Range-reference determination of lymphocyte subsets in Moroccan blood donors

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Abstract

Background: Information on lymphocyte populations (T, B, and Natural killer cells) and subpopulations (CD4 and CD8) in Morocco is scarce if not inexistent.

Objective: To establish a reference value of these cells in 242 Moroccan young adult blood donors by flow cytometry.

Results: Smokers had significantly higher total leukocyte count ($p < 0.001$), total lymphocyte count ($p < 0.0001$) and higher CD3+CD4+ cells ($p < 0.0001$). The percentage of CD3-CD56+ subsets was affected by smoking ($p < 0.01$).

Our analysis positively correlate with previous observations of an increase of absolute CD4+ T cells, with no changes in other lymphocyte subset cells in smokers.

The lymphocyte subpopulation distributions for all antigens were found to be similar to those reported in Saudi and Italian adults, while higher levels were reported for the same gender in other countries, especially Ghana and Kuwait.

Conclusion: The international classification standards of the HIV-infected subjects according to their rates of CD4 are applicable to the present study's population.

Key words: Young Moroccan adults, flow cytometry; Lymphocyte counts, cigarette effects

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Introduction

Human peripheral blood immunologic cell counts may vary among healthy individuals in terms of age, sex, stress, race, smoking habits, diet and physical exercise. [1, 2, 3, 4, 5, 6] These variations may also be due to pathologies that involve the immune system (infections, acquired immunodeficiency syndromes, immuno-heamatological and auto-immune disorders), medication or during immunotherapy.[7] Past research has demonstrated country-related differences of CD4 ranges, which are essential biomarkers in the following and the monitoring responses to antiviral therapies in Human Immunodeficiency Virus-infected individuals.[8]

In this regard, it is of great importance to establish a referential range of lymphocyte subsets (more importantly CD4) for each population; thus, differences between normal and pathologic values can be established, knowing that these pathologic values cannot be reliably quantified if they are not rigorously controlled and compared to the normal ones. These values may be determined through outcome measures such as flow cytometry, being such an easy, straightforward method for immuno-phenotyping lymphocyte subsets. Therefore, our study was carried out to establish reference value for lymphocyte subsets in a group of healthy Moroccan blood donors by flow cytometry.

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Methods

Study population

242 healthy military blood donors were the informants in this study. They were all considered free of clinical infection; check-up of blood pressure, pulse rate, and hemoglobin were controlled in the Rabat Military Hospital Blood Transfusion Center. All donors were screened for syphilis, HIV[1,+2], viral hepatitis B and C infections by routine serological tests respectively Biokit hemaglutination assay, Biorad HIV[1,+2], Enzygnot AgHBs and Murex HCV and were negative. Blood samples were collected in EDTA-tubes and used within 6 hours of storage at temperature room. Specimens including 222 males and 20 females were analyzed, as well.

Flow Cytometry

Dual labeled monoclonal antibodies (CD3-Fluorescein IsothioCyanate (FITC)/ CD4-PhycoErythrin (PE), CD3-FITC/CD8-PE, CD3-FITC/CD56-PE, CD3-FITC/CD19-PE, CD45-FITC/CD14-PE and IgG1-FITC/IgG2a-PE isotype control (opticlones from Immunotech, Beckmann Coulter, Marseille, France) were used for the direct immunostaining. 10 µl of every MAb was added to 50 µl of whole blood adjusted to 5000 cells/µl, incubated 10 minutes in dark at room temperature. Afterwards, the cells were lysed with NH_4Cl for 30 minutes and were immediately analyzed by flow cytometer (Beckman Coulter: Epics XL equipped with Argon laser excitation at 488 nm).

