



## VALUES OF LYMPHOCYTE SUBPOPULATIONS IN HEALTHY MACEDONIAN CHILDREN UNDER THE AGE OF FIVE

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### ABSTRACT

**Background:** The effects of demographic factors on a wide range of immunological variables demonstrate the importance of having normative data representative of particular patient population. There was no lymphocyte subpopulation data for Macedonian children and the purpose of this study was to establish such a data.

**Subject and methods:** The study population consists of 87 healthy children. Subjects were grouped into four age categories as follows: group 1 age range 5d-10d (n=15); group 2 age range 1 mo-1 yr (n=18); group 3 age 1yr-2 yr(n=20) and group 4 age 2yr-5 yr(n=34)

Monoclonal antibodies labeled with fluorochromes and immunofluorescent microscopy were used to detect cells bearing specific cell markers.

**Results:** The mean lymphocyte cell count gradually fell with increasing age from  $6,65 \times 10^9/l$  in group 1, to  $5,67 \times 10^9/l$  in group 2,  $4,55 \times 10^9/l$  in group 3, and to  $4,14 \times 10^9/l$  in group 4. Absolute values of CD3, CD4 and CD20 positive cells decreased gradually with age. Significant differences in mean absolute values were observed for absolute lymphocyte counts between groups 1/2 and 1/4 ( $P < 0,01$ ) and groups 2/4 ( $P < 0,05$ ); for CD3 positive lymphocytes between groups 1/3 ( $P < 0,05$ ) and 1/4 ( $P < 0,01$ ); for CD4 positive lymphocytes between groups 1/3 ( $P < 0,05$ ) and 1/4 ( $P < 0,01$ ) and for CD20 positive lymphocytes between groups 1/3 and 3/4 ( $P < 0,05$ ) and groups 1/4 ( $P < 0,01$ ). Significant difference for CD4/CD8 ratio and for percentage values of different lymphocyte subpopulations between the different age groups was not found.

**Conclusion:** This data may serve as a reference range for studies of Macedonian pediatric subjects.

**Keywords:** lymphocyte subpopulation, children,

### INTRODUCTION

Imunophenotyping of blood lymphocytes is an important tool in the diagnosis of immunological and hematological disorders. Because of the maturation and expansion of the immune system in the first years of life, the relative and the absolute size of lymphocyte subpopulations vary during childhood. Adult reference values cannot be used as discrepancies are constantly observed between normal values in children and those in adults. [1, 2, 3] It has been suggested that human immune system is functionally less mature at birth and within first years of life, and undergoes

process of sequential development that is both programmed genetically and stimulated external by antigen exposure and nutrition. [4 - 9]

The effects of demographic factors on a wide range of immunologic variables demonstrate the importance of having normative data representative of particular patient population. There was no detailed lymphocyte subpopulation data for Macedonian children and the purpose of this study was to establish data for the relative and the absolute size of some most important lymphocyte subpopulations in Macedonian children in the first five years of life.

### Subjects and methods

The study population consists of 87 healthy children. Subjects were grouped into four age categories as follows: group 1 age range 5d-10d (n=15); group 2 age range 1 mo-1 yr (n=18); group 3 age 1yr-2 yr (n=20) and group 4 age 2yr-5 yr (n=34). Blood samples were obtained from peripheral blood of healthy newborn infants who were delivered normally at term and had no evidence of infection or congenital anomaly and from healthy children who did not have history of chronic or recurrent illness, acute infection or were not under medications. None of the children had received any blood product transfusions.

### Sample preparation and analysis

Peripheral venous blood samples were obtained in collection tubes containing heparin and stored at room temperature for no longer than 10 hours before staining. Mononuclear cells were separated from heparinized whole blood samples by Ficoll-Paque gradient-density centrifugation. Monoclonal antibodies labeled with fluorochromes and immunofluorescent microscopy were used to detect cells bearing specific cell markers. A total blood count including differential blood count was performed. Absolute counts were delivered by using the following formula:

Absolute count = WBC (cell  $\times 10^9/l$ )  $\times$  %lymphocytes  $\times$  %antigen positive

T cells were defined as those cells expressing the CD3 antigen, subpopulation of helper cells as those expressing CD4 antigen and subpopulation of suppressor-cytotoxic cells as those cells expressing CD8 antigen. B cells were defined as those expressing CD19 and CD20 antigen, whereas NK cells were defined as those CD16 positive. In each age group the sum of lymphocyte lineage percentages was:

$$\%T + \%B + \%NK = 100\% \pm 5\%$$

Mann-Whitney analysis of variance by rank was used for evaluation of the differences between groups.

