

Full Length Research Paper

## Lymphocyte subsets reference values for healthy adults in Togo

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Received February 5, 2012; Accepted May 23, 2012

The aim of this study was to establish the lymphocyte subsets reference values for healthy adults in Togo. A total of 139 subjects (100 males, 39 females) with hematological normal ranges were selected among 1349 voluntary blood donors who were otherwise included in a parallel study to establish the hematological reference values in Togo. All of them were negative for HIV, HBV, HCV infections and malaria. The dual-platform method was used to perform lymphocyte subsets counts. Reference ranges by gender (males vs females) were as follows: total T-lymphocytes (1071-2850 cells/ $\mu$ l vs 1185-2600 cells/ $\mu$ l), CD4-lymphocytes (595-1750 cells/ $\mu$ l vs 675-1650 cells/ $\mu$ l), CD8-lymphocytes (420-1300 cells/ $\mu$ l vs 450-1100 cells/ $\mu$ l), B-lymphocytes (160-558 cells/ $\mu$ l vs 153-700 cells/ $\mu$ l) and NK-lymphocytes (114-560 cells/ $\mu$ l vs 105-550 cells/ $\mu$ l). The range of the CD4:CD8 ratio was 0.9-2.1 in males and 1.1-2.3 in females. The CD8 cells count was lower and CD4:CD8 ratio was higher in females than in males. The B-lymphocyte count was higher than the NK-lymphocyte count unlike some literature data.

**Key words:** lymphocyte subsets, reference values, healthy adults, Togo

### INTRODUCTION

It is conceivable that the immune system is partly shaped by the nature and the amount of antigenic stimulations encountered throughout the body. Many of these stimulations are partly directly linked to the environment. Thus, environmental factors, various endemic infestations, and poor nutrition have been suggested as possible causes for the differences between populations in lymphocyte subsets (Chng *et al.*, 2004). Accordingly, the lymphocyte subsets values will not be the same in the different regions of the world. The race parameter should also be a consideration in determining reference intervals for immunophenotyping lymphocyte subsets (Howard *et al.*, 1996; Uppal *et al.*, 2003). In Africa, unfavorable

environmental factors, helminthic infestation, poor nutrition and viral infections are frequent. Those phenomena would be the basis of the stronger antigenic stimulation than in westernized countries.

In the past, the lymphocyte subsets were studied in European and American countries and those values were first used in Africa as references. Nowadays along with the expansion of flow cytometry on the African continent lymphocyte studies are performed in African countries leading to various results (Böhler *et al.*, 2007; Kibaya *et al.*, 2008; Klose *et al.*, 2007; Tsegaye *et al.*, 1999; Menard *et al.*, 2003; Lugada *et al.*, 2004; Urassa *et al.*, 2003).

According to these data, it seems important to establish the reference lymphocyte subsets values for the different countries. In Togo, country of Western Africa of essentially tropical climate, we have established lymphocyte subsets reference values by studying the voluntary blood donors recruited in National Blood

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Transfusion Center of Lomé (Togo).

## Subjects

Between August and September 2008, 139 subjects (100 males and 39 females) which gave their informed consent to this study were chosen among 1349 voluntary blood donors who were retained for the study to establish the hematological reference values in Togo. Accordingly, the patients included had a normal lymphocyte count, i.e. ranging from 1500 to 4100 /mm<sup>3</sup>. All the subjects had given less than three blood donations. All of them lived in urban zone. All of them were tested negative for HIV, HBV, HCV viral infections and malaria.

## Blood collection

All samples were collected between 07:00 and 09:00 am at the National Blood Transfusion Center of Lomé (Togo) and were analyzed on the day of collection.

Whole blood was collected with a Vacutainer system in 5 ml EDTA tubes. The serology-tests of HIV, HBV and HCV were done by ELISA (Enzyme-Linked-Immunosorbent Assay) system by the National Blood Transfusion Center of Lomé. Malaria was tested by thick drop method.

## Total lymphocyte count

A Sysmex SF-3000 automated hematology analyzer was used for total lymphocyte count.

## Flow cytometric analysis

Lymphocyte cells were analyzed on a FACSCalibur Flow cytometer (Becton Dickinson Immunocytometry Systems) with the following combinations of monoclonal antibodies (MAb): Simultest Control IgG1-FITC/IgG1-PE, CD3-FITC/CD4-PE/CD45-PerCP, CD3-FITC/CD8-PE/CD45-PerCP, CD3-FITC/CD19-PE/CD45-PerCP and CD3-FITC/CD16-56-PE/CD45-PerCP. All monoclonal antibodies were purchased from Becton Dickinson. In brief, 50µl of whole blood was mixed and incubated at room temperature for 20 min with 10µl of each MAb combination. RBC were then lysed by adding 1 ml of fluorescence activated cell sorter lysing solution (Becton Dickinson). After vortexing, tubes were incubated in the dark at room temperature for 10 min and centrifuged at 1500 rpm for 5 min. The cell pellet was washed once with 1 ml of FACS Flow<sup>®</sup>, resuspended in 500µl of FACS Flow, and analyzed with Cell QuestPro software (Becton Dickinson).

