

## Establishment of Reference Values of CD4 and CD8 Lymphocyte Subsets in Healthy Nigerian Adults

D. K. Oladepo, E. O. Idigbe, R. A. Audu, U. S. Inyang, G. E. Imade, A. O. Philip, G. O. Okafor, D. Olaleye, S. B. Mohammed, N. N. Odunukwe, T. O. Harry, M. Edyong-Ekpa, J. Idoko, A. Z. Musa, A. Adedeji, A. Nasidi, Y. Ya'aba and K. Ibrahim

*Clin. Vaccine Immunol.* 2009, 16(9):1374. DOI:  
10.1128/CVI.00378-08.

Published Ahead of Print 29 July 2009.

---

Updated information and services can be found at:  
<http://cvi.asm.org/content/16/9/1374>

---

*These include:*

**REFERENCES**

This article cites 13 articles, 1 of which can be accessed free at:  
<http://cvi.asm.org/content/16/9/1374#ref-list-1>

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](#)

---

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>  
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

## Establishment of Reference Values of CD4 and CD8 Lymphocyte Subsets in Healthy Nigerian Adults<sup>∇</sup>

D. K. Oladepo,<sup>1\*</sup> E. O. Idigbe,<sup>2</sup> R. A. Audu,<sup>2</sup> U. S. Inyang,<sup>1</sup> G. E. Imade,<sup>3</sup> A. O. Philip,<sup>4</sup>  
G. O. Okafor,<sup>5</sup> D. Olaleye,<sup>6</sup> S. B. Mohammed,<sup>1</sup> N. N. Odunukwe,<sup>2</sup> T. O. Harry,<sup>7</sup>  
M. Edyong-Ekpa,<sup>8</sup> J. Idoko,<sup>3</sup> A. Z. Musa,<sup>2</sup> A. Adedeji,<sup>9</sup> A. Nasidi,<sup>9</sup>  
Y. Ya'aba,<sup>1</sup> and K. Ibrahim<sup>1</sup>

*National Institute for Pharmaceutical Research and Development, Idu, Abuja,<sup>1</sup> Nigerian Institute of Medical Research, Yaba, Lagos,<sup>2</sup> Jos University Teaching Hospital, Jos,<sup>3</sup> Uthman Danfodio University Teaching Hospital, Sokoto,<sup>4</sup> University of Nigeria Teaching Hospital, Enugu,<sup>5</sup> University College Hospital, Ibadan,<sup>6</sup> University of Maiduguri Teaching Hospital, Maiduguri,<sup>7</sup> University ofuyo Teaching Hospital, Uyo,<sup>8</sup> and Federal Ministry of Health, Abuja,<sup>9</sup> Nigeria*

Received 17 October 2008/Returned for modification 12 December 2008/Accepted 3 July 2009

**A total of 2,570 apparently healthy human immunodeficiency virus-negative adults from the six geopolitical zones in the country were enrolled in our study in 2006. The samples were assayed using the Cyflow technique. Data were analyzed using the Statistical Package for Social Scientists (SPSS). The majority (64%) of the participants had CD4 counts within the range of 501 to 1,000 cells/ $\mu$ l. The reference range for CD4 was 365 to 1,571 cells/ $\mu$ l, while the reference range for CD8 was 145 to 884 cells/ $\mu$ l.**

In Nigeria, although country-specific reference ranges for some hematological measures have been determined (3, 5, 6), national data for CD4 reference values are still not available. Prior to a few recent monocenter studies carried out among defined populations of healthy Nigerians (2, 13), CD4 reference range values from literature based on studies in western countries were largely employed for clinical decision making.

However, as the access to treatment increased in Nigeria, it became critically necessary to determine on a national level the reference values for CD4 cell counts and the factors that may affect it. This was necessary to inform the clinicians of the required minimal range for the initiation of antiretroviral therapy, and also for accurate monitoring of responses to therapy and other treatment outcomes.

The national study reported in this document was a multicenter study conducted among healthy human immunodeficiency virus (HIV)-negative adult Nigerians, in eight sites across the six geopolitical zones of the country. Therefore, the objective of this study was to establish the normal reference values of CD4 and CD8, as well as CD4/CD8 lymphocyte ratios, indigenous to Nigeria.