### RESULTS

The mean lymphocyte cell count gradually fell with increasing age from  $6,65 \times 10^9/l$  in group 1, to  $5,67 \times 10^9/l$  in group 2,  $4,55 \times 10^9/l$  in group 3, and to  $4,14 \times 10^9/l$  in group 4. The mean, standard deviation and median absolute lymphocyte subpopulations values are summarized in Table 1. The statistical P values for variance between the different groups are presented in Table 2. Considerable differences in mean absolute values were observed for absolute lymphocyte counts between groups 1/2 and 1/4 ( $P < 0,01$ ) and groups 2/4 ( $P < 0,05$ ); for CD3 antigen positive lymphocytes between

groups 1/3 ( $P < 0,05$ ) and 1/4 ( $P < 0,01$ ); for CD4 antigen positive lymphocytes between groups 1/3 ( $P < 0,05$ ) and 1/4 ( $P < 0,01$ ) and for CD20 antigen positive lymphocytes between groups 1/3 and 3/4 ( $P < 0,05$ ) and groups 1/4 ( $P < 0,01$ ). Trends of absolute lymphocyte count and of the absolute number of major lymphocyte populations and subpopulations in the first five years of life are shown on Figure 1 and 2.

The mean, standard deviation and median percentage values for different lymphocyte subpopulations are summarized in Table 3. Significant difference for CD4/CD8 ratio and for percentage values of different lymphocyte subpopulations between the different age groups was not found.

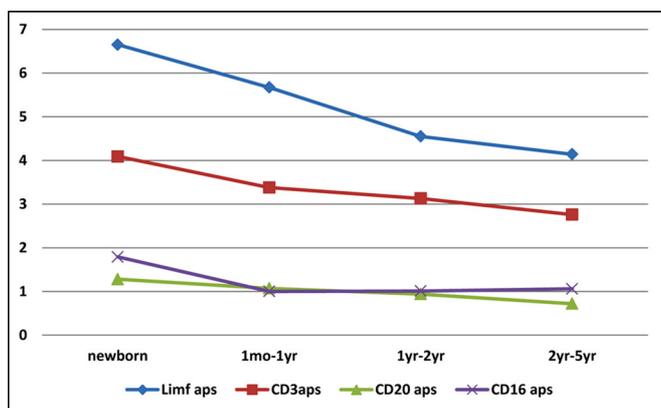
**Table 1.** Absolute values of lymphocyte subpopulations

	Group 1 mean±SD	Med	Group 2 mean±SD	Med	Group 3 mean±SD	Med	Group 4 mean±SD	Med
Limf aps	6,65±1,97	6,72	5,67±2,18	5,75	4,55±1,45	4,21	4,14±1,47	3,24
CD3	4,09±1,34	4,55	3,38±1,64	3,35	3,13±1,11	2,80	2,76±0,99	2,38
CD4	2,73±0,88	2,99	2,41±1,00	2,69	2,14±0,88	1,82	1,82±0,67	1,62
CD8	1,55±0,60	1,41	1,26±0,60	1,14	1,18±0,35	1,16	1,12±0,48	1,06
CD16	1,79±2,52	0,94	1,00±0,64	0,78	1,01±0,87	0,69	1,06±1,64	0,69
CD19	1,13±0,56	0,96	1,03±0,63	0,78	0,96±0,63	0,71	0,77±0,38	0,71
CD20	1,28±0,57	1,08	1,07±0,57	0,84	0,94±0,35	0,82	0,72±0,37	0,78

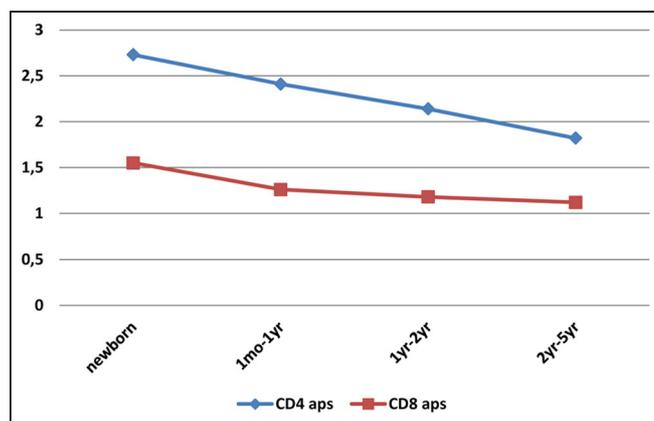
**Table 2.** P values of differences in the absolute lymphocyte subpopulation values between the different age groups

