Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2026.65.010146

CD4/CD8 T-Lymphocyte Ratio as a Potential Marker of Immune Restoration in HIV-Positive Patients with an Undetectable Viral Load at the Yaoundé University Teaching Hospital

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ARTICLE INFO

Received: February 25, 2026

Published: March 24, 2026

Citation: Mbongue Mikangue CA. CD4/CD8 T-Lymphocyte Ratio as a Potential Marker of Immune Restoration in HIV-Positive Patients with an Undetectable Viral Load at the Yaoundé University Teaching Hospital Biomed J Sci & Tech Res 65(1)-2026. BJSTR. MS.ID.010146.

ABSTRACT

Background: The purpose of antiretroviral triple therapy (ART) is to make the viral load undetectable, which leads to an immune restoration that results in an increase in the level of CD4 T lymphocytes>500 cells/mm3. However, despite an increase in these cells, it was observed that CD8 T lymphocyte levels remained elevated in some people living with HIV and under ART. The objective of this study was to study the CD4:CD8 lymphocyte ratio as a predictive marker for immune restoration in HIV-positive ARV patients with an undetectable viral load in Yaoundé.

Methods: This was a cross-sectional study, conducted at the Yaoundé University Teaching Hospital (YUTH) between September 2018 and February 2019. Demographic and clinical data, T-cell profile, viral load and seroprevalence for HBV and HCV infections were determined by appropriate methods. The data obtained were analysed using Epi-info version 7.2.0.1. and presented as percentages. A $p < 0.05$ value was considered significant.

Results: A total of 94 PHAs agreed to take part in the study. The average age was 43.55 9.79 years, and female participants were the majority 68.09%. The most commonly used treatment line was Zidovudine-Lamivudine-Nevirapine at 32.62%. The average CD4 LT rate was 859.86 61 cells/mm3 (>500 cells/mm3, but 71.28% of participants had a CD4:CD8<1 ratio. The presence of HBV infection was found at 13.83% and only in patients with a CD4:CD8<1 ratio, the difference was statistically significant.

Conclusion: A CD4:CD8 < 1 ratio points to the suspicion of opportunistic infection, and the presence of HBV infection in this class demonstrates the importance of observing the CD4:CD8 T lymphocyte ratio as a marker of therapeutic success under TARV.

Keywords: ART; CD4:CD8 Ratio; HIV; LT CD4

Abbreviations: ART: Antiretroviral Triple Therapy; YUTH: Yaoundé University Teaching Hospital; HIV: Human Immunodeficiency Virus; AIDS: Acquired Immunodeficiency Syndrome; PVL: Plasma Viral Load; CTA: Approved Treatment Center; CSCDD: Center for the Studies and Control of Communicable Diseases

Introduction

The Human Immunodeficiency Virus (HIV), under certain conditions, is responsible for Acquired Immunodeficiency Syndrome (AIDS) [1]. The African continent is the most affected, and according to the 2017 UNAIDS annual report, there were 36.9 million people living with HIV (PLHIV) worldwide, more than 70% of whom were in sub-Saharan Africa [2]. With a prevalence of 3.4% according to the latest estimates provided by the National AIDS Control Committee (CNLS) [3], HIV/AIDS remains a public health problem in Cameroon [4,5]. CD4 T lymphocytes (CD4 T cells) are essential markers of immune status, as they coordinate the immune response. However, these are the main target cells of HIV because they express the CD4 molecule on their surface, a high-affinity receptor for glycoprotein (gp) 120 of the HIV membrane envelope. The CD4 T cell count tends to decrease as the infection progresses, associated with an increase

in the CD8 T lymphocyte (CD8 T cell) count. Measuring the CD4 T cell count assesses the extent of the immunological damage already sustained, hence its role as a primary therapeutic indicator [6]. Plasma viral load (PVL), which is the plasma quantification of the HIV genome, is currently the best way to assess HIV replication and the main indicator of the response to antiviral treatment in the body [7]. When initiating antiretroviral therapy (ART), it is important to compare the virological response with the immunological response for a better evaluation of treatment efficacy.

Indeed, the goal of triple antiretroviral therapy is to achieve an undetectable viral load. It has therefore been observed that in patients on ART with an undetectable viral load, a CD4 T-cell count >500 cells/mm^3 is observed. However, CD8 T-cell counts remained elevated in these people living with HIV (PLHIV), which could lead to immune hyperactivity, exposing patients to chronic inflammation responsible for comorbidities, opportunistic infections, and, in the long term, a decrease in the effectiveness of the immune response [8-11]. In this context, the objective of our work was to study the CD4:CD8 T-cell ratio and compare it to the various classic indicators of immune reconstitution, namely viral load and CD4 T-cell count.

Materials and Methods

Sampling

We conducted a cross-sectional study at the Yaoundé University Teaching Hospital (YUTH) from September 2018 to February 2019. Sampling was non-probability, conducted at the Approved Treatment Center (CTA), and participants were selected from medical records. Only the records of patients with virological success and on ART who had data meeting the study criteria were included, and sociodemographic characteristics, clinical parameters, and biological parameters were collected. Participants were included in the study after obtaining their informed consent.

