

## Short Report

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# Determination of T-lymphocyte Subsets in a North African Population (Tunisia): Establishment of Normal Ranges and Results in HIV-Infected Individuals

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## INTRODUCTION

Human Immunodeficiency Virus (HIV) infection is characterized by a depletion of the CD4+ T-lymphocytes. Absolute counts of CD3+CD4+ cells have been used in monitoring the progression of this disease and in assessing clinical trials of drugs developed to treat HIV infection and related complications [6,10]. In 1993, the definition of AIDS was revised to include HIV-infected persons whose CD4+ T-lymphocytes were  $\leq 200$  per  $\mu\text{l}$ , even in the absence of opportunistic infections or neoplasm.

Both the CDC (Center for Disease Control) and WHO (World Health Organization) have emphasized the need to study lymphocyte subsets in normal populations [3]. It is thus necessary for a testing laboratory to establish normal ranges for these subsets in its local population. A number of studies have been done on lymphocyte subsets

in healthy individuals. There is, however, a paucity of published data about Tunisians and North Africans in general. Therefore, our first objective in the present study was the establishment of normal ranges for CD3+CD4+ and CD3+CD8+ cells in a healthy Tunisian population. After that, the second step was the assessment of lymphocyte subsets in HIV infected individuals.

## MATERIALS AND METHODS

### Subjects

The population studied comprised 52 healthy blood donors of both sexes, aged from 19 to 56, and 53 HIV-1 seropositive patients, also of both sexes and aged from 23 to 57.

### Flow cytometric analysis

Aliquots of blood samples were stained with a panel of TriTEST reagents, using TruCount Absolute Count tubes (Becton Dickinson). Data were collected on the cytometer (FacsCalibur, Becton Dickinson) using MultiSet software.

## RESULTS AND DISCUSSION

Tables 1 and 2 show data on lymphocyte subsets for the healthy donors and the HIV-1 seropositive patients. Among the 53 seropositive

Table 1  
Lymphocyte subsets in percentages for the healthy donors and the HIV-1 seropositive patients

	Healthy donors			HIV seropositives			$\chi^2$	P
	Average	DS	Min-max	Average	DS	Min-max		
CD3+	71.8	6	58-83	75	15.8	25-94.4	0.0019	NS
CD3+CD4+	43.2	7.6	29-66	12.3	9.25	0.244-32	1.7969	< 10 <sup>-3</sup>
CD3+CD8+	24.1	7.6	12-40	58.7	15.6	21.3-98	0.8462	< 10 <sup>-3</sup>
CD4:CD8 ratio	1.93	0.8	0.72-4.55	0.24	0.2	0.004-0.9	6.166	< 10 <sup>-3</sup>

Table 2  
Lymphocyte subsets in absolute counts for the healthy donors and the HIV-1 seropositive patients

	Healthy donors			HIV seropositives			$\chi^2$	P
	Average	DS	Min-max	Average	DS	Min-max		
CD3+	1345	353	632-1937	1229	400	54-4894	0.0081	NS
CD3+CD4+	799	245	388-1597	227	309	0-1347	1.80	< 10 <sup>-3</sup>
CD3+CD8+	470	202	170-1140	840	679	43-2855	0.346	< 10 <sup>-3</sup>

Table 3  
Distribution of patients according to their clinico-biological stage (CDC classification; 1993)

Clinical category	Number of patients	Biological category	Number of patients	Average of CD3+CD4+ cells/mm <sup>3</sup>
A	2 (5.13%)	A1	0	0
		A2	2 (5.13%)	331
		A3	0	0
B	17 (43.6%)	B1	4 (10.3%)	991
		B2	5 (12.8%)	291
		B3	8 (20.5%)	75
C	20 (51.3%)	C1	1 (2.56%)	591
		C2	2 (5.13%)	271
		C3	17 (43.6%)	57

subjects, 39 are classified according to clinical stage (we have no clinical data for the others), using the CDC classification (1993), and the results are shown on Table 3.

This report provides data on CD3+, CD3+CD4+ and CD3+CD8+ lymphocyte counts, and CD4:CD8 ratios, in an HIV seronegative North African (Tunisian) population (Tables 1 and 2). There is little published data of this type.

When we compare these Tunisian normal values to results obtained in other countries, we note that:

Both the percentage and the absolute count of CD3+ and CD3+CD4+ cells are very similar to those published in French [2], Belgian [7] and

Australian studies [3]. Both the percentage and the absolute count of CD3+CD8+ cells are slightly decreased compared to those obtained in the study of Blanc et al. [2] using the same cytometer mark, the same reagents and the same analysis method, but the differences are not significant.

Finally, when we consider the CD4:CD8 ratio calculated from the percentages of CD3+CD4+ and CD3+CD8+ cells, we note that it is much higher than the value reported by Hannet et al. [7] (average = 1.2; min = 1; max = 1.5) and could be explained by the high CD8+ value in this Belgian study.

Comparing HIV seronegative patients with HIV seropositive subjects, the latter group has significantly lower percentages and numbers of CD3+CD4+ cells ( $p < 10^{-3}$ ), higher numbers of CD3+CD8+ cells ( $p < 10^{-3}$ ) and a lower CD4:CD8 ratio ( $p < 10^{-3}$ ). There is no difference in the percentage and the number of CD3+ cells between these two groups.

These results are in accord with those obtained all over the world. The depletion of CD3+CD4+ cells is not clearly explained at present; however, an increase of the CD3+CD8+ cells is explained by expansion of the cytotoxic T-lymphocyte population during HIV infection.

We used, in our laboratory, the CD38 marker in a two-stain combination with CD8, in HIV seropositive samples, and we found that nearly all of the CD3+CD8+ cells were CD38 positive, concluding that these cytotoxic T-lymphocytes were activated, most probably by the virus.

Considering the distribution of HIV seropositive patients according to their clinico-biological stage (CDC classification; 1993), we note that the majority of patients are in the second (43.6%) and the third (51.3%) clinical categories (B and C), and most of them are in the third biological category (20.5% B3; 43.6% C3). The paucity of subjects of the first clinical category (A) in our data can be explained by the fact that the HIV seropositive patients are diagnosed late, and when they come to our laboratory for a T-lymphocytes subsets count, they are already at the B or C category. The lack of antiretroviral medicines and the natural progression of the disease also explain the bigger number of patients in the third biological categories with an average number of 75 CD3+CD4+ cell/mm<sup>3</sup> for the 20.5% patients (B3 category) and 57 CD3+CD4+ cell/mm<sup>3</sup> for the 43.6% patients (C3 category).

## CONCLUSION

In Tunisia, HIV infection is relatively not extensive: among the 10 million inhabitants, the total number of declared seropositive individuals since the beginning of the epidemic until now, is

one thousand (0.01%); two hundred of them have AIDS and every year, there is a mean of 46 new declared cases. HIV infection is not a major problem for Public Health in Tunisia, ahead of other pathologies like hepatitis or cancers. However, the extent of the epidemic in the world causes us to be worried about the future. Therefore, we started in our laboratory by establishing normal ranges for T-lymphocytes subsets counts that could be a reference for all North Africa. Following this, we can monitor the efficacy of treatment available to our patients.

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