

## ORIGINAL ARTICLE

# Peripheral blood CD4<sup>+</sup> T cell count predicts recurrence of condyloma acuminatum after photodynamic therapy in HIV-positive patients

Li Gu<sup>1</sup> | Shu Zhou<sup>1</sup> | Zhinan Shi<sup>1</sup> | Xiaoyu Zhai<sup>1</sup> | Lique Gu<sup>1</sup> | Bingrong Zhou<sup>2</sup> | Hui Hua<sup>1</sup>

<sup>1</sup>Department of Dermatology, Nantong Third People's Hospital Affiliated to Nantong University, Nantong, China

<sup>2</sup>Department of Dermatology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

## Correspondence

Hui Hua, Department of Dermatology, Nantong Third People's Hospital Affiliated to Nantong University, Nantong 226001, China.

Email: 214972881@qq.com

Bingrong Zhou, Department of Dermatology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China.

Email: zhoubingrong@njmu.edu.cn

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## Abstract

**Background:** Few studies have reported postoperative relapse of condyloma acuminatum (CA) after 5-aminolevulinic acid photodynamic therapy (ALA-PDT) in human immunodeficiency virus (HIV) positive patients.

**Methods:** The clinical data of HIV-positive CA patients treated with ALA-PDT from October 2018 to December 2019 were analyzed retrospectively. Univariate and multivariate Cox regression was used to analyze the variables related to postoperative recurrence. Pearson correlation test was employed to analyze the correlation between CD4<sup>+</sup> T cell count and postoperative recurrence rate. Kaplan–Meier method was used to compare the CA recurrence after ALA-PDT in low CD4 group and high CD4 group.

**Results:** A total of 38 HIV-positive patients with CA were included in the study. Among them, 26 patients experienced CA recurrence within 6 months, and the recurrence rate was 68.4%. CD4<sup>+</sup> T cell count was 187.0 (79.0–596.0) cells/μl in relapsed patients and 406.0 (89.0–612.0) cells/μl in non-relapsed patients, showing a statistically significant difference ( $p = .005$ ). Pearson correlation coefficient analysis revealed a negative correlation between CD4<sup>+</sup> T cell count and postoperative recurrence rate ( $p = .005$ ,  $r = -.443$ ). Univariate regression analysis showed that CD4<sup>+</sup> T cell count was correlated with postoperative recurrence, hazard ratio (HR) was 0.99 [95% Confidence interval (CI) = 0.99–1.0,  $p = .012$ ]. Multivariate Cox regression analysis showed that with the low CD4<sup>+</sup> T cell count as the reference, the high CD4<sup>+</sup> T cell count was negatively correlated with postoperative recurrence (HR = 0.09, 95% CI 0.01–0.87,  $p = .038$ ).

**Conclusions:** Peripheral blood CD4<sup>+</sup> T cell count can predict the CA recurrence rate after ALA-PDT in HIV-positive patients.

## KEYWORDS

5-aminolevulinic acid photodynamic therapy, condyloma acuminatum, HIV, recurrence, retrospective analysis

Li Gu and Shu Zhou contributed to the work equally and should be regarded as co-first authors. Bingrong Zhou and Hui Hua are Co-Corresponding authors.

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## 1 | INTRODUCTION

Condyloma acuminatum (CA), a most common sexually transmitted disease worldwide, is a verrucous hyperplasia of genital and perianal areas caused by human papilloma virus (HPV) infection.<sup>1</sup> The prevalence of CA in people infected with human immunodeficiency virus (HIV) is much higher than that in the general population.<sup>2</sup> HIV-positive patients have a reduced ability to eliminate HPV and the resultant prolonged duration of HPV infection.<sup>3,4</sup> Persistent high-risk HPV (especially HPV16) infection is also an important risk factor for anal cancer.<sup>5,6</sup>

The traditional physical therapeutic methods of CA include surgical resection, microwave, electrocoagulation, cryotherapy, and CO<sub>2</sub> laser therapy. The recurrence rate is about 20%–70% within 6 months after these physical treatments.<sup>7,8</sup> Compared with these methods, 5-aminolevulinic acid photodynamic therapy (ALA-PDT) can effectively reduce the recurrence rate of CA (10.29%), by using photosensitizers, light, and oxygen to produce cytotoxic reactive oxygen species (ROS) and eliminate the subclinical and latent HPV infection.<sup>7,9</sup> Xu et al.<sup>10</sup> found that the CA recurrence rate in HIV-positive patients treated with ALA-PDT was approximately 29%. Although this rate is lower than that of other physical therapy methods, it is significantly higher than that of the normal population.<sup>11</sup> At present, there is no study on the clinical factors predicting the CA recurrence after ALA-PDT treatment in HIV-positive patients.

CD4<sup>+</sup> T lymphocytes (CD4 cells), the main target cells of HIV infection, play a central role in immune responses.<sup>12</sup> CD4 cell count reflects the host's defense against pathogens, infections, and diseases,<sup>13</sup> and therefore has a strong association with HIV progression and prognosis.<sup>14</sup> Maintaining sufficient CD4 cells helps to reduce the risk of comorbidity during HIV infection.<sup>13,15</sup> An American study shows that the recurrence rate of anal condyloma in HIV-positive patients with high preoperative CD4 cells was significantly lower than those with low preoperative CD4 count after surgical resection of CA lesions.<sup>16</sup>

The clinical application of ALA-PDT in HIV-positive CA patients is rarely reported, and there lacks analysis of clinical factors related to the recurrence rate after treatment in this population. In this study, we retrospectively analyzed the CA recurrence rate within 6 months after ALA-PDT in 38 HIV-positive patients and analyzed the significance of preoperative CD4 cell count in predicting the prognosis of HIV-positive CA patients treated with photodynamic therapy.