Blood Samples

At the CTA, for each participant, venous blood was collected from the crook of the elbow into 5 ml EDTA tubes. Each collection day, the samples were transported to the Center for the Studies and Control of YUTH from September 2018 to February 2019. Sampling was non-probability, conducted at the Approved Treatment Center (CTA), and participants were selected from medical records. Only the records of patients with virological success and on ART, with data meeting the study criteria, were included, and sociodemographic characteristics, clinical parameters, and biological parameters were collected. Participants were included in the study after obtaining their informed consent.

Blood Samples

At the CTA, for each participant, venous blood was collected from the crook of the elbow in 5 ml EDTA tubes. Each collection day, the samples were transported to the Center for the Studies and Control of Communicable Diseases (CSCCD) of the Faculty of Medicine and Biomedical Sciences for measurement of T-cell counts, viral load, and testing for HBV and HCV infections.

Determination of HBV and HCV Viral Serology

This was performed using the cassette immunochromography technique according to the manufacturers' instructions (HBV 5 in 1 Combtest (S/P) Nantong, Diagnos Biotechnologyco., China) for HBV and (One Step Rapid Test HCV AB Test Cassettes (S/P) Hightop Boitech, China) for HCV.

Determination of Viral Load

This was performed using the Cobas Ampliprep /Cobas Taqman 96 platform (Roche Diagnostics Branchburg, New Jersey USA), strictly adhering to the manufacturer's instructions. The limit of detection was <40 copies/ml [12].

CD4 and CD8 Lymphotyping

Measurements of the various T cell subpopulations (CD3, CD4, and CD8) were performed using immune phenotyping. Fifty microliters (50 µl) of whole blood from each sample were used for this analysis, using the BD FACS Count analyzer (BD Biosciences, San Jose, California, USA), according to the manufacturer's instructions.

Interpretations

The collected data were interpreted according to the following criteria for each variable: - Virological success was defined as a CVP < 50 copies/ml, Immunological success was defined as a CD4:CD8 T-cell ratio ≥1, - Immunological failure was defined as a CD4:CD8 T-cell ratio < 1. After database cleaning, the data were analyzed using Epi-info software version 7.2.0.1. Descriptive data are presented as counts and percentages. Fisher's exact test was used for all comparisons, and a p-value < 0.05 was considered statistically significant.

Ethical and Administrative Considerations

The research protocol received administrative authorization from the Yaoundé University Teaching Hospital Directorate, reference number 245/AR/CHUY/DG/DGA/CAPRC. Participants were included in the study after obtaining their consent. Standard measures necessary to guarantee the confidentiality of the information collected in the records were taken. Only patient file numbers were recorded, and access to the data was secured by an encrypted password.

Results

Demographic Characteristics

During the study period, a total of 94 people living with HIV (PLHIV) on ART, followed at the Yaoundé University Hospital, who met the selection criteria, agreed to participate in the study. Female participants were in the majority, 68.09% (n=64), and male participants represented 31.01% (n=30), for a sex ratio of 0.46. The mean

age was 43.55 ± 9.79 years, and the most represented age group was 41-50 years (47.87%) (Table 1).

Table 1: Demographic Parameters.

Variables	Number (n= 94)	Percentage (%)
Sex		
Female	64	68.09
Male	30	31.01
Age Group(per year)		
< 20	5	5.32
21-30	4	4.26
31-40	19	20.21
41-50	45	47.87
51-60	20	21.28
61-70	1	1.06

Clinical and Biological Parameters

All 94 patients were confirmed to have virological success (viral load <50 copies/ml) and were at clinical stage II, according to the WHO classification. Hepatitis B virus (HBV) coinfection was 13.83% (n=13) with p=0.80. These participants were serotyped: 91 cases of HIV-1M (96.81%) and 3 cases of HIV-2 (3.19%). Patients were on ART protocols according to national guidelines, and the most common first-line protocol was Zidovudine-Lamivudine-Nevirapine (AZT+3TC+NVP) with 31 patients (32.62%), while 6 people were on a second-line protocol (Table 2).

Table 2: Clinical Parameters.

	Number(n=94)	Percentage (%)
Serological type		
VIH-1 M	91	96.81
VIH-2	3	3.19
HBV Coinfection		
Absence	81	86.17
Présence	13	13.83
Duration of Treatment (per year)		
<10	52	55
≥10	42	45
ART Treatment Protocol		
AZT + 3TC + NVP	31	32.97
AZT + 3TC + EFV	28	29.78
TDF + 3TC + EFV	7	7.44
TDF+3TC+NVP	22	23.40
AZT+3TC+ATV/r	4	4.25
TDF+3TC/ATV/r	2	2.12

Characterization of T Cell Counts

All 94 patients had a high CD4 T cell count (>500 cells/mm^3), with a median count of 859 cells/mm^3. Twenty-seven (27) patients, or 28.72%, had a CD4/CD8 T lymphocyte ratio ≥ 1 (with a median CD4 T cell count of 868 cells/mm^3 and a median CD8 T cell count of 831 cells/mm^3), and 67 patients, or 71.28%, had a CD4/CD8 T lymphocyte ratio < 1 (with a median CD4 T cell count of 855 cells/mm^3 and a median CD8 T cell count of 1007 cells/mm^3) (Table 3).