This project was carried out as a cross-sectional study among apparently healthy Nigerians aged 18 years and older who tested HIV negative at voluntary counseling and testing site sites. Exclusion criteria included pregnancy, sickle cell anemia, or clinical illness.

A 5-ml sample of blood was collected from each participant by venipuncture into a Vacutainer EDTA bottle. These samples were retested using the Genie II kit (Bio-Rad), which is a rapid HIV serology test kit. Only samples that were confirmed negative were assayed for CD4 and CD8 cell counts concur-

rently using the Cyflow technique, with an instrument known as the Cyflow counter (Partec). This instrument is for counting and analyzing particles and cells. The first step in the measurement of cells is staining with a fluorescent dye. The fluorescent molecules are taken up by the cells. The cells are individually illuminated by light of a defined wavelength. The light activates the fluorescent molecules so that they emit light of a characteristic color (wavelength). This fluorescent light is filtered out, and its intensity is measured by a ploidy analyzer for each single cell. The fluorescence light intensity emitted by a labeled cell is proportional to its CD4 or CD8 content. For cell counting or concentration determination, the sample volume detector measures exactly 0.2 ml of the sample volume. Each fluorescent cell in this volume is counted, from which the ploidy analyzer determines the concentration, or the number of cells per ml.

Data were entered and analyzed using the Statistical Package for the Social Scientist (SPSS). The mean, median, and standard deviation values were calculated for the CD4 and CD8 counts and the CD4/CD8 ratios. The reference ranges were determined using the 2.5 and 97.5 percentiles of CD4<sup>+</sup>, CD8, and CD4/CD8 parameters between males and females.

The Kruskal-Wallis H test was used to compare the distributions of the hematological parameters among the age groups and marital statuses.

A total of 2,570 participants were enrolled in this exercise; data collated on the demographic characteristics of the participants are reflected on Table 1. Data were generated on the sex, age, marital status, occupation, and geographical zone of each participant. Among the participants, 53% were males, and the age distributions showed that the majority (34.7%) were within the age group of 26 to 35 years. The marital status data of the participants showed that 49.5% were married, while an evaluation of their occupational status indicated that most (41.9%) were employed, with a distribution of 32.4% in the public sector and 9.5% in the private sector.

The percentage distribution of participants within various

\* Corresponding author. Mailing address: National Institute for Pharmaceutical Research and Development, P.M.B. 21, Garki, Abuja, Nigeria. Phone: 23483855848. Fax: 2348033855848. E-mail: daudaoladepo@yahoo.com.

<sup>∇</sup> Published ahead of print on 29 July 2009.

