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Original article

B and T lymphocytes in rabbits change according to the sex and throughout the year

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Abstract

The European rabbit (*Oryctolagus cuniculus*) is a good model in biomedicine used in research on several human diseases. The reference values of B and T cells and their subpopulations are very important to understand how the adaptive immune system is responding to infectious agents. The aim of this study was to determine values of B and T cells and their subpopulations in Polish mixed-breed rabbits, considering seasons of the year and sex. The study was performed on 200 Polish mixed-breed rabbits and the percentage of B and T lymphocytes was measured cytometrically using mouse anti-rabbit antibodies. The study revealed that the season of the year and sex of the animals affected the percentage of B- and T-cells and their subpopulations in peripheral blood. Statistically significant values of CD19+ B-cells in spring and autumn, of T CD5+ cells in spring and winter, of T CD4+ in spring, summer, autumn and winter, of T CD8+ in winter and of T CD25+ in spring were noted. Generally the highest values were found mainly in warm part of the year, while the lowest in colder months. A statistical significance was also observed between males and females – changes were found in T $CD4^+$ and T CD25+ lymphocytes in spring, T CD8+ cells in winter and higher percentage was generally obtained in females than in males. The only exception was the T CD5+ subpopulation in which no differences were observed between the sexes and throughout the year. This is the first paper on adaptive immune system cell values in the European rabbit of domestic breeds.

Key words: rabbit, B and T lymphocytes, reference values

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Introduction

The European Rabbit (*Oryctolagus cuniculus*) is a commonly used laboratory animal, along with mice, rats and