202016].

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

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