Absolute values of lymphocyte subsets were obtained according to the dual-platform counting technology. This method combines the percentage of positive cell subsets obtained by flow cytometry and the absolute cell count obtained by automated hematology analyzer to derive the

absolute value of each subset (Böhler et al., 2007;

Brando et al., 2000; Jentsch-Ullrich et al., 2005). T lymphocyte cells were identified by the expression of CD3, T subpopulation by the coexpression of CD4 or CD8. B-lymphocytes were defined by the expression of CD19 and natural killer (NK) lymphocytes were identified by the positivity of CD16 and/or CD56 without expression of CD3.

## Statistical analysis

Data were recorded in Excel software and analyzed with Epi Info 3.3.2 software. The median and 95th percentile ranges values were calculated for each parameter. Kruskal-Wallis test for two groups was used to compare the parameters by gender and difference was statistically significant if  $p < 0.05$ .

## RESULTS

Among the 139 blood donors, 100 (71.9%) were males and 39 (28.1%) females. Their age ranged from 19 to 57 years with means of 27.4±6.7 years for males and 28.9±7.6 years for females.

Table 1 shows the medians and the 95th percentile ranges of total lymphocyte count and percentage and absolute counts of lymphocyte subsets by gender.

The 95th percentile ranges of absolute T-CD4 lymphocytes count were 595-1750 cells/µl for males and 975-1650 cells/µl for females with respectively medians of 1045 and 1100 cells/µl which is not significant. The median of absolute T-CD8 lymphocytes count for males and females were respectively 726 cells/µl (range: 420-1300 cells/µl) and 660 cells/µl (range: 450-1100 cells/µl). The T-CD8 lymphocytes counts were lower in females than males ( $p < 0.05$ ). The CD4/CD8 ratio was higher in females (1.63) than males (1.41) ( $p = 0.00$ ).

The median of absolute B-lymphocyte count for males was 322 cells/µl (160-558 cells/µl) and 308 cells/µl (153-700 cells/µl) for females. The difference was not statistically significant between gender.

In males and females, the absolute median NK-cells count and range were respectively 234 cells/µl (114-560 cells/µl) and 242 cells/µl (105-560 cells/µl). No statistical difference was found between genders.

## DISCUSSION

The reference values of the main circulating lymphocyte subsets were established by many studies throughout the world and have shown some variability according to geography and methodology.

The tables 2 and 3 show the comparative values of lymphocyte subsets in African's countries and other

**Table 1:** Median Percentage and absolute values (cells/ $\mu$ l) of lymphocyte subsets and total lymphocyte counts ( $\times 10^9$ /l) by gender

			All (n=139)	Males (100)	Females (39)	p
	Lymphocyte	count	2.3 (1.5-4.1)	2.3 (1.5-4.1)	2.3 (1.5-3.9)	0.79
T cells	CD3	%	<b>75</b> (60-82)	76 (60-82)	75 (64-81)	0.17
		count	<b>1820 (1071-2850)</b>	<b>1837 (1071-2850)</b>	<b>1805 (1185-2600)</b>	0.98
	CD3+CD4+	%	45 (35-54)	45 (35-54)	46 (37-51)	0.13
		count	<b>1085 (595-1750)</b>	<b>1077 (595-1750)</b>	<b>1100 (675-1650)</b>	0.29
	CD3+CD8+	%	31 (21-40)	31 (22-40)*	29 (21-37)	<0.01*
		count	<b>742 (420-1300)</b>	<b>760 (420-1300)*</b>	<b>697 (450-1100)</b>	0.04*
	CD4/CD8 ratio		1.4 (0.9-2.3)	1.4 (0.9-2.1)*	1.6 (1.1-2.3)	<0.01*
B cells	%		13 (9-25)	13 (9-24)	14 (9-25)	0.41
	count		<b>328 (153-700)</b>	<b>322 (160-558)</b>	<b>342 (153-700)</b>	0.58
NK cells	%		11 (6-21)	10 (6-21)	11 (7-20)	0.70
	count		<b>263 (105-560)</b>	<b>261 (114-560)</b>	<b>270 (105-550)</b>	0.59

\*:  $p < 0.05$ : Statistically significant difference between males and females

The ranges are in brackets

countries respectively.