## 2 | METHODS

### 2.1 | Study design and subjects

This study retrospectively analyzed the clinical data of 38 HIV-positive CA patients treated between October 2018 and December 2019 in Nantong Third People's Hospital Affiliated to Nantong University, a tertiary infectious disease hospital located in the central city of eastern China. The Department of Dermatology receives

about 100 referred CA patients per year. The study was approved by the Ethics Committee of Nantong Third People's Hospital Affiliated to Nantong University (No. EL2020003). Inclusion criteria: (1) HIV/AIDS confirmed by enzyme-linked immunosorbent assay and Western blotting, and CA meeting the diagnostic criteria in the clinical guidelines for the diagnosis and treatment of CA<sup>17</sup>; (2) no local and/or systemic treatment within 6 months before surgery. Exclusion criteria: (1) skin allergy, porphyria, hypersensitivity to porphyrins; (2) pregnancy or lactation; (3) incomplete data; (4) failure to complete at least three sessions of ALA-PDT. Peripheral blood collection and CD4 cell count detection were also performed in all patients before the first ALA-PDT treatment.

### 2.2 | Sample collection and laboratory tests

**Peripheral venous blood collection:** Two tubes of 2 ml fasting morning EDTA anticoagulant venous blood (one for CD4 cell count and the other for blood routine) was collected. The percentage of CD4 cells was determined by flow cytometry (FACSCalibur, BD Biosciences) within 24 h. Based on the lymphocyte count in blood routine, CD4 cell count was calculated by multiplying the total lymphocyte count by the percentage of CD4 cells.

**HPV specimen collection and typing:** All wart surfaces of different anatomical parts of the patient were brushed to obtain specimens. Patients with intraanal warts were asked to lie on the examination table in the knee-chest position and specimens were obtained using cell brush with the aid of anal dilator. The specimen was placed in the elution tube with special cell preservation solution, and then stored at 2–8°C in the refrigerator for no more than 48 h. HPV typing was performed according to the manufacturer's instructions using the human papillomavirus typing test kit (Shanghai Tellgen Life Science Co., Ltd.), which can detect 17 high-risk types (HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV59, HPV66, HPV68, HPV82) and 10 low-risk types (HPV6, HPV11, HPV40, HPV42, HPV43, HPV44, HPV55, HPV61, HPV81, HPV83).

### 2.3 | ALA-PDT treatment

After routine disinfection with 0.5% iodophor in the wart area, 2% lidocaine was applied for local infiltration anesthesia. The visible warts were removed with CO<sub>2</sub> laser apparatus (Jilin Keying Laser Technology Co. Ltd., jc-100d) with a power of 1–4 w and the normal tissue 0.5 cm away from the warts was cauterized to the superficial dermis. ALA-PDT was performed immediately after operation. ALA powder (Shanghai Fudan Zhangjiang Bio-Pharmaceutical Co., Ltd., 118mg/bottle) was allocated to 20% ALA solution or gel by saline or gel. For patients with warts in the exposed areas such as perianal and foreskin, ALA solution was adopted. The visible skin lesions and the surrounding skin with a radius of 1.0 cm was covered with a thin sterile absorbent cotton soaked with 20% ALA solution, and

then wrapped with preservative film. For patients with warts in both the exposed areas and the anus, ALA gel was employed. The finger cuff embolus was made from rubber gloves by filling in cotton balls. Then, 20% ALA gel was evenly applied on the finger cuff embolus, and then multiple sterile absorbent cottons impregnated with 20% ALA gel were attached. Using the anal dilator, the finger cuff embolus was put into the anus and preservative film was used as the covering. After 3–5 h of packaging, the covering and cotton sheet were removed to fully expose the ALA dressing area, which was then irradiated with a red LED lamp therapeutic instrument for 20 min (LED PDT instrument, Wuhan Yage Optic and Electronic Technique Co. Ltd., 635 nm, 80 mW/cm<sup>2</sup>), with a total energy density of 96 J/cm<sup>2</sup>. The interval between each treatment was 7–10 days. In each treatment, if there existed warts, CO<sub>2</sub> laser cauterization was conducted prior to ALA-PDT treatment; if no warts were found, ALA-PDT treatment was performed directly. All patients received a minimum of three times of photodynamic therapy. If no visible warts were found 7–10 days after the end of the three photodynamic therapies, the white acetate test was performed again. If the test result was positive, photodynamic therapy was continued until it turned negative 7–10 days after the last treatment. If the result was negative, the treatment ended. If visible warts were still found 7–10 days after the end of three photodynamic treatments, additional CO<sub>2</sub> laser and ALA-PDT sessions were performed until there were no visible warts 7–10 days after the last treatment, and then acetic acid white test was conducted. The treatment end point was determined as mentioned above. All patients were followed up for 6 months after the treatment, once a week in the first month and once a month thereafter. Patients were advised not to have the high-risk sexual behavior after diagnosis especially during treatment and follow-up. During the follow-up, if no new warts were found in and around the original lesion, and the acetic acid white test was negative, the case was considered clinically cured; if new warts appeared at or around the original lesion area and the acetic acid white test was positive, a recurrence was determined.

## 2.4 | Data collection

Collected were the general clinical data of patients, including CD4 cell count, number of photodynamic drugs per course (vial), age, marital status, ALA incubation time (h), frequency of sexual intercourse (less than once a week, once a week, twice a week, thrice a week), anal intercourse (no, yes), type of sexual intercourse (same sex, opposite sex, bisexual), lesion location (perianal, genital, perianal + genital), affected area (skin, mucosa, skin + mucosa), combined underlying diseases (hepatitis, kidney disease, autoimmune diseases, hypertension, diabetes), combined use of drugs (topical application of antiviral drug: recombinant human interferon-2b gel, Hefei Zhaoke Pharmaceutical Co., Ltd; oral immune-boosting drug: thymosin enteric coated tablets, Xi'an Desai Biological Pharmaceutical Co., Ltd; topical wart removal lotion: the empirical formula of our hospital consisting of 30 g of radix isatidis, kidney bean root, equisetum and

cyperus, 20 g of raw coix seed, purslane and white fresh skin, 10 g of hive, asarum, safflower and angelica dahurica, and 6 g of alum) and HPV types (low-risk, low-risk + high-risk), number of CO<sub>2</sub> laser treatments, times of photodynamic therapies. The recurrence time, location, and area of CA were also recorded during follow-up.