TABLE 1. Demographic characteristics of study participants<sup>a</sup>

Characteristic	Total no. (%) of patients	No. (%) of patients		Mean (SD)			P value		
		Male	Female	CD4 (cells/ $\mu$ l)	CD8 (cells/ $\mu$ l)	CD4/CD8 ratio	CD4	CD8	CD4/CD8 ratio
Sex	2,570	1,363 (53)	1,207 (47)	847 (307)	435 (191)	2.3 (2.2)	0.001	0.001	0.002
Age (yr)							0.008	>0.05	>0.05
18–25	818 (32.0)	402 (29.6)	416 (34.6)	861 (288)	448 (193)	2.2 (1.6)			
26–35	889 (34.7)	461 (34.0)	428 (35.6)	836 (300)	432 (188)	2.3 (3.0)			
36–45	495 (19.3)	286 (21.1)	209 (17.4)	826 (313)	424 (182)	2.2 (1.0)			
46–60	331 (12.9)	189 (13.9)	142 (11.8)	876 (343)	426 (192)	2.5 (2.2)			
$\geq$ 60	26 (1.0)	19 (1.4)	7 (0.6)	774 (433)	457 (284)	1.8 (1.7)			
Marital status							>0.05	>0.05	0.029
Married	1,139 (49.5)	596 (49.2)	543 (49.8)	844 (315)	447 (201)	2.2 (1.3)			
Single	1,110 (48.2)	607 (50.1)	503 (46.1)	852 (286)	433 (187)	2.3 (3.0)			
Widowed	41 (1.8)	5 (0.4)	36 (3.3)	990 (387)	416 (140)	2.5 (1.0)			
Divorced or separated	13 (0.6)	4 (0.3)	9 (0.8)	1,022 (689)	332 (213)	5.3 (8.2)			
Occupation							>0.05	>0.05	0.007
Student	949 (39.5)	435 (34.4)	514 (45)	844 (285)	448 (190)	2.1 (1.5)			
Unemployed	446 (18.6)	238 (18.8)	208 (18.2)	847 (338)	440 (199)	2.2 (1.7)			
Employed	1,009 (41.9)	590 (58.5)	419 (41.5)	859 (319)	429 (194)	2.4 (2.9)			
Zone							0.0001	0.0001	0.0001
North East	395 (15.4)	174 (12.8)	221 (18.3)	804 (245)	501 (180)	1.7 (0.4)			
North West	399 (15.5)	265 (19.4)	134 (11.1)	783 (233)	396 (143)	2.1 (0.7)			
North Central	398 (15.5)	209 (15.3)	189 (15.7)	801 (307)	398 (200)	2.8 (4.4)			
South West	581 (22.6)	315 (23.1)	266 (22.0)	879 (313)	409 (172)	2.4 (1.6)			
South East	394 (15.3)	198 (14.5)	196 (16.2)	908 (329)	424 (166)	2.4 (1.1)			
South South	403 (15.7)	202 (14.8)	201 (16.7)	889 (368)	496 (255)	2.1 (2.2)			

<sup>a</sup> Some of the row totals do not add up to the total figure of 2,570 because of missing data. Subjects with missing values were left out of the analysis for each variable.

ranges of CD4 cell counts is illustrated in Fig. 1. The majority (64%) of the participants had CD4 counts within the range of 501 to 1,000 cells/ $\mu$ l. While 22% had CD4 counts within the range of 1,001 to 1,500 cells/ $\mu$ l, 8.3% had a range of 351 to 500 cells/ $\mu$ l. Table 2 shows that the estimated mean CD4 cell count for the study was 847 ( $\pm$ 307) cells/ $\mu$ l. The reference range was 365 to 1,571 cells/ $\mu$ l. The estimated mean CD8 count was 435 ( $\pm$ 191) cells/ $\mu$ l, while the reference range was 145 to 884 cells/ $\mu$ l. Similarly, the mean CD4/CD8 ratio for the entire study population was 2.3 ( $\pm$ 2.2), with an estimated reference range of 0.7 to 5.3. The differences observed between the two sexes were statistically significant ( $P < 0.05$ ).

The distribution of mean CD4 counts among different age groups showed that the 18- to 25-year age group had a significantly higher CD4 cell count of 861 cells/ $\mu$ l with a lower standard deviation ( $\pm$ 288), while the lowest count of 774 ( $\pm$ 433) cells/ $\mu$ l was recorded among those older than 60 years of age (Table 1). However, there was no significant difference in the distributions of CD8 counts and CD4/CD8 ratios between the different age groups. Table 1 also shows the distribution of the T-lymphocyte cells among the subjects with different marital status. Though the lowest mean CD4 count observed was among the married subjects and the highest was among the separated and divorced subjects, the difference was not statistically significant. However, the mean CD4/CD8 ratio among the separated and divorced subjects was significantly higher than those among the other groups.

The effect of participants' occupation on the distribution of CD4 and CD8 counts as well as on that of the CD4/CD8 ratios

was also recorded as shown in Table 1. There was no significant difference in the distribution of the mean CD4 and CD8 counts among the students, the unemployed, and the employed. However, the employed had a significantly higher CD4/CD8 ratio. Whereas, within the zones, the southern zones had significantly higher CD4 counts, even though the North Central zone had the highest CD4/CD8 ratio (0.0001).

The absolute CD4 T-lymphocyte count is one of the best surrogate markers for assessing the risk of progression to AIDS among HIV-infected individuals. This marker is also useful in determining the risk of developing certain AIDS-related opportunistic infections and the time for initiating antiretroviral and prophylactic antimicrobial therapies (5). The laboratory staging of HIV infection is also based on this marker. It became necessary to establish the reference values for CD4 and immunological markers among healthy HIV-negative Nigerians, which will provide a guide for the initiation of antiretroviral therapy in Nigeria.