Determination of absolute lymphocytes subsets by single-platform is known to yield lower CVs. This method was used by different countries such as Burkina-Faso (Böhler et al., 2007), China (Chng et al., 2004; Kam et al., 1996). Some other countries (Germany (Jentsch-Ullrich et al., 2005), Italia (Santagostino et al., 1999)) have used dual-platform to determine the lymphocytes subsets. The main advantage of dual-platform is its lower cost. Besides, the comparison of both methods in Burkina-Faso has shown that the utilization of CD45 for selecting the mature cells in the dual-platform, gives similar result as a single-platform for evaluating the absolute lymphocytes subsets (Böhler et al., 2007).

In our study, the range of absolute total T-lymphocytes count (1071-2850 cells/ $\mu$ l) was similar to the studies done in Kenya (744-2634 cells/ $\mu$ l) (Kibaya et al., 2008), Burkina-Faso (1069-2921 cells/ $\mu$ l) (Klose et al., 2007) and Koweit (830-2710 cells/ $\mu$ l) (Kaaba et al., 2002). They were higher than studies done in Germany (780-2240 cells/ $\mu$ l) (Jentsch-Ullrich et al., 2005), Italy (605-2460 cells/ $\mu$ l) (Santagostino et al., 1999) and Switzerland (536-1787 cells/ $\mu$ l) (Bisset et al., 2004). The percentage of total T-lymphocytes (60-82%) and the T-lymphocytes subpopulations (35-54% for CD4 lymphocyte and 21-40% for CD8 lymphocyte) were similar to those of some other studies (Kibaya et al., 2008; Klose et al., 2007; Tsegaye et al., 1999; Jentsch-Ullrich et al., 2005; Kam et al., 1996; Santagostino et al., 1999; Kaaba et al., 2002; Bisset et al., 2004).

The median absolute CD4-T lymphocyte count was 1085 cells/ $\mu$ l (range of 595-1750 cells/ $\mu$ l). This result was similar to those found in Burkina-Faso. The absolute CD4 counts found in Burkina-Faso were among the highest ever reported in the literature. The authors discussed the influence of genetic factor in this unexpected finding (Klose et al., 2007). Our range values were similar to those found in Burkina-Faso, and could indeed possibly be linked to genetic factor. To our knowledge, no study has yet addressed the question of the influence of the genetic factors on lymphocytes subsets values.

The median absolute CD8-lymphocyte count in males and females were respectively 760 cells/ $\mu$ l and 697 cells/ $\mu$ l. The CD4:CD8 ratio was lower in males than in females (1.4 and 1.6,  $p < 0.05$ ). Thus, we confirm the lower absolute values of CD8-lymphocytes and the higher ratio CD4/CD8 found in females which were observed elsewhere in studies for the reference values of lymphocytes subsets in the world. (Kibaya et al., 2008; Klose et al., 2007; Tsegaye et al., 1999; Menard D, 2003; Lugada et al., 2004; Kaaba et al., 2002).

The absolute values of B-lymphocytes are 153-700 cells/ $\mu$ l globally, 160-558 cells/ $\mu$ l in males and 153-700 cells/ $\mu$ l in females. Our results were close to those of studies done in Kenya (93-627 cells/ $\mu$ l) (Kibaya et al., 2008), Burkina-Faso (148-658 cells/ $\mu$ l) (Klose et al., 2007) and Kuwait (50-610 cells/ $\mu$ l) (Kaaba et al., 2002). Low results were found in studies done in Ethiopia (51-419 cells/ $\mu$ l) (Tsegaye et al., 1999), Germany (80-490 cells/ $\mu$ l) (Jentsch-Ullrich et al., 2005) and Switzerland (72-460 cells/ $\mu$ l) (Bisset et al., 2004). The values of B-

Table 2: Median Percentage and absolute counts of lymphocyte subsets (cells/ $\mu$ l) by gender in some Africa countries

	Ethiopia [14]			Kenya [9]			Burkina - Faso [10]		
	All (142)	Males (92)	Females (50)	All (1293)	Males (848)	Females (445)	All (186)	Males (97)	Females (89)
Lymphocyte	1857	1857	1856	1950	1860	2160		NA	NA
	(1032-3432)	(1098-3487)	(1098-3487)	(1140-3454)	(1120-3160)	(1290-3957)			
CD3	1555	1564	1539	1415	1293	1660	1801	1690	1890
	(854-2556)	(696-2738)	(871-2413)	(744-2634)	(711-2351)	(851-2742)*	(1069-2921)	(1036-2963)	(1176-2921)
CD3+CD4+	775	753	816	810	744	810	1082	989	1169
	(366-1235)	(306-1249)	(456-1368)	(421-1550)	(407-1340)	(421-1550)*	(631-1696)	(546-1696)	(716-1749)*
CD3+CD8+	747	777	692	486	454	549	600	595	620
	(311-1618)	(318-1249)	(273-1418)	(210-1081)	(195-1046)	(210-1081)*	(328-1167)	(324-1164)	(328-1202)
CD4/CD8 ratio	1.2 (0.4-2.4)	1.1 (0.4-2.1)	1.3 (0.6-2.7)*	1.7 (0.9-3.3)	1.6 (0.8-3.3)	1.7 (0.9-3.3)	1.7 (0.9-2.8)	1.6 (0.9-2.5)	1.7 (1.1-3.1)*
B cells	191	184	203	244	218	295	349	351	329
	(51-419)	(31-420)	(61-471)	(93-627)	(86-551)	(122-716)*	(148-658)	(141 - 633)	(181-716)
NK cells	250	277	277	253	252	255	352	408	313
	(75-581)	(56-639)	(85-871)	(83-739)	(82-752)	(84-716)*	(150-1047)	(185-1294)	(141-681)