## 2.5 | Statistical analysis

Data analysis was performed using empower stats (X & Y solutions) and R software (version 3.5.2). Categorical variables were presented as numbers (%), normally distributed data as mean ± standard deviation, and skewed continuous data as median (min–max). Among-group comparison was performed by  $\chi^2$  test. Pearson correlation analysis was applied to analyze the correlation between peripheral blood CD4 cell count and postoperative recurrence rate. ROC curves were used to evaluate the efficacy of peripheral blood CD4 cell count in predicting recurrence after ALA-PDT, and the area under the ROC curve (AUC) was used to determine the discriminating ability of CD4 in predicting recurrence. Univariate Cox regression analysis was performed to identify variables that might be related to postoperative recurrence, and then multivariate Cox regression analysis was conducted to further confirm these variables. Based on the median CD4 cell count of the enrolled patients, the population was divided into “high CD4 group” (CD4 count > 213 cells/ $\mu$ l) and “low CD4 group” (CD4 count  $\leq$  213 cells/ $\mu$ l). The postoperative recurrence curves of the two groups were drawn using Kaplan–Meier method, and the recurrences after photodynamic therapy in the two groups were compared by the Log-rank test.  $p < .05$  was considered statistically significant.

## 3 | RESULTS

### 3.1 | Clinical characteristics of patients

The clinical data of 56 HIV-positive CA patients were analyzed. All patients were men. Among them, four patients failed to complete all three sessions of ALA-PDT, eight patients had received other treatments including CO<sub>2</sub> laser treatment in other hospitals within 6 months before the treatment at our hospital, and six patients presented with incomplete follow-up clinical data. Finally, 38 patients were enrolled in the study. A total of 26 patients recurred within 6 months after the end of treatment, and the recurrence rate was 68.4%. Table 1 shows the comparison of clinical characteristics between the patients with and without recurrence. There was no significant difference between the recurrent and non-recurrent patients in baseline clinical characteristics (age, marital status, frequency of sexual intercourse, anal intercourse, type of sexual intercourse, lesion location, affected area, combined underlying diseases, HPV types), photodynamic treatment parameters (number of photodynamic drugs per course, ALA incubation time, times of photodynamic therapies), and synergistic therapy (number of

TABLE 1 Demographic and clinical characteristics of 38 HIV-positive CA patients

Characteristics	All (n = 38)	No recurrence (n = 12)	Recurrence (n = 26)	Standardize diff. (95% CI)	p Value
CD4 cell count (cells/ $\mu$ l)	213.0 (79.0–612.0)	406.0 (89.0–612.0)	187.0 (79.0–596.0)	1.0 (0.3, 1.7)	.005
Number of photodynamic drugs per course(vial)	4.0 (1.0–7.0)	4.0 (2.0–7.0)	4.0 (1.0–6.0)	0.3 (–0.4, 1.0)	.351
Age (years)	37.5 (18.0–84.0)	42.0 (19.0–84.0)	34.5 (18.0–72.0)	0.1 (–0.6, 0.7)	.861
Marital status					
Unmarried	16 (42.1%)	4 (33.3%)	12 (46.2%)	0.3 (–0.4, 1.0)	.457
Married	22 (57.9%)	8 (66.7%)	14 (53.8%)		
ALA incubation time(h)					
<4	9 (23.7%)	3 (25.0%)	6 (23.1%)	0.0 (–0.6, 0.7)	.897
> = 4	29 (76.3%)	9 (75.0%)	20 (76.9%)		
Frequency of sexual intercourse					
Less than once a week	12 (31.6%)	4 (33.3%)	8 (30.8%)	0.7 (–0.0, 1.4)	.432
Once a week	5 (13.2%)	2 (16.7%)	3 (11.5%)		
Twice a week	16 (42.1%)	6 (50.0%)	10 (38.5%)		
Thrice a week	5 (13.2%)	0 (0.0%)	5 (19.2%)		
Anal intercourse					
No	3 (7.9%)	1 (8.3%)	2 (7.7%)	0.0 (–0.7, 0.7)	.946
Yes	35 (92.1%)	11 (91.7%)	24 (92.3%)		
Type of sexual intercourse					
Same sex	15 (39.5%)	4 (33.3%)	11 (42.3%)	0.2 (–0.5, 0.9)	.869
Opposite sex	3 (7.9%)	1 (8.3%)	2 (7.7%)		
Bisexual	20 (52.6%)	7 (58.3%)	13 (50.0%)		
Lesion location					
Perianal	24 (63.2%)	7 (58.3%)	17 (65.4%)	0.5 (–0.2, 1.2)	.379
Genital	8 (21.1%)	4 (33.3%)	4 (15.4%)		
Perianal + genital	6 (15.8%)	1 (8.3%)	5 (19.2%)		
Affected area					
Skin	9 (23.7%)	2 (16.7%)	7 (26.9%)	0.5 (–0.2, 1.2)	.317
Mucosa	5 (13.2%)	3 (25.0%)	2 (7.7%)		
Skin + mucosa	24 (63.2%)	7 (58.3%)	17 (65.4%)		
Combined underlying diseases					
With	2 (5.3%)	1 (8.3%)	1 (3.8%)	0.2 (–0.5, 0.9)	.565
Without	36 (94.7%)	11 (91.7%)	25 (96.2%)		
Combined use of drugs					
Topical wart removal lotion	14 (36.8%)	2 (16.7%)	12 (46.2%)	0.8 (0.1, 1.5)	.226
Topical application of antiviral drugs	3 (7.9%)	2 (16.7%)	1 (3.8%)		
Oral immune-boosting drugs	9 (23.7%)	4 (33.3%)	5 (19.2%)		
No drug combination	12 (31.6%)	4 (33.3%)	8 (30.8%)		
HPV types					
Low-risk	3 (7.9%)	1 (8.3%)	2 (7.7%)	0.4 (–0.2, 1.1)	.728
Low-risk + high-risk	6 (15.8%)	1 (8.3%)	5 (19.2%)		
Not detected	28 (73.7%)	10 (83.3%)	18 (69.2%)		
Negative	1 (2.6%)	0 (0.0%)	1 (3.8%)		

