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# Immunohematological Reference Ranges for Adults from the Central African Republic

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**A survey was carried out on 150 healthy adults to establish hematological reference ranges for human immunodeficiency virus (HIV)-negative adults from the Central African Republic (CAR). Immunohematological mean values, medians, and 95th-percentile reference ranges were established. Mean values were as follows: leukocyte (WBC) counts, 5.28 × $10^9$/liter (males) and 5.11 × $10^9$/liter (females); erythrocyte counts, 5.20 × $10^{12}$/liter (males) and 4.50 × $10^{12}$/liter (females); hemoglobin, 15.1 g/dl (males) and 12.5 g/dl (females); hematocrit, 45% (males) and 37% (females); lymphocytes, 2,587/μl (males) and 2,466/μl (females); CD4 T cells, 927/μl (males) and 940/μl (females); CD8 T cells, 898/μl (males) and 716/μl (females); and CD4/CD8 T-cell ratio, 1.13 (males) and 1.41 (females). We concluded that (i) the WBC and hemoglobin values of healthy HIV-negative adults from the CAR are lower than the reference values currently used in the CAR and (ii) the absolute CD4 T-cell counts of healthy HIV-negative adults from the CAR are similar to values for Europeans but the absolute CD8 T-cell counts are much higher. Thus, the CD4/CD8 T-cell ratios for healthy adults from the CAR are significantly reduced compared to the ratios for healthy Europeans.**

Hematological reference values for adults from the Central African Republic (CAR) have never been established. The values that are currently used in Central African countries are adopted from textbooks that mainly refer to Caucasian subjects (20). Similarly, the immunological reference values used in the CAR are derived from non-Central African subjects. CD4 T cells are important in the monitoring of human immunodeficiency virus (HIV) infection progression, and immunological reference values for parameters such as total lymphocytes and their subpopulations need to be established for adults from the CAR (7, 8, 17). At the end of 2000, the prevalence of HIV infection was estimated as 15% of the population (about 500,000 cases of HIV infection) (Ministry of Health, unpublished data).

Several factors affect immunohematological parameters, including genetics, dietary patterns, sex, age, and altitude (9, 20). These factors differ depending on the population and geographical area studied, and radical differences have been reported in immunohematological parameters worldwide. For example, low CD4 T-cell counts in Asians (11) and Chinese (3, 4), low CD4/CD8 T-cell ratios in Saudi Arabians (16), and leucopenia in Sierra Leoneans (15) have been observed.

Thus, the reference values that have been validated for nonadults from the CAR could be misleading when used for other patients from the CAR. Given this background, we performed a study to establish immunohematological reference values for future use in the CAR.

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## MATERIALS AND METHODS

**Subjects.** A total of 150 adults from the CAR (68 men [45.3%] aged from 17 to 58 years and 82 women [54.7%] aged from 16 to 47 years) were enrolled in this study. Individuals included were healthy adults between 15 and 60 years of age presenting for HIV testing at the HIV testing and counseling center. They were checked by physicians and were included in the study if they were found to be HIV negative. Patients were not enrolled if they were pregnant, had been vaccinated in the previous 3 months, were receiving medical treatment, or had a recent or current infection.

**Blood collection and HIV serology.** Whole blood was collected with a Vacutainer system in 10-ml tubes containing EDTA. HIV status was determined with plasma samples by an enzyme-linked immunosorbent assay with Vidas HIV DUO kit (BioMérieux France, Marçy l'Etoile, France) and Determine test (Abbott). All samples were collected between 7:00 and 12:00 a.m. at the Pasteur Institute laboratory and were analyzed on the day of collection.

**Hematological analysis.** A Coulter AcT Diff 2 analyzer that was standardized against a 4C plus blood control was used for whole-blood analysis of hematological parameters. The machine automatically dilutes a whole-blood sample of 29.6 μl, lyses, and counts and gives a printout result of absolute numbers of leukocytes (WBC) (expressed as number of cells × [$10^9$] per liter), erythrocytes (RBC) (number of cells × [$10^{12}$] per liter), platelets (number of cells × [$10^9$] per liter), lymphocytes (number of cells × [$10^9$] per liter), mononuclear cells (number of cells × [$10^9$] per liter), and granulocytes (number of cells × [$10^9$] per liter). Differentiation between neutrophils, eosinophils, and basophils was not made. In addition, hemoglobin (in grams per deciliter) and hematocrit (in percent) were measured by the analyzer.

