

Inverted CD4⁺/CD8⁺ ratio associated with AIDS event and death in HIV- 1 infected individuals in Nasarawa State, Nigeria

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Abstract: The current guidelines for the use of antiretroviral therapy in Nigeria places emphasis on the use of CD4⁺ enumeration to take decision of initiating antiretroviral therapy and HIV disease monitoring. CD4⁺ counts are known to be inherently inconsistent and therefore could be misleading. This study was undertaken to analyze the CD4⁺/CD8⁺ ratio vis-à-vis CD4⁺ in HIV-1 infected individual in North Central Nigeria and to correlate these immunological parameters to AIDS event and death in relation to gender and age of patients. Cell counts were carried out using a Fluorescence Activated Cell Sorter (FACS) that quantifies CD4⁺ and CD8⁺ T lymphocytes as absolute numbers of lymphocytes per μ L of blood and the CD4⁺/CD8⁺ T lymphocyte ratio recorded in an automated fashion. A total of 290 HIV-1 positive persons were enrolled for this study. The median CD4⁺/CD8⁺ ratio were 0.05, 0.27, 0.64 in patients with CD4⁺ counts of <50, 51-200, >350 respectively. CD4⁺/CD8⁺ of 0.05 and 0.27 were corresponding predictors of AIDS-related event and death. Patients with >0.64 are predictive of better disease prognosis and low progression to AIDS. The CD4⁺/CD8⁺ were minimally higher in female patients with a median CD4⁺/CD8 ratio of 0.27. The age distribution of our patients at point of first entry was not found to influence CD4⁺/CD8⁺ ratios. These findings provide basic and critical CD4⁺/CD8⁺ cut-off values in predicting HIV disease progression and an alternative to absolute CD4⁺ counts at predicting the onset of HIV related disease. These data are useful to determine when intervention with antiretroviral therapy is needed and to determine the likelihood of virological failure.

Key Words: CD4⁺/CD8⁺ ratio, HIV/AIDS, anti-retroviral therapy, Nigeria

Introduction

Nigeria has the largest HIV/AIDS epidemic in sub-Saharan Africa (UNAIDS, 2007). The Government of Nigeria and other international agencies are vigorously instituting programs to increase the awareness of HIV/AIDS infection in the country. Unfortunately, this has not translated into change in behavior as the rate of infection continues to rise unabated (Raufa, 2000; Nwokoji & Ajuwon, 2004; Udonwa *et al.*, 2004). The current guidelines for the use of antiretroviral therapy in Nigeria places emphasis on the use of CD4⁺ enumeration to take decision of initiating antiretroviral therapy and HIV disease monitoring (FMOH, 2001, 2004; Weidle *et al.*, 2002). Therefore, the extent of immunosuppression and the probability of developing an AIDS-related complication in HIV-infected individuals are usually measured by the absolute number of CD4⁺ positive T-cells. CD4⁺ counts are known to be inherently inconsistent as many other factors like vaccination, diurnal variation and inter-laboratory differences may affect it and therefore could be misleading

(Hoover, 1993; Bartlett & Gallant, 2004). With inherent weaknesses of the dependence on CD4⁺ counts, several researchers have sought for other alternatives in monitoring HIV/AIDS in Nigeria (Akinola *et al.*, 2004; Erhabor *et al.*, 2005).

Other immunological changes in HIV infection include a transient increase in CD8⁺ cells and inversion of CD4⁺/CD8⁺ ratio (Cooper *et al.*, 1988; Pedersen *et al.*, 1990; Normann *et al.*, 2004). This is one of the best surrogate markers for the assessment of the risk for progression to AIDS in HIV infected individuals (Li *et al.*, 2002). The ratio of CD4⁺ cells to CD8⁺ in healthy people is between 0.9 and 1.9. The CD4⁺/CD8⁺ ratio observed among adult Indians is 0.94 which is closer to the Chinese and Ethiopians values but lower than the ratio of 1.36 reported in North American populations (Yeni *et al.*, 2002). Low CD4⁺/CD8⁺ ratios have also been reported in Saudi Arabians (Shahabuddin, 1995). The CD4⁺/CD8⁺ values for healthy adults in Central African Republic (CAR) have been reported to be significantly reduced compared to the ratio for healthy Europeans (Menard *et al.*, 2003).