TABLE 1 (Continued)

Characteristics	All (n = 38)	No recurrence (n = 12)	Recurrence (n = 26)	Standardize diff. (95% CI)	p Value
Number of CO <sub>2</sub> laser treatments					
1	7 (18.4%)	5 (41.7%)	2 (7.7%)	1.3 (0.6, 2.1)	.056
2	10 (26.3%)	1 (8.3%)	9 (34.6%)		
3	5 (13.2%)	1 (8.3%)	4 (15.4%)		
4	5 (13.2%)	3 (25.0%)	2 (7.7%)		
5	9 (23.7%)	2 (16.7%)	7 (26.9%)		
6	2 (5.3%)	0 (0.0%)	2 (7.7%)		
Times of photodynamic therapies					
Three times	12 (31.6%)	2 (16.7%)	10 (38.5%)	0.5 (−0.2, 1.2)	.179
More than three times	26 (68.4%)	10 (83.3%)	16 (61.5%)		

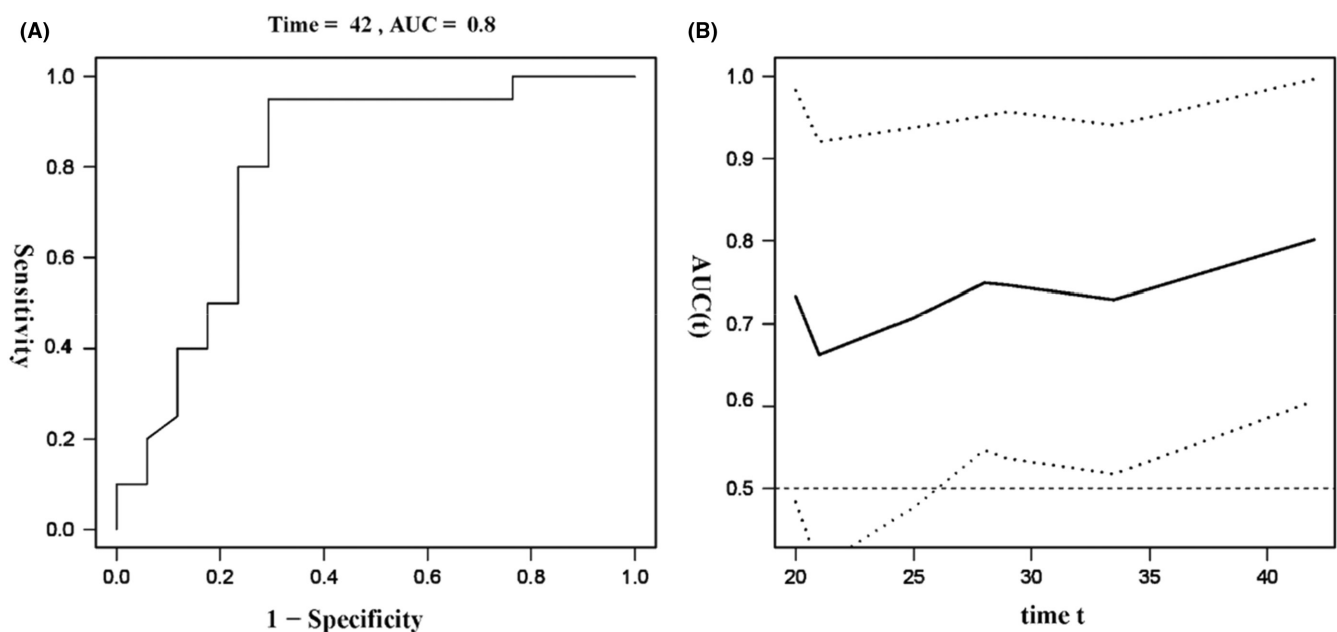


FIGURE 1 ROC (A) and AUC curve (B) of CD4 cell count predicting CA recurrence after photodynamic therapy in HIV-positive patients

CO<sub>2</sub> laser treatments, combined use of drugs) ( $p > .05$ ). The mean CD4 cell count was 213.0 (79.0–612.0) cells/ $\mu$ l in patients before treatment. The CD4 cell count was 406.0 (89.0–612.0) cells/ $\mu$ l in patients without recurrence, and 187.0 (79.0–596.0) cells/ $\mu$ l in patients with recurrence, presenting statistically significant difference ( $p = .005$ ). Only 10 patients were tested for HPV typing, and there was no significant difference in HPV types (including not detected) between the recurrent and non-recurrent patients ( $p > .05$ ). Among those without recurrence, 16.7% received topical recombinant human interferon  $\alpha$ -2b gel and 33.3% received oral thymosin; among those with recurrence, 3.8% received topical recombinant human interferon  $\alpha$ -2b gel and 19.2% received oral thymosin, showing no statistically significant difference ( $p > .05$ ). (Table 1).