**Table 3:** Percentage and absolute counts (cells/ $\mu$ l) of lymphocyte subsets in some other countries of the world

			Germany [4]	Italy [13]	Switzerland [1]	Kuwait [7]	Chine [8]
	Lymphocyte	count				1400-3620	
T cells	CD3	%	53-83	60-87	54,90-84,03	64-85	54,8-83
		count	<b>780-2240</b>	<b>605-2460</b>	<b>536-1787</b>	<b>830-2710</b>	<b>672-2368</b>
	CD3+CD4+	%	30-59	32-61	32,53-62,88	34-54	23,1-51
		count	<b>490-1640</b>	<b>493-1666</b>	<b>309-1139</b>	<b>450-1650</b>	<b>292-1366</b>
	CD3+CD8+	%	10-40	14-43	11,55-38,60	20-42	17,9-47,5
		count	<b>170-880</b>	<b>224-1112</b>	<b>137-823</b>	<b>290-1170</b>	<b>240-1028</b>
	CD4/CD8 ratio		0,9-5,0	NA	NA	1,5 $\pm$ 0,35	NA
	B cells	%	5-21	5-20	4,70-19,13	44713,00	5,1-20,7
		count	<b>80-490</b>	<b>72-520</b>	<b>72-460</b>	<b>50-610</b>	<b>82-560</b>
	NK cells	%	5-32	4-28	5,35-30,93	4-22	7,1-38
count		<b>80-690</b>	<b>73-654</b>	<b>77-427</b>	<b>60-580</b>	<b>130-938</b>	

lymphocytes found in Africa and Kuwait were higher than those in the Caucasian studies. This difference can be due to the impact of environment on antigenic stimulations. We think that the reduction of some diseases due to the environment might influence the values of lymphocytes subsets in the underdeveloped countries.

The absolute values of NK-lymphocyte in our study were 105-560 cells/ $\mu$ l globally, 114-560 cells/ $\mu$ l in males and 105-550 cells/ $\mu$ l in females. Comparable values were found in Ethiopia (75-581 cells/ $\mu$ l) (*Tsegaye et al., 1999*), Kuwait (60-580 cells/ $\mu$ l) (*Kaaba et al., 2002*) and Switzerland (77-427 cells/ $\mu$ l) (*Bisset et al., 2004*). The values found in Burkina-Faso (185-1294 cells/ $\mu$ l) (*Klose et al., 2007*) were higher than all other studies found in the literature (*Kibaya et al., 2008; Tsegaye et al., 1999; Jentsch-Ulrich et al., 2005; Kam et al., 1996; Santagostino et al., 1999; Kaaba et al., 2002; Bisset et al., 2004*).

Also, our B-lymphocytes counts were higher than the NK-lymphocytes cells one globally or by gender. In literature, only the study done in Kenya showed this particularity only in males (*Kibaya et al., 2008*). We do not know how to explain this different result. May be some future studies can give an explanation to this observation.

The lower values of lymphocyte subsets in Caucasian (*Jentsch-Ulrich et al., 2005; Santagostino et al., 1999; Bisset, 2004*) and the higher one in Black African population (*Kibaya et al., 2008; Klose et al., 2007*) and in

Kuwait (*Kaaba et al., 2002*) could lead to the hypothesis that the race and ethnic group can be added to environmental phenomenon in the diversity of the values of the lymphocyte subsets. In the same area or country, we could have different values of lymphocyte subsets.

## CONCLUSION

Our results and those of other countries show that lymphocytes subpopulation values are different according to geography. The difference can be related with environment, antigenic stimulations or genetic characters. The assessment of relevant reference values obtained from the local national population is thus necessary to properly monitor patients, notably in AIDS setting.

## ACKNOWLEDGMENTS

We acknowledge all the staff of the National Blood Transfusion Center of Lomé where subjects were received and serological tests were performed, the technicians of PERFECT-LABO laboratory where the total blood cell count with differential was performed. We also acknowledge all the personnel of BIOLIM laboratory in Medecine Faculty in University of Lomé and all them of Hematology Laboratory in Henri-Mondor Hospital for their support.

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