**Flow cytometric analysis.** Lymphocyte subsets were analyzed on a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) with two monoclonal antibodies (aCD4 and aCD8; Becton Dickinson Immunocytometry Systems). In brief, 100 μl of whole blood was mixed and incubated at room temperature for 20 min with 10 μl of aCD4 and aCD8. RBC were then lysed by adding 2 ml of fluorescence-activated cell sorter lysing solution (Becton Dickinson Immunocytometry Systems). After vortexing, tubes were incubated in the dark at room temperature for 10 min and centrifuged at 300 × *g* for 5 min. The cell pellet was washed once with 2 ml of Facs Flow solution (Becton Dickinson Immunocytometry Systems), resuspended in 500 μl of Facs Flow solution, and analyzed with the FACSCalibur's CellQuest software (Becton Dickinson Immunocytometry Systems). The FACSCalibur was calibrated with fluorescent beads (CaliBrite; Becton Dickinson Immunocytometry Systems) and Auto-Comp software (Becton Dickinson Immunocytometry Systems) weekly. By using quality control (Multicheck; Becton Dickinson Immunocytometry Sys-

TABLE 1. Means, medians, and 95th-percentile reference ranges of hematological parameters and WBC subset absolute counts for 150 HIV-negative adults from the CAR

| Subject group (*n*) and parameter | Value for group | | |
|---|---|---|---|
| | Mean ± SD (*P*)[a] | Median | 95% Range |
| Male (68) | | | |
| WBC ($10^9$/liter) | 5.28 ± 1.57 | 5.05 | 2.9 ± 8.3 |
| RBC ($10^{12}$/liter) | 5.20 ± 0.49 | 5.14 | 4.50 ± 6.10 |
| Hemoglobin level (g/dl) | 15.1 ± 1.3 | 14.9 | 12.3 ± 17.3 |
| Hematocrit (%) | 45 ± 4 | 45 | 39 ± 52 |
| Platelet ($10^9$/liter) | 230 ± 70 | 225 | 124 ± 378 |
| Lymphocyte[b] | 2,587 ± 767 | 2,471 | 1,512 ± 4,224 |
| CD4 T cell[b] | 927 ± 349 | 851 | 380 ± 1,617 |
| CD8 T cell[b] | 898 ± 336 | 828 | 267 ± 1,545 |
| CD4/CD8 T-cell ratio | 1.13 ± 0.45 | 1.03 | 0.34 ± 1.88 |
| Female (82) | | | |
| WBC ($10^9$/liter) | 5.11 ± 1.38 (NS) | 4.90 | 2.7 ± 8.0 |
| RBC ($10^{12}$/liter) | 4.50 ± 0.49 ($<10^{-6}$) | 4.50 | 3.42 ± 5.44 |
| Hemoglobin level (g/dl) | 12.5 ± 1.3 ($<10^{-6}$) | 12.5 | 9.1 ± 14.9 |
| Hematocrit (%) | 37 ± 4 ($<10^{-6}$) | 38 | 28 ± 44 |
| Platelet ($10^9$/liter) | 235 ± 68 (NS) | 228 | 117 ± 382 |
| Lymphocyte[b] | 2,466 ± 595 (NS) | 2,404 | 1,452 ± 3,680 |
| CD4 T cell[b] | 940 ± 291 (NS) | 912 | 386 ± 1,454 |
| CD8 T cell[b] | 716 ± 255 ($<10^{-4}$) | 646 | 226 ± 1,225 |
| CD4/CD8 T-cell ratio | 1.41 ± 0.49 ($<10^{-4}$) | 1.33 | 0.60 ± 2.27 |

[a] Values in parentheses are *P* values (Mann-Whitney U test) for comparisons of means for male and female subjects. NS, not significant.
[b] Values are absolute cell counts.

tems), the accuracy of the technique was assessed every 6 months. Each week, furthermore, divided clinical samples were tested to assess the intralaboratory coefficient of variation.

**Statistical analysis.** Data were entered and analyzed with Epi Info 6.04 software. The mean, median, and standard deviation values were calculated for each immunohematological parameter. The 95th-percentile reference ranges were determined by using 2.5 and 97.5 percentiles. The Mann-Whitney U test was used to compare the distribution of immunohematological parameters between genders. Means comparisons between our study and others were performed by using analysis of variance with the Fischer test.