### 3.2 | Correlation between CD4 cell count and recurrence rate after ALA-PDT

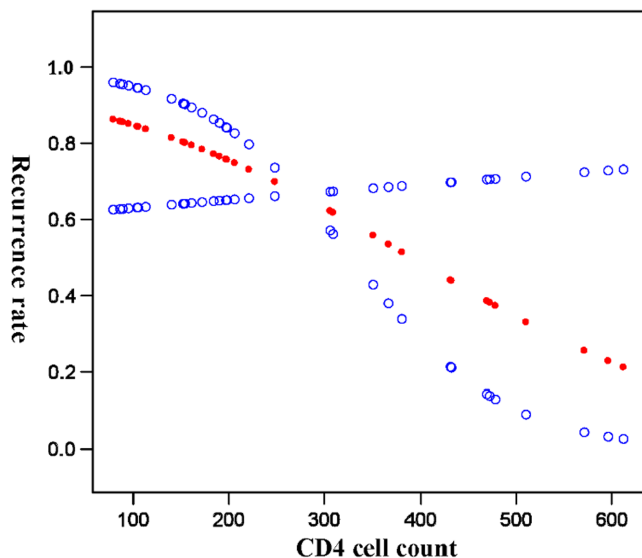
Pearson correlation analysis showed that there was a correlation between CD4 cell count and postoperative recurrence rate, with a correlation coefficient of  $-0.443$  ( $p = .005$ ). ROC analysis indicated that CD4 cell count (AUC = 0.8, 95% CI: 0.498–0.861) was significant in predicting postoperative recurrence (Figure 1). Univariate Cox regression analysis showed that CD4 cell count was significantly correlated with postoperative recurrence (HR = 0.99, 95% CI = 0.99–1.0,  $p = .012$ , Table 2). With the low CD4 group (CD4 count  $\leq 213$  cells/ $\mu$ l) as the reference, the high CD4 group (CD4 count  $> 213$  cells/ $\mu$ l) was negatively correlated with recurrence (HR = 0.11, 95% CI 0.02–0.59,  $p = .011$ ). Univariate analysis also showed that correlation between

**TABLE 2** Univariate and multivariate Cox regression analysis of factors associated with CA recurrence after photodynamic treatment in HIV-positive patients

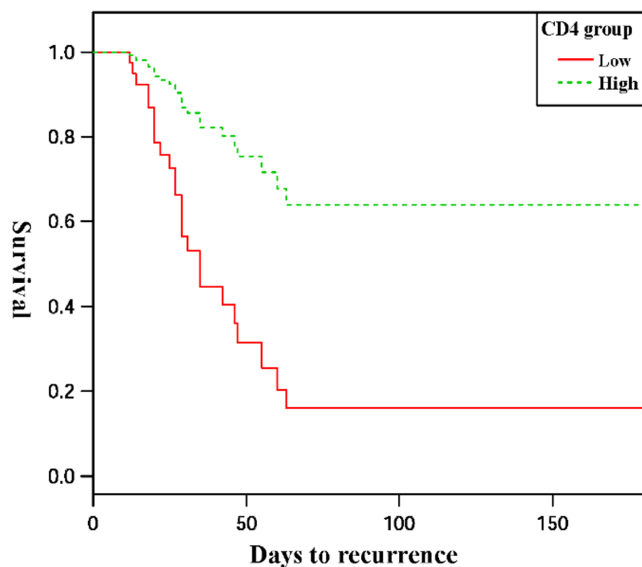
Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p Value	HR (95% CI)	p Value
CD4 cell count	0.99 (0.99, 1.00)	.012		
CD4 numerical grouping				
Low CD4 group	1		1	
High CD4 group	0.11 (0.02, 0.59)	.011	0.09 (0.01, 0.87)	.038
Combined underlying diseases				
With	1			
Without	2.27 (0.13, 39.73)	.574		
Affected area				
Skin	1		1	
Mucosa	0.19 (0.02, 2.06)	.172	0.24 (0.00, 13.00)	.484
Skin + mucosa	0.69 (0.11, 4.20)	.691	0.51 (0.02, 15.35)	.699
Lesion location				
Perianal	1			
Genital	0.41 (0.08, 2.13)	.290		
Perianal + genital	2.06 (0.20, 20.96)	.542		
ALA incubation time(h)				
<4	1			
> = 4	1.11 (0.23, 5.47)	.897		
Combined use of drugs				
Topical wart removal lotion	1		1	
Topical application of antiviral drugs	0.08 (0.00, 1.41)	.085	0.05 (0.00, 2.21)	.123
Oral immune-boosting drugs	0.21 (0.03, 1.53)	.123	0.60 (0.03, 12.86)	.742
No drug combination	0.25 (0.04, 1.77)	.166	0.43 (0.02, 8.30)	.576
Number of photodynamic drugs per course(vial)	0.80 (0.51, 1.26)	.343		
Age (years)	1.00 (0.96, 1.04)	.856		
Times of photodynamic therapies				
Three times	1		1	
More than three times	0.32 (0.06, 1.77)	.192	0.07 (0.00, 1.57)	.095
Marital status				
Unmarried	1			
Married	0.58 (0.14, 2.43)	.459		
Number of CO <sub>2</sub> laser treatments				
<1	1		1	
> = 1	8.57 (1.36, 54.15)	.022	13.97 (0.40, 486.47)	.145

the number of CO<sub>2</sub> laser treatments and recurrence was statistically significant ( $p < .05$ ). As shown in [Table 2](#), after the adjustment of the variables including times of photodynamic therapies, number of CO<sub>2</sub> laser treatments, affected area, and combined use of drugs, CD4 cell count was still significantly correlated with postoperative recurrence (HR = 0.09, 95%CI = 0.01–0.87,  $p = .038$ ). Multivariate Cox regression analysis demonstrated that CD4 cell count was the only independent risk factor for recurrence, and other indices had no significant relationship with recurrence ( $p > .05$ ). As shown in [Figure 2](#),

the relationship between CD4 cell count and postoperative recurrence rate was fitted by smooth curve. By adjusting the variables including times of photodynamic therapies, number of CO<sub>2</sub> laser treatments, affected area and combined use of drugs, there existed a basically downward and smooth curve between CD4 cell count and postoperative recurrence rate, indicating a dominant negative effect of preoperative peripheral blood CD4 cell count on postoperative recurrence rate. According to the coefficient of determination ( $R^2 = .257$ ), it means that 25.7% of postoperative recurrence was



**FIGURE 2** The relationship between CD4 cell count and postoperative recurrence rate is fitted by smooth curve. The adjusted variables are times of photodynamic therapies, number of CO<sub>2</sub> laser treatments, affected area and combined use of drugs. The small red dots represent the median recurrence probability, and the blue dots represent the corresponding 95% credibility interval.