Since Nigeria is a very heterogeneous country, care was taken to have a fairly even distribution of both sexes and marital statuses. There was unbiased representation of the unemployed, employed, and student populations. All adult age groups were represented, and every geopolitical zone was fairly represented except for the South West, which enrolled more participants. However, this difference was adequately catered for in the statistical analysis of the data generated to prevent any bias. The majority of the participants had CD4 counts of  $>$ 350 cells/ $\mu$ l. The significant difference seen in the distribution of CD4 counts between the northern and southern zones

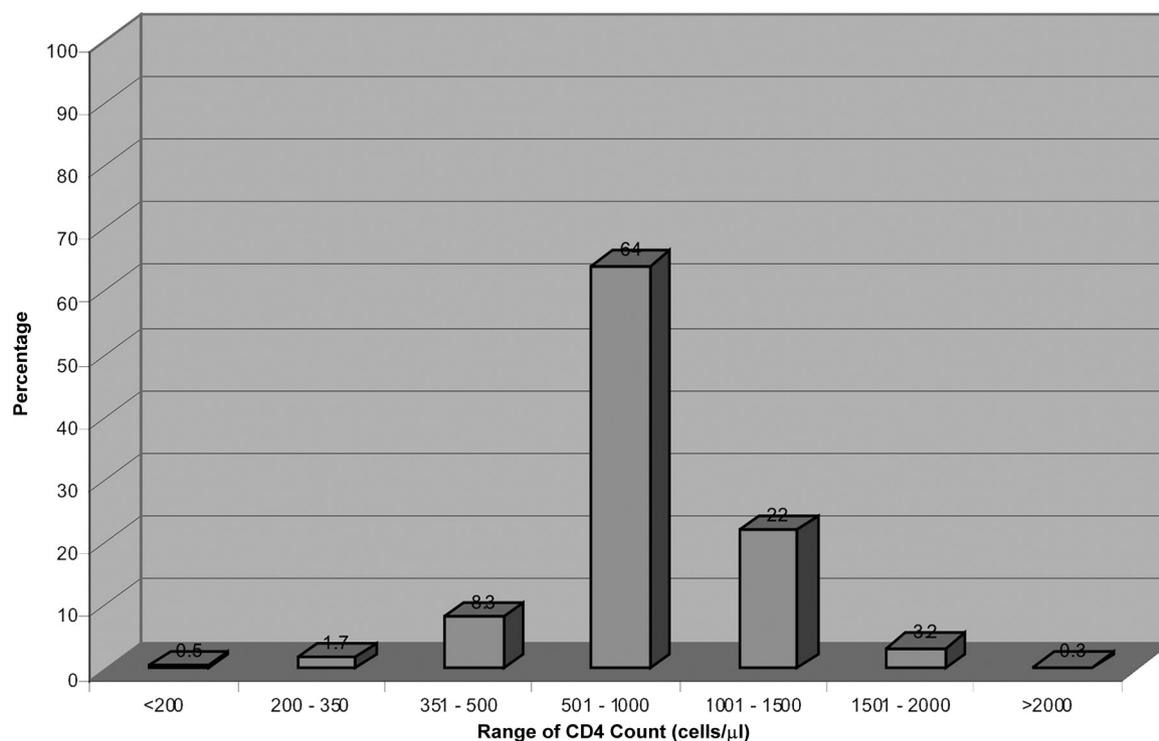


FIG. 1. Distribution of CD4 counts for all subjects.

of the country confirms the heterogeneous nature of Nigeria. This observation could be attributed to differences in environmental, genetic, or nutritional factors (12). Despite these differences, all zones had similar distributions in the CD4 ranges, whereby the highest frequency occurred at the 501 to 1,000 cells/μl range. This showed that the CD4 T lymphocytes were equally distributed among Nigerians irrespective of the zone. The methodological variation was controlled, since all study sites used the same type of instrument for analyzing the samples and the analysis of the results was done centrally; in addition, count check beads were used for internal control, and exchange of samples within 6 hours between sites was effected for external control. Similar to earlier studies carried out in Nigeria (1), there was no significant difference between the CD4 cell counts when compared by age groups. This is consistent with findings from India, Kuwait, and Central African Republic (9, 12).