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CD4⁺ counts <50 cells/ μ l and between 51-199 cells/ μ l, when compared with patients with CD4⁺ counts >350 cells/ μ l have been identified as factors associated with AIDS events before highly active anti-retroviral treatment (HAART) prescription (Hogg *et al.*, 2001). Consideration of CD4⁺ level in terms of CD4⁺/CD8⁺ ratio can be a good alternative to absolute CD4⁺ count in determining disease status in HIV infected individuals and predicting response to HAART (Bonnet *et al.*, 2005). This study was carried out to determine the correlation between CD4⁺/CD8⁺ ratios and CD4⁺ counts among HIV-1 infected individuals in an attempt to describe critical cut-off values that are predictive of HIV disease status.

Materials and methods

Study site and population

This study was conducted between July 2005 and September 2006 among western blot confirmed HIV-1 positive patients who attended the Virology Laboratory, Innovative Biotech, in Keffi, Nasarawa State, Nigeria, for voluntary HIV counselling and testing or for other health needs. The patients enrolled in this study were anti-retroviral naïve and at their first seropositive visit. CD4⁺ and CD8⁺ test results are recorded in the laboratory in a computer database in an anonymous fashion.

Our laboratory is located in north central Nigeria and is a complete diagnostic and research virology facility that performs testing for a full range of human pathogens of viral origin including human immunodeficiency virus, hepatitis viruses, respiratory and enteric viruses. We use real-time molecular techniques and serological assays such as Western blot for HIV, rapid detection of viral antigens and antibodies. We support local health care delivery and other health care organizations through the provision of exceptional laboratory services. The laboratory is continuously modifying its test algorithm in order to keep at breast of current developments and provide better services to

our patients. The catchment population of our laboratory is North Central Nigeria although we do receive clinical specimens from the whole country. The patients enrolled in this study were selected randomly and who met the criteria stated above. HIV-2 seropositive patients were not included in this study.

Fluorescence Activated Cell Sorter analysis of T-lymphocyte profile

CD4⁺ and CD8⁺ values were measured using a Fluorescence Activated Cell Sorter (FACS) system (Becton Dickenson FACSCount, Canada). Briefly, this system quantifies CD4⁺ and CD8⁺ T lymphocytes as absolute numbers of lymphocytes per μ L of blood. The CD4⁺/CD8⁺ T lymphocyte ratio is recorded in an automated fashion using the manufacturer's protocol and reagents.

Data analysis

The Pearson coefficient (r) was used to estimate the correlation of CD4⁺ and CD4⁺/CD8⁺ ratios after recording the data on a Microsoft Excel worksheet on a Windows '98 platform. The median and interquartile ranges of the CD4⁺/CD8⁺ ratios were also determined. The interpretation of CD4⁺/CD8⁺ ratios is dependent on the AIDS defining absolute CD4⁺ counts that are already known (Hogg *et al.*, 2001).

Ethical consideration

The study protocol was approved by the Innovative Biotech Research Committee and informed consent was obtained from all patients prior to blood collection.

Results

A total of 290 HIV-1 positive patients were recruited for the study. Their corresponding CD4⁺/CD8⁺ ratio vis-à-vis CD4⁺ cell counts and distribution by gender are summarised in Table 1. The distribution of CD4⁺/CD8⁺ ratios and their corresponding CD4⁺ cell values by age are summarised in Table 2.

Table 1: CD4⁺/CD8⁺ ratio vis-à-vis CD4⁺ cell absolute counts and their distribution by gender

Category	Gender		Total	CD4 ⁺	CD4 ⁺ Range	CD4 ⁺ /CD8 ⁺ Ratio	Median CD4 ⁺ /CD8 ⁺ Ratio	Pearson coefficient (r)
	Male	Female						
A	45	41	86	CD4 ⁺ <50	1-43	0.01-0.09	0.05 (0.11)	1
B	20	74	94	CD4 ⁺ =51-200	139-199	0.07-0.046	0.27 (0.029)	1
C	46	64	110	CD4 ⁺ >350	368-755	0.41-0.87	0.64 (0.47)	1
Total	111	179	290					