	1/2	1/3	1/4	2/3	2/4	3/4
Limf aps	ns	p<0,01	p<0,01	ns	p<0,05	ns
CD3aps	ns	p<0,05	p<0,01	ns	ns	ns
CD4 aps	ns	p<0,05	p<0,01	ns	ns	ns
CD8 aps	ns	ns	ns	ns	ns	ns
CD16 aps	ns	ns	ns	ns	ns	ns
CD19 aps	ns	ns	ns	ns	ns	ns
CD20 aps	ns	p<0,05	p<0,01	ns	ns	p<0,05
CD4/CD8	ns	ns	ns	ns	ns	ns

**Fig. 1.** Changes in absolute lymphocyte counts of major lymphocyte populations



**Fig. 2.** CD4 and CD8 T cells development in Macedonian children



**Table 3.** Percentage values of lymphocyte subpopulations

CD	Group 1		Group 2		Group 3		Group 4	
	mean±SD	Med	mean±SD	Med	mean±SD	Med	mean±SD	Med
CD3	63,33±9,11	65,00	64,33±10,53	63,50	66,40±9,98	64,00	67,02±9,18	70,00
CD4	43,14±11,50	40,00	43,11±11,50	40,00	45,70±10,03	42,00	44,11±7,24	46,00
CD8	24,07±5,46	23,50	22,05±4,00	21,50	26,50±3,23	26,00	27,08±6,04	26,00
CD4/CD8	1,76±0,37	1,71	1,82±0,27	1,80	1,64± 0,32	1,53	1,66±0,30	1,55
CD16	17,69±6,52	18,00	18,11±6,76	18,00	20,13±8,53	18,00	20,06±3,14	20,00
CD19	18,35±4,28	19,50	17,72±5,10	18,00	20,00±6,50	19,00	19,12±5,13	19,00
CD20	19,35±3,85	19,00	18,29±3,65	18,00	19,10±4,42	20,00	17,54±4,67	16,00

## DISCUSSION

This study of lymphocyte subpopulations in children under the age of five represents first reported data of cellular immunity in healthy Macedonian children to date. This data demonstrates differences in absolute lymphocyte counts related to age. These findings contribute to the literature concerning normative data on children immunology.

It is well known that the total number of lymphocyte and the absolute number of different lymphocyte subpopulations gradually fall with age [11-17]. Results in our study also show trends of declining of the total number of lymphocytes and absolute counts of CD3, CD4 and CD20 positive lymphocytes.

Children who live in poor economic conditions and in environment with high antigen density have higher lymphocyte cell counts. [1, 10, 11, 12]. The data of the total number of lymphocytes and CD3 lymphocyte counts in our study shows lower values than in the studies of those children.

Absolute number of CD8 positive lymphocytes in our study remains constant without significant difference among the different age groups which is also found in other studies [4]. This constant number of CD8 positive lymphocytes suggests that the suppressor- cytotoxic function in early childhood is one of the most mature functions and one who is less influenced by antigen exposure or nutrition.

Many studies have reported peak of absolute number

of CD16 positive lymphocytes in cord blood and in first 2 months of life [8, 9] where other studies have shown increasing number of NK cells with aging [6]. In our study number of CD16 positive lymphocytes remains constant, with slow, not significant declining during the first 5 years of life.

Significant difference in percentage values of different lymphocyte subpopulations among different age groups in our study was not found. These results, together with the results of absolute lymphocyte subpopulations counts suggested that changes in the absolute size of lymphocyte subpopulations are not always consistent with changes in their relative size. This demonstrates that the relative counts of lymphocyte subpopulations do not reflect their actual size and are therefore of limited value. Also, influence of genetics and differences in antigen exposure and nutritional habits of different population groups cannot be undermined, therefore we find that is important reference ranges for different population group to be established

## CONCLUSION

This study on distribution of lymphocyte subpopulations helps to enhance our knowledge about cell phenotypes in infants and small children, and contributes to the correct interpretation of laboratory results for children with primary and secondary immune disorders. This data may serve as a reference range for studies of Macedonian pediatric subjects regarding method used.

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