**Ethics.** Informed consent was obtained from each participant.

## RESULTS

Table 1 shows the means, medians, and 95th-percentile reference ranges according to gender for the hematological parameters and WBC subset absolute counts. The distributions of the RBC parameters (median hemoglobin, hematocrit, and RBC) were statistically different by gender, women having lower values than men ($P < 10^{-6}$). No gender-specific differences were observed for WBC or platelets.

The various WBC subset values were not significantly different between men and women except for the CD8 T-cell count, which was lower ($P < 10^{-4}$) in women, and (consequently) the CD4/CD8 T-cell ratio, which was lower ($P < 10^{-4}$) in men. No significant differences were found between age classes (under 20 years of age, between 20 and 29 years, between 30 and 39 years, and above 40 years) concerning the different parameters studied.

## DISCUSSION

The peripheral blood absolute CD4 cell count, CD4 percentage, and CD4/CD8 ratio values are among the best surrogate markers for the assessment of the risk for progression to AIDS in HIV-infected individuals. They are clinically useful in assessing the risk of developing certain AIDS-related opportunistic infections and for timing the initiation of antiretroviral and prophylactic antimicrobial therapies (5). Current guidelines for the initiation of antiretroviral therapy and preventive therapy against *Pneumocystis carinii* and *Mycobacterium avium* complex are based on serial assessments of the CD4 lymphocyte count. The quantification of CD4 lymphocyte counts as a surrogate marker of the stage of HIV infection and the overall degree of immunosuppression are widely used in the stratification and follow-up of HIV-infected individuals in clinical trials (7).

Therefore, it was necessary to establish hematological reference ranges among healthy adults from the CAR, since reference ranges derived from North American or European populations may not be representative of those in African people and since the CAR is one of the countries in Central Africa with the highest prevalence of HIV-1 infection. This study presents the preliminary data needed to establish normal hematological reference ranges to facilitate the interpretation of AIDS-related research studies in Africa. The finding of significant gender differences for the RBC parameters (RBC, hemoglobin, and hematocrit), as shown in Table 1, is consistent with the well-established fact that men have higher values for RBC, hemoglobin, and hematocrit than women, with the difference partly due to the influence of the androgen hormone on erythropoiesis and also due to menstrual blood loss in women. No differences between the genders with regard to WBC and platelet counts were observed. The general absence of gender differences for WBC counts is in agreement with other reports (2, 15, 18). We found that the values for WBC (males, $2.9 \times 10^9$ to $8.3 \times 10^9$/liter; females, $2.7 \times 10^9$ to $8.0 \times 10^9$/liter) and hemoglobin (males, 12.3 to 17.3 g/dl; females, 9.1 to 14.9 g/dl) were significantly lower in our study than those established in Ethiopia (18), Europe (18), and the United States (20).

Low values for these hematological parameters have also been reported from other African countries, and our values are similar to those observed for Madagascar (13). Lymphocyte counts for adults from the CAR are significantly higher than those found in studies conducted in Ethiopia (18), Saudi Arabia (1), Madagascar (13), and The Netherlands (18). In contrast, higher counts were reported for Uganda (19) and Cameroon (21), but a statistical analysis could not be made. As previously described (6, 14), differences in WBC subset values between age classes were not significant; therefore, it was possible to group the results for adolescents and young adults with those for others. CD4 T-cell counts for adults from the CAR are similar to those for adults from Europe or Cameroon. But they are significantly higher than those observed in Ethiopia (means = 775/μl) (18) and significantly lower than those observed in Uganda (means = 1,256/μl) (19). As observed in other African countries, CD8 T-cell values and CD4/CD8 T-cell ratios were similar to those reported for African adults (10). In general, this study confirms previous reports of high CD8 T-cell counts in Africans (12, 18, 19, 20). The high prevalence of infection and nutritional factors have been implicated as possible contributors to the reduced CD4/CD8 T-cell ratios (20).

With regard to gender differences for lymphocyte subsets, women from the CAR were found to have significantly lower CD8 T-cell counts and higher CD4/CD8 T-cell ratios than men. Higher CD4/CD8 T-cell ratios in women have also been reported in Uganda (19) and Ethiopia (18), but the difference was mainly due to higher CD4 counts in women.

We propose that in the absence of other data, the present immunohematological reference ranges for adults from the CAR should be used in the clinical management of patients from the CAR.

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