In this study, contrary to reports from the earlier limited studies carried out in Nigeria (1), females were found to have significantly higher values of absolute CD4 counts, although

the results of this study agree with the reportedly higher CD4/CD8 ratios in females. This observation has also been reported in several other countries among Africans and Caucasians (9, 12), like Uganda and Ethiopia (10). A sex hormone effect is one possible explanation for the reported difference in CD4 counts between genders that has been suggested (10).

This comprehensive study involving all the geopolitical zones of the country has established the reference range for CD4 counts in Nigeria to be from 365 to 1,571 cells/μl. This is similar to earlier reports based on studies of smaller isolated populations in the country (1, 3, 7), though the range from this study is wider. The lower cutoff point obtained in this study is higher than the WHO recommended cutoff point of 350 cells/μl for initiating therapy. Comparing the mean CD4 counts obtained with values reported from other parts of the world, except for Botswana and India (4), which reported lower values, the other countries, from East Africa, Tanzania and Uganda (11); from West Africa, Ghana (2); Central African Republic (12); as well as those in Europe (13), had mean values greater than what was recorded in this study.

TABLE 2. Reference ranges of the CD4 and CD8 counts and the CD4/CD8 ratio of study participants<sup>a</sup>

Study group	CD4 (cells/μl)			CD8 (cells/μl)			CD4/CD8 ratio		
	Mean (SD)	Median	Reference range	Mean (SD)	Median	Reference range	Mean (SD)	Median	Reference range
Male	782 (272)	746	351–1,455	422 (184)	417	155–863	2.1 (1.4)	1.9	0.7–5.1
Female	920 (327)	892	383–1,654	450 (197)	401	133–919	2.4 (2.8)	2	0.8–5.8
Male and female	847 (307)	812	365–1,571	435 (191)		145–884	2.3 (2.2)	1.9	0.7–5.3

<sup>a</sup> *P* value is 0.0001 for CD4, 0.001 for CD8, and 0.002 for CD4/CD8. *P* values of <0.05 were computed with the Mann-Whitney U test, comparing the distribution of the immunologic parameters between the sexes.

Meanwhile, the mean CD4 count of 847 cells/ $\mu$ l in this study is similar to the mean value of 828 cells/ $\mu$ l recorded in an earlier study in Nigeria (1). A variety of factors have been reported to influence population differences in the CD4 counts. In a study of health of Asian and non-Asian populations living in the United States, the CD4 cell counts were found to be significantly different (6); race was found to significantly affect the CD4 counts. This confirms the heterogeneity of different populations. Interlaboratory variability and the use of different methods in the measurement of absolute CD4 cell counts could possibly account for the observed differences. Despite the differences in the mean CD4 and CD8 cell counts in all of the studies referred to earlier, the CD4/CD8 ratios were all greater than 1, as is expected in healthy adult individuals with no overt infections. It is worth noting that the CD4/CD8 ratio among Nigerian adults was higher than those reported for all the other countries mentioned earlier. This shows that Nigerians generally have more-competent immune systems. This is unexpected, given the relatively poor environmental sanitation of the country, which should enhance infections, thereby lowering the immune system due to morbidity.

Those aged 60 years had the lowest mean CD4 count. This might explain why the elderly people fall ill more often than the younger ones, who have more-competent immune systems. Looking at marital status, it was observed that the married subjects had the lowest mean CD4 counts and, consequently, have a significantly lower CD4/CD8 ratio. Since a number of factors are associated with low CD4 cell counts, including psychological stress (8), it is plausible that the married population undergo more stress than the unmarried counterparts, resulting in the observed trend.

From the results of this widespread and comprehensive national study, it is concluded that the normal reference range for the CD4 counts for healthy adult Nigerians is from 365 to 1,571 cells/ $\mu$ l. This range could be useful in enrolling HIV patients into the ART program and as a basis for making clinical decisions.

We are grateful to the National AIDS and STD Control Programme (NASCP) and the National Agency for the Control of AIDS (NACA) in Nigeria, which provided the grant that funded this project.