Table 3: Characterization of T Cell Rates.

Components of the CD4/CD8 Ratio	Median (cells/mm³)	CD4:CD8 Lymphocyte Ratio	Number (n=94)	Percentage (%)
LT CD4	868	≥1	67	71.28
LT CD8	831			
LT CD4	855	<1	27	28.72
LT CD8	1007			

By correlating treatment duration, HIV/HBV coinfection, and CD4:CD8 ratio, 24 of the 42 patients with a treatment duration > 10 years had a CD4:CD8 T-cell ratio ≥ 1. This result is statistically significant (p=0.01). The 13 HIV/HBV co-infected patients were diagnosed in those with a ratio < 1, and no co-infections were diagnosed in patients with a ratio ≥ 1. This result is statistically significant (p=0.01) (Table 4).

Table 4: CD4/CD8 Ratio According to HIV/HBV Coinfection.

Ratio CD4 :CD8	HIV/HBV Coinfection	Mean	Number (n=94)	(%)	*p value*
<1	Présence	850.38 ± 88.13	13	13.83	0.001
≥1	Absence	861.06 ± 86.83	81	86.17	

Discussion

The mean age of the patients in our study was 43.55 ± 9.79 years, with a range of 19 to 63 years. We also had a female predominance at 68.09. This agrees with the 2011 data from the Demographic and Health Survey and Multiple Indicator Cluster Survey in Cameroon, which gave us a female prevalence of 5.6%, and the 15-49 age group as being the most exposed to sexually transmitted infections because it is the most sexually active [13]. 96.70% of the PLHIV in our sample were infected with HIV-1M. This predominance of HIV-1M could be explained by the fact that it is group M of HIV type 1 that is the most widespread in the world and is responsible for the current pandemic [14]. For reasons of availability, and because it is highly active, as recommended by the World Health Organization [3], the most used therapeutic protocol was AZT/3TC/NVP. Our study found that all patients had a CD4 T-cell count >500 cells/mm^3, which could explain the efficacy of triple therapy [3]. However, this study also presented two classes of CD4:CD8 T-cell ratios: 28.72% and 71.28% for CD4:CD8 ≥ 1 and CD4:CD8 < 1, respectively. Our data are close to those of Tang et al. in 2016, whose CD4:CD8 < 1 ratio was 77.4% [15], which could demonstrate immunological failure. Indeed, most of our patients had been on ART for a long time, which makes HIV infection chronic. This chronicity is responsible for immune sene scence and chronic T-cell activation/ inflammation, with fibrotic destruction of lymph nodes and lymphoid tissue [16].

In the present study, the seroprevalence of HBV infection was 13.83% (13 cases). This seroprevalence is high considering that our participants had virological success associated with a CD4 T cell count >500 cells/mm^3. The presence of HIV/HBV coinfection could not be justified if only the CD4 T cell count is considered as a marker of immunological recovery. Indeed, it was only observed in patients with a CD4:CD8 ratio < 1, a ratio that suggests the suspicion of an opportunistic infection. Therefore, based on this T lymphocyte ratio, immunological recovery and immunological success can be better assessed [17]. According to Happi Mbakam, et al. who worked in Cameroon in 2017 at the same hospital, the CD4:CD8 ratio can be predictive of immunological dysfunction associated with disease progression and thus be used as an immunological marker in the monitoring of people living with HIV (PLHIV) [12].

Conclusion

Our study included PLHIV with virological success and on ART for at least one year. It was found that although all patients had a CD4 T-cell count >500 cells/mm^3, HBV infection was observed only in the class with a CD4/CD8 T-cell ratio <1. A CD4/CD8 ratio <1 suggests the suspicion of an opportunistic infection, and the presence of HBV infection in this class demonstrates the importance of observing the CD4/CD8 T-cell ratio as a marker of therapeutic success under ART.

Acknowledgement

We would like to thank the management of the Yaoundé University Teaching Hospital for allowing us to conduct the study in this institution, as well as all its staff and the patients for their availability and cooperation in this research.

Conflict of Interest

The study was fylly funded by the authors and they do not declare no conflit of interest.

Authors Contributions

- **Study design:** Mbongue-Mkangue CA
- **Field data collection:** Mbongue-Mikangue CA
- **Data analysis and / or interpretation:** Mbongue-Mikangue CA
- **Manuscript revision:** Mbongue-Mikangue CA
- **Approval final version:** Mbongue-Mikangue CA.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2026.65.010146

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