**FIGURE 3** Kaplan-Meier curve of recurrence rate after photodynamic therapies based on high/low CD4 cell count (low CD4 group: CD4 cell count  $\leq 213$  cells/ $\mu$ l; high CD4 group: CD4 cell count  $> 213$  cells/ $\mu$ l).

affected by preoperative CD4 cell count. Figure 3 shows the recurrence of patients in the high and low CD4 groups. Compared with patients in the “low CD4” group, patients in the “high CD4” group had a lower recurrence rate ( $p = .015$ , log rank test) (adjusted variables: times of photodynamic therapies, number of CO<sub>2</sub> laser treatments, affected area, and combined use of drugs).

## 4 | DISCUSSION

ALA-PDT has been shown to reduce the recurrence of CA in HIV-positive patients,<sup>9,10</sup> but its application in this population is still challenging, and there lack research on the prognostic factors. This study retrospectively analyzed the clinical data of 38 HIV-positive CA patients. The results indicated that CD4 cell count was an important clinical predictor of CA recurrence in HIV-positive population after ALA-PDT.

Sebastian g et al. studied 63 intractable anal CA patients undergoing surgery and found the recurrence rate in the patients with normal immune function was 27%, while it was as high as 66% in the low immune function population (including HIV seropositive, leukemia, idiopathic lymphopenia syndrome or organ transplantation patients).<sup>16</sup> Similarly, our present study showed a postoperative recurrence rate of 68.4% in HIV-positive CA patients treated with ALA-PDT. The recurrence rate of CA after treatment is proved to be strongly correlated with the systemic and local immune function. Yet, in Xu et al.'s study reporting 41 cases of HIV-positive homosexual men with anal warts treated with three sessions of ALA-PDT, the recurrence rate was 29% after 6 months of follow-up,<sup>10</sup> which was significantly lower than that in the present study. We believe the possible reasons for the relatively high postoperative recurrence rate in this study are as follows: First, a large proportion of patients in this study were referred from other hospitals, indicating their conditions were relatively complex and difficult to treat; Second, in Xu et al.'s study, all lesions were located in the mucosa, while in this study, only 13.2% lesions were in the mucosa, and the proportion of skin and mucosal lesions was 63.2%. HPV lesions are commonly classified as either skin- or mucosal-type, depending on the tissue origin.<sup>18</sup> The CA lesions on both the skin and mucosa suggest the infection of multiple types of HPV.<sup>19</sup> A recent published study showed that the infection of multiple HPV types is an important clinical predictor of recurrence after photodynamic therapy.<sup>11</sup> Third, the preoperative mean CD4 cell count of the patients in our study was lower than that in Xu et al.'s study. In this study, the median CD4 cell count of HIV-positive recurrent and non-recurrent patients was 213.0 cells/ $\mu$ l, and almost half of the patients had a CD4 count lower than 200 cells/ $\mu$ l. In Xu et al.'s study, only 34% of patients with HIV-positive CA had a lower-than-200 cells/ $\mu$ l CD4 cell count, and these patients received combined antiretroviral therapy (cART) before operation.

HIV-infected people usually have cellular immune dysfunction, and the immune function can be evaluated quantitatively by CD4 cell count.<sup>20</sup> The CD4 cell count in healthy adults ranges from 500 to 1500 cells/ $\mu$ l. A CD4 cell count lower than 200 cells/ $\mu$ l indicates severely damaged immune system, poor abilities to clear infection in the body, and an increased risk of opportunistic infections and cancer in HIV-infected population.<sup>21,22</sup> HIV-positive patients are more prone to CA recurrence than immunocompetent patients, which could be explained by the fact that HIV infection diminishes the number and function of CD4 cells, and hence reduces their ability of spontaneous HPV clearance.<sup>23</sup> In addition, our



data showed that CD4 cell count was closely related to the recurrence after CA photodynamic surgery, suggesting the important role of CD4 cells in photodynamic clearance of HPV infection in HIV-positive patients, which is consistent with Mistrangelo's conclusion.<sup>24</sup> Moreover, some researchers believe that patients with CD4 count lower than 200 cells/ $\mu$ l have a higher CA recurrence rate after surgical treatment, and cART treatment can help reduce postoperative recurrence.<sup>25</sup> These results suggest that it is possible to significantly reduce the CA recurrence rate after ALA-PDT if CD4 cell count is regulated before operation or at the initiation of the anti-HIV therapy and the patient's systemic immune function is improved. Yet, this supposition needs to be confirmed by further prospective studies.

Studies have shown that the inhibition of local cellular immune function is the main cause of persistent HPV infection and recurrence.<sup>26,27</sup> HPV infection can directly cause local immunosuppression by inhibiting the activity of dendritic cells, reducing the number or function of T cell subsets, interfering with the expression of immune cytokines such as IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , IL-2, IL-4, IL-10, and IL-12.<sup>27-29</sup> The advantage of ALA-PDT in the treatment of CA may be related to the activation of local-specific immunity. Giomi et al.<sup>30</sup> have found that local immune responses dominated by CD4 cells and dendritic cells play a leading role in the regression of skin lesions after 5 weeks of ALA-PDT treatment. Xie et al.<sup>26</sup> have found that the number of CD4 cells and IFN- $\gamma$  expression increase in patients with CA after a 4-h ALA-PDT treatment, and the numbers of CD3<sup>+</sup> T lymphocytes and dendritic cells, and the expression levels of IFN- $\alpha$  and IFN- $\beta$  increase after a 24-h treatment. It further confirms that ALA-PDT enhances the antiviral immune response that is mainly mediated by CD4 cells in the early stage of treatment. Shi et al.<sup>31</sup> have first used ALA-PDT to treat Majocchi's granuloma, confirming that ALA-PDT exerts an antifungal effect by activating the immune response mediated by CD4 cells. In conclusion, ALA-PDT can counter CA through activating local immunity, and CD4 cells play a major role in this process. Moreover, some studies have shown that photodynamic therapy does not affect peripheral blood T-cell level or function in the treatment of multiple actinic keratosis.<sup>32</sup> This may indicate that ALA-PDT treatment does not affect peripheral blood CD4 cells. However, the effect of ALA-PDT treatment on peripheral blood T cells of CA has not been reported, and should be analyzed in future research.