Females had a minimally higher CD4⁺/CD8⁺ ratios when compared with males (Table 1). The age distribution was not found to influence CD4⁺/CD8⁺ ratios (Table 2)

predicting AIDS event and death. Whether our logic may not apply to all individuals is subject to be answered in a more robust study nationwide. Consequently, decisions made on the

Table 2: The Distribution of CD4⁺/CD8⁺ ratio by age

Median CD4 ⁺ /CD8 ⁺ ratio	CD4 ⁺	Age group-1	Age group-2	Age group-3	Total
		20-40years	41-60years	>61years	
0.05	CD4 ⁺ <50	54	23	3	80
0.27	CD4 ⁺ =51-200	78	18	1	97
0.64	CD4 ⁺ >350	83	19	11	113
	Total	215	60	15	290

Discussion

It has been established that HIV-1 infection is characterized by an inverted CD4⁺/CD8⁺ T-cell ratio (Cooper *et al.*, 1988; Pedersen *et al.*, 1990), but the basic critical cut-off values/ratios to predict AIDS event are not known and its use to independently predict time to AIDS event and death has not been fully explored in our setting (Margolick *et al.*, 2006). To our knowledge, this is the first study in Nigeria to examine the phenomenon of CD4⁺/CD8⁺ inversion in predicting HIV disease status.

Our result indicates CD4⁺/CD8⁺ ratios of 0.05 and 0.27 as indicators for AIDS event and death with those below 0.05 being closer to end-point HIV infection. If CD4⁺ counts of 200 are a good starting point for HIV therapy, then CD4⁺/CD8⁺ ratio of 0.27 can be taken as a cut-off ratio to take decisions on the commencements of HAART in Nigeria. Also, if CD4⁺>350/ μ L is considered, then CD4⁺/CD8⁺ ratio of >0.64 could be used to take crucial decisions depending on which have comparative advantage for the patient. We therefore propose the use of these values in monitoring disease progression. On average, low CD4⁺/CD8⁺ ratios identify a group of individuals who are more likely to be in ill health. In patients with CD4⁺/CD8⁺ of >0.64 (correlating with CD4⁺>350/ μ L) the clinical progression to AIDS is low. This correlates with the findings of Stephenson (2002). This study therefore has determined critical cut-off CD4⁺/CD8⁺ ratios necessary to predict AIDS event and disease progression to cut down overdependence of CD4⁺ count which is known to have inherent controversies (Hoover, 1993; Johnson, 2000). CD4⁺/CD8⁺ ratios are likely to be a good alternative surrogate marker for

basis of CD4⁺/CD8⁺ ratios should be interpreted with caution particularly decisions that could prove potentially damaging, such as starting antiretroviral medications (Mayer *et al.*, 2003).

The CD4⁺/CD8⁺ ratio is rarely less than 1.0 in HIV negative individuals, but may drop as low as 0.1 in patients with recent HIV infection or very advanced disease (Li *et al.*, 2002). There is almost always substantial recovery of this ratio, even without antiretroviral therapy, during the second to third month of HIV infection, which then persists for some time. The CD4⁺/CD8⁺ ratio will generally gradually decline over years of HIV infection in the absence of antiretroviral therapy (Bonnet *et al.*, 2005). With therapy this ratio may again rise to above 1.0 - a recovery rarely seen in patients with more advanced HIV infection (Cooper *et al.*, 1988; Pedersen *et al.*, 1990; Normann *et al.*, 2004).

In this study, there was no obvious gender preponderance with regards to these ratios. This may be as a result of other factors that we did not investigate. Previous studies had shown female sex associated with increased percentage of CD4⁺ cells (Tollerud *et al.*, 1989). Also, from our study, it is difficult to say exactly which age group of patients is likely to present with lower CD4⁺/CD8⁺ ratios. The reason for this is not immediately obvious. Tollerud *et al.* (1989) had found that age was independently associated with an increased percentage of CD4⁺ cells. Further research to properly document and better define these situations is needed. In conclusion, we have found critical cut-off CD4⁺/CD8⁺ ratios that correlate with CD4⁺ values that are predictive of AIDS event and death. The lower the CD4⁺/CD8⁺ ratio (0.05 and 0.27 or below), the worse the damage to the immune system. CD4⁺/CD8⁺ ratios of >0.64 area

predictors for low disease progression and may be employed in routine HIV/AIDS monitoring in Nigeria.

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