We are also grateful to all who volunteered to participate in this study.

#### REFERENCES

1. Aina, O., J. Dadik, M. Charurat, P. Amangaman, S. Gurundi, E. Mang, R. Guyit, N. Lar, P. Datong, C. Daniyam, P. Kanki, A. Abimiku, et al. 2005. Reference values of CD4 T-lymphocytes in human immunodeficiency virus-negative adult Nigerians. *Clin. Diagn. Lab. Immunol.* **12**:525–530.
2. Ampofo, W., K. Torpey, Y. D. Mukadi, K. Koram, K. Nolan, R. Amenyah, E. Kaitoo, P. Antevi, D. Ofori-Adjei, and P. Lamptey. 2006. Normal CD4+ T-lymphocyte levels in HIV seronegative individuals in the Manyaliya Krobo communities in the eastern region of Ghana. *Viral Immunol.* **19**:260–262.
3. Audu, R. A., E. O. Idigbe, A. S. Akanmu, A. G. Mafe, J. Onyewuche, and C. T. Oparaugo. 2007. Values of CD4+ T-lymphocyte in apparently healthy individuals in Lagos, Nigeria. *Eur. J. Sci. Res.* **16**:168–173.
4. Coetzee, M. J., P. N. Badenhorst, J. I. De Wit, and G. Joubert. 1994. Haematological condition of the San (Bushmen) relocated from Namibia to South Africa. *S. Afr. Med. J.* **84**:416–420.
5. Idoko, J. A., M. O. Njoku, M. D. Sirisena, and D. Jelpe. 2001. CD4+ T-lymphocyte counts in human immunodeficiency virus (HIV) infected healthy Nigeria population. *Niger. Med. Pract.* **39**:53–56.
6. Levin, A., G. Brubaker, J. S. Shao, D. Kumby, T. R. O'Brien, J. J. Geodert, K. W. Stauss, W. A. Blattner, and I. Hannet. 1996. Determination of T-lymphocyte subsets on site in rural Tanzania: results in HIV-1 infected and non-infected individuals. *Int. J. STD AIDS* **7**:288–291.
7. Menard, D., M. J. Madana, M. B. Tothy, E. K. Kelembho, G. Gresenguet, and A. Talarmin. 2003. Immuno hematological reference ranges for adults from Central African Republic. *Clin. Diagn. Lab. Immunol.* **10**:443–445.
8. Messele, T., M. Abdulkadir, A. L. Fontanet, B. Petros, D. Hamann, M. Koot, M. T. Roos, P. T. Schellkens, F. Miedema, and T. F. Rinke De Wit. 1999. Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. *Clin. Exp. Immunol.* **115**:442–450.
9. Njoku, M. O., N. D. Sirisena, J. A. Idoko, and D. Jelpe. 2003. CD4+ T-lymphocyte counts in patients with human immunodeficiency virus type 1 (HIV-1) and healthy population in Jos, Nigeria. *Postgrad. Med. J.* **10**:135–139.
10. Prins, M. J., J. R. Robertson, R. P. Brettler, I. H. Aguado, B. Broers, F. Boufassa, D. J. Foldberg, R. Zangerle, R. A. Coutinho, and A. Vandean Hoek. 1999. Do gender differences in CD4 counts matter? *AIDS* **13**:2361–2364.
11. Reichert, T., M. De Bruyere, V. Deneys, T. Totterman, P. Lydyard, F. Yuksel, H. Chapel, D. Jewell, L. Vand Hore, J. Lindern, and L. Buchner. 1991. Lymphocyte subset reference ranges in adult Caucasians. *Clin. Immunol. Immunopathol.* **60**:190–208.
12. Tugume, S. B., E. M. Piwowar, T. Lutalo, P. N. Mugenyi, R. M. Grant, F. W. Mangeni, K. Pattishall, and E. Katongole-Mbidde. 1995. Hematological reference ranges among healthy Ugandans. *Clin. Diagn. Lab. Immunol.* **2**:233–235.
13. Uppal, S. S., S. Verma, and P. S. Dhot. 2003. Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity and smoking. *Cytometry* **52B**:32–36.