To reduce the recurrence rate after CA treatment, some clinicians give oral immune-boosting drugs and/or topical application of antiviral drugs to enhance systemic or local immunity of the patients.<sup>33</sup> Studies have shown that thymosin can increase the level of CD4 cells in HIV-infected patients, thereby to improve non-specific immunity, alleviate clinical symptoms, and prevent reinfection, which further confirms the association between CD4 cell count and the prognosis of HIV patients.<sup>34</sup> However, our data failed to confirm the effect of thymosin on the CA recurrence rate of HIV patients after photodynamic therapy, which may be related to the small sample size (only 9 patients took oral thymosin). Also, these patients began oral thymosin treatment after ALA-PDT.

Due to the low CD4 level before operation and the late start of thymosin treatment, their immune function might not recover to a desirable state. Hence, we believe that early preoperative correction of patients' immune status and improvement of CD4 cell count are of significance in preventing CA recurrence in these patients. Furthermore, the results also showed that postoperative combination with topical recombinant human interferon  $\alpha$ -2b gel had no effect on recurrence. A meta-analysis of 11 clinical studies also reached a similar conclusion. For HIV-positive patients with anal warts, there was no significant difference in the recurrence rate between electrocautery combined with intralesional injection of interferon and electrocautery alone during the 6-month follow-up.<sup>35</sup> Consistently, another study also found no clinical benefits of topical interferon ointments in the treatment of CA in HIV-positive patients.<sup>36</sup> The systemic immunosuppression in HIV-positive CA patients affects their local immune response.<sup>37</sup> Whether topical recombinant human interferon  $\alpha$ -2b gel can cooperate with ALA-PDT to eliminate HPV virus and further reduce the recurrence rate after photodynamic therapy needs further investigation.

Our study has several limitations. First, this is a retrospective study with a follow-up of merely 6 months and a relatively small sample size. Second, we did not distinguish and rule out the re-infection cases from all recurrent cases. Third, viral load was not detected in all the included cases. Fourth, the study cannot completely exclude the overreported or underreported sexual behaviors, which may lead to the misclassification of exposure bias in our estimation. Fifth, most patients did not undergo HPV typing test before photodynamic therapy. Sixth, no female subjects were included in this study, which might bring with selection bias. Seventh, histopathological examination and immunohistochemical examination were not performed to evaluate local cellular immune function. Additionally, changes in peripheral blood CD4 after treatment were not detected in this study.

In conclusion, CD4 cell count can help to predict CA recurrence in HIV-positive patients after photodynamic therapy. Our data suggest that the systemic and local immune status has a significant impact on the treatment outcome of CA.

## AUTHOR CONTRIBUTIONS

Li Gu and Shu Zhou reviewed the literature and drafted the manuscript. Zhinan Shi, Xiaoyu Zhai, and Liqun Gu helped in data acquisition and statistical analysis. Bingrong Zhou and Hui Hua revised the manuscript. All authors have approved the submission of this manuscript.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## REFERENCES