The panel used was the same as recommended by the CDC for performing CD4+ T-cell determinations in individuals infected with HIV.[9] Lymphocyte analysis was carried out with a particular attention to the purity of the lymphocyte gate: more than 95% was the maintained purity level. T Lymphocytes were defined with CD3 expression. As to the lymphocyte sub-populations, only cells with double staining were considered positive for T4 and T8 cells respectively CD3+CD4+ and CD3+CD8+ cells. B and NK (Natural Killer) cells were CD19+CD3- and CD56+CD3- respectively.

We resorted to a dual-platform approach to generate absolute lymphocyte subset cell counts using a hematology analyzer (ACT-10). The absolute values for T4 and T8 cells were obtained by multiplying the percentage of lymphocytes which co-express CD3 and CD4, CD3 and CD8 and that the value obtained by the absolute lymphocyte count. CD19 and CD56 absolute values were obtained by multiplying their percentages by the absolute lymphocyte count. The lymphocyte recovery and the consistency of some check sums were rigorously performed as internal quality controls.

Analytical tools

Differences between means of males and females, smokers and non-smokers were compared by Kruskal-Wallis test. The percentages were compared by ANOVA. The data of hematological studies were used for comparison. *p*-values less than 0,05 were considered to reflect the significance level.

Ethical consideration

All donnors gave informed consent and the donation was voluntary.

Results

The age of the scrutinized population ranged between 19 and 49 years with a means value of 27,8 years. The distribution of individuals according to their sex and smoking habits is shown in table I: The predominant sex in the studied group was male. Indeed, the sex ration M/F is 11,1: Table 1 shows that 71% of individuals are non-smokers.

Table 1: Study population characteristics

Characteristics	Males	Female	Total
Smokers	69	2	71
Non-smokers	151	20	171
Total population	220	22	242

Table 2 shows the complete blood count for the study group. The absolute counts of human leukocyte and total lymphocyte in the study population are respectively 6,822x103±1,687x103 and 1876±500 cells/µl. No statistically significant differences were found between males and females.

Table 2: Complete blood count data in 242 blood donors

Parameters	Mean	Minimum	Maximum
WBC (10^3)	6,8	3700	11700
RBC (10^6/µl)	5,1	3,9	13,8
Hgb (g/dl)	15,0	11,6	18
PCV (%)	45,6	4,0	95,9
MCV (fL)	88,5	20,6	105,8
MCHC (g/dl)	31,7	3,1	30,6
Plt (10^3/µl)	215,4	24	374

WBC: Whole blood cells; RBC: Red blood cells; Hgb: haemoglobin; PCV: Hematocrit or packed cell volume; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; Plt: Platelets, Ly: Lymphocytes.

The relative and absolute numbers of lymphocyte subpopulations with their corresponding standard deviations are given in table 3. For normal values of various lymphocyte components, we found that the mean values of 1876 cells/µl (28,44%) for total lymphocytes, 871 cells/µl (53%) for CD3+CD4+ cells, 637,54 cells/µl (33%) for CD3+CD8+ cells, 211 cells/µl (11,73%) for CD3-CD56+ and 159 cells/µl (5,26%) for CD19.

Table 3: Relative and absolute lymphocyte subset population counts

Parameters	Mean	Minimum	Maximum
Ly (%)	28,44	11	47
Ly/µl	1876	900	3600
CD3(%)	74,3	52	89
CD4(%)	52,9	23	68
CD8(%)	32,9	17	57
CD56(%)	11,7	2	34
CD19(%)	5,26	1	12
CD3 cells/µl	1431,5	605	3026
CD4 cells/µl	871,8	363	2278
CD8 cells/µl	637,5	252	1512
CD56 cells/µl	211	300	1124
CD19 cells/µl1	59	3	319

Absolute and relative counts of lymphocytes and lymphocyte subpopulations in non-smokers and smokers are given in table 4. With the exception of CD3/CD56+ lymphocytes (p= 0.01), no significant differences between smokers and non-smokers were identified for the percentages among all investigated subpopulations.

Table 4: Absolute and relative counts of lymphocytes and lymphocyte subpopulations in non-smokers and smokers

	Smokers	Non-smokers	p-value
	Nb: 71	171	
WBC (10^3)	7,54±1,74	6,55±2,58	p<0,001
Ly (%)	29,82±6	27,87±7	p=0,04
Ly	2208±562	1797,8±443	p<0,001
CD3(%)	74,0±6,13	74,5±6,9	p=0,29
CD4(%)	45,18±8,05	45,5±6,9	p=0,38
CD8(%)	31,11±6,55	33,75±6,53	p=0,20
CD56(%)	12,15±5,9	10,61±6,95	p=0,01
CD19(%)	7,79±4,2	7,04±3,8	p=0,20
CD3	1639±480	1346±379	p<0,0001
CD4	998±820	731±224	p<0,0001
CD8	692±258	615±211	p<0,052
CD56	234±170	221±127	p=0,84
CD19	145±98	124±71	p=0,17

The *p* values were determined by the Kruskall-Wallis test.

In contrast, absolute leukocyte counts according to smoking status showed several significant differences in lymphocyte subpopulations between smokers and non-smokers. In addition to an increased number of peripheral white blood cells, smokers also had a higher total count of blood lymphocytes. This increase is mostly due to an increase of T lymphocytes (CD3+), especially of CD4+ lymphocytes. Statistically, no significant increase could be detected for CD8+ cells, B and NK lymphocytes.

Discussion

The present study was undertaken to determine relative and absolute numbers in human peripheral blood lymphocyte subsets among 242 Moroccan blood donors to establish a reference range. Our population was relatively young, with 90% of male sex and 70% non-smokers. This is not surprising for Moroccan military population, because the majority of the Moroccan military people are males, retiring at 50 years old. All individuals had a normal complete blood count. To the best of our knowledge, this is the first study reported for the Moroccan population concerning relative and absolute baseline lymphocyte/µl subsets.

The number of the circulating white blood cells was shown to be influenced by smoking habits, which confirmed the previous data concerning the impacts of cigarette smoke on blood cells. Indeed, leukocytosis in smokers has been well established based on cross-sectionaland longitudinal studies in the general population. [10,11] Moreover, the absolute CD4 lymphocyte counts were higher in smokers than in non smokers. In contrast, the other lymphocyte subpopulations investigated were not affected by smoking. The smoking effect has been reported to increase the number of T cells and mainly CD4 peripheral blood lymphocytes. [11,12]

When we compared lymphocyte subset counts (CD4 and CD8) found in the present study with other published series in other countries such as Saudi Arabia and Italy similar ranges were found. [13,14] In contrast to other countries, especially Ghana and Kuwait, higher level of CD4 cells was reported for the same gender.[15,16] In those studies, the smoking habits of healthy control groups were not reported. Despite using the same panel of monoclonal antibodies as recommended by the CDC [9] observed differences might be due to differences in the

methodological variables which were not subject to any restrictive criteria due to a lack of a wide consensus such as lysing solutions, time and temperature of sample incubation, fixative employment and staining technique. [14,17,18] We have used a dual platform to determine the lymphocyte subset values; but did not use triple or quadruple staining, which might reduce inter-laboratory variations as reported in many studies [19,20] In addition, these differences of lymphocyte numbers can be associated with sex and age. Population parameters, however, were not well defined.

Conclusion

This study cannot be representative of Moroccan population. However, one can consider the values that we found as normal values in a young representative sample and provide the background level of immunity in the general population aged 19-49 years. CD4 counts offer a measure of a person's immune function and can also be used to monitor trends in infection in the young population over time. The smoking habits of healthy control groups, however need to be taken into account especially when comparing the numbers of T cells and CD4 lymphocyte subpopulations from patients with different diseases to normal controls. Moreover, the value added of the present study resides in its finding vis-à-vis the age group of people most affected with HIV.

Through this study, we concluded that the international standards of classification of the HIV-infected subjects according to their rates of CD4 are applicable to the young Moroccans population.

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