1. Ao C, Xie J, Wang L, et al. 5-aminolevulinic acid photodynamic therapy for anal canal condyloma acuminatum: A series of 19 cases and literature review. *Photodiagnosis Photodyn Ther*. 2018;23:230-234.
2. de Pokomandy A, Rouleau D, Ghattas G, et al. Prevalence, clearance, and incidence of anal human papillomavirus infection in HIV-infected men: the HIPVIRG cohort study. *J Infect Dis*. 2009;199(7):965-973.
3. Fan S, Li P, Ouyang L, et al. Anal human papillomavirus infection among MSM attending university in China: implications for vaccination. *Vaccines (Basel)*. 2020;8(2):175.
4. Li X, Li M, Yang Y, et al. Anal HPV/HIV co-infection among men who have sex with men: a cross-sectional survey from three cities in China. *Sci Rep*. 2016;6:21368.
5. Arnold JD, Byrne ME, Monroe AK, Abbott SE. The risk of anal carcinoma after anogenital warts in adults living with HIV. *JAMA Dermatol*. 2021;157(3):283-289.
6. Wu PF, Hang JF, Strong C, et al. Anal human papillomavirus and its associations with abnormal anal cytology among men who have sex with men. *Sci Rep*. 2020;10(1):3165.
7. Hu S, Yang Y, Jiang B, et al. Treatment of condyloma acuminatum using the combination of laser ablation and ALA-PDT. *Photodiagnosis Photodyn Ther*. 2019;25:193-196.
8. Szeimies RM, Schleyer V, Moll I, Stocker M, Landthaler M, Karrer S. Adjuvant photodynamic therapy does not prevent recurrence of condylomata acuminata after carbon dioxide laser ablation-A phase III, prospective, randomized, bicentric, double-blind study. *Dermatol Surg*. 2009;35(5):757-764.
9. Zhu X, Chen H, Cai L, Yu Z, Cai L. Decrease recurrence rate of condylomata acuminata by photodynamic therapy combined with CO<sub>2</sub> laser in mainland China: a meta-analysis. *Dermatology*. 2012;225(4):364-370.
10. Xu J, Xiang L, Chen J, et al. The combination treatment using CO<sub>2</sub> laser and photodynamic therapy for HIV seropositive men with intraanal warts. *Photodiagnosis Photodyn Ther*. 2013;10(2):186-193.
11. Hua H, Zhou S, Gu L, Shi Z, Gu L, Zhou B. Multiple-type HPV infection predicting condyloma acuminatum recurrence after aminolevulinic acid photodynamic therapy. *Photodiagnosis Photodyn Ther*. 2021;36:102538.
12. Shah Z, Jan R, Kumam P, Deebani W, Shutaywi M. Fractional Dynamics of HIV with Source Term for the Supply of New CD4(+) T-Cells Depending on the Viral Load via Caputo-Fabrizio Derivative. *Molecules*. 2021;26(6):1806.
13. Ye Y, Burkholder GA, Wiener HW, Aslibekyan S, Khan A, Shrestha S. CD4 trajectory models and onset of non-AIDS-defining anal genital warts, precancer, and cancer in people living with HIV INFECTION-1. *Sex Transm Dis*. 2020;47(9):628-633.
14. Parsa N, Zaheri PM, Hewitt RG, Karimi Akhormeh A, Taravatmanesh S, Wallin L. The rapid CD4 + T-lymphocyte decline and human immunodeficiency virus progression in females compared to males. *Sci Rep*. 2020;10(1):16816.
15. Bordoni V, Brando B, Piselli P, et al. Naïve/effector CD4 T cell ratio as a useful predictive marker of immune reconstitution in late presenter HIV patients: a multicenter study. *PLoS One*. 2019;14(12):e0225415.
16. de la Fuente SG, Ludwig KA, Mantyh CR. Preoperative immune status determines anal condyloma recurrence after surgical excision. *Dis Colon Rectum*. 2003;46(3):367-373.
17. Gilson R, Nugent D, Werner RN, Ballesteros J, Ross J. 2019 IUSTI-Europe guideline for the management of anogenital warts. *J Eur Acad Dermatol Venereol*. 2020;34(8):1644-1653.
18. de Villiers EM. Cross-roads in the classification of papillomaviruses. *Virology*. 2013;445(1-2):2-10.
19. Cong X, Sun R, Zhang X, Wang Y, Wang L, Yu Y. Correlation of human papillomavirus types with clinical features of patients with condyloma acuminatum in China. *Int J Dermatol*. 2016;55(7):775-780.
20. Meys R, Gotch FM, Bunker CB. Human papillomavirus in the era of highly active antiretroviral therapy for human immunodeficiency virus: an immune reconstitution-associated disease? *Br J Dermatol*. 2010;162(1):6-11.
21. Greenblatt R, Bacchetti P, Boylan R, et al. Genetic and clinical predictors of CD4 lymphocyte recovery during suppressive antiretroviral therapy: whole exome sequencing and antiretroviral therapy response phenotypes. *PLoS One*. 2019;14(8):e0219201.
22. Yirga AA, Melesse SF, Mwambi HG, Ayele DG. Negative binomial mixed models for analyzing longitudinal CD4 count data. *Sci Rep*. 2020;10(1):16742.
23. Lima MD, Braz-Silva PH, Pereira SM, Riera C, Coelho AC, Gallottini M. Oral and cervical HPV infection in HIV-positive and HIV-negative women attending a sexual health clinic in São Paulo. *Brazil Int J Gynaecol Obstet*. 2014;126(1):33-36.
24. Mistrangelo M, Cornaglia S, Pizzio M, et al. Immunostimulation to reduce recurrence after surgery for anal condyloma acuminata: a prospective randomized controlled trial. *Colorectal Dis*. 2010;12(8):799-803.
25. Mistrangelo M, Dal Conte I, Volpato S, et al. Current treatments for anal condylomata acuminata. *Minerva Chir*. 2018;73(1):100-106.
26. Xie F, Yu HS, Wang R, et al. Photodynamic therapy for genital warts causes activation of local immunity. *J Cutan Med Surg*. 2019;23(4):370-379.
27. Einstein MH, Schiller JT, Viscidi RP, et al. Clinician's guide to human papillomavirus immunology: knowns and unknowns. *Lancet Infect Dis*. 2009;9(6):347-356.
28. Veasey JV, Campaner AB, Lellis RF. Aspects of Langerhans cells and TNF- $\alpha$  in the cutaneous immunity of anogenital warts. *An Bras Dermatol*. 2020;95(2):144-149.
29. Cao Y, Zhao J, Lei Z, et al. Local accumulation of FOXP3+ regulatory T cells: evidence for an immune evasion mechanism in patients with large condylomata acuminata. *J Immunol*. 2008;180(11):7681-7686.
30. Giomi B, Pagnini F, Cappuccini A, Bianchi B, Tiradritti L, Zuccati G. Immunological activity of photodynamic therapy for genital warts. *Br J Dermatol*. 2011;164(2):448-451.
31. Shi L, Wu Q, Yang J, et al. ALA-PDT successfully treated Majocchi's granuloma by directly killing Trichophyton tonsurans and recruiting T lymphocytes. *Photodiagnosis Photodyn Ther*. 2021;35:102328.
32. Reginato E, Gruber-Wackernagel A, Wolf P. Methyl aminolevulinic acid photodynamic therapy for actinic keratosis does not affect peripheral regulatory T-cell level or function. *Photodermatol Photoimmunol Photomed*. 2015;31(5):274-278.
33. Fleshner PR, Freilich MI. Adjuvant interferon for anal condyloma. A prospective, randomized trial. *Dis Colon Rectum*. 1994;37(12):1255-1259.
34. Liu X, Liu Y, Wang L, Hu L, Liu D, Li J. Analysis of the prophylactic effect of thymosin drugs on COVID-19 for 435 medical staff: a hospital-based retrospective study. *J Med Virol*. 2021;93(3):1573-1580.

35. Werner RN, Westfechtel L, Dressler C, Nast A. Anogenital warts and other HPV-associated anogenital lesions in the HIV-positive patient: a systematic review and meta-analysis of the efficacy and safety of interventions assessed in controlled clinical trials. *Sex Transm Infect.* 2017;93(8):543-550.
36. Brockmeyer NH, Poffhoff A, Bader A, et al. Treatment of condylomata acuminata with pegylated interferon alfa-2b in HIV-infected patients. *Eur J Med Res.* 2006;11(1):27-32.
37. Arany I, Tyring SK. Systemic immunosuppression by HIV infection influences HPV transcription and thus local immune responses in condyloma acuminatum. *Int J STD AIDS.* 1998;9(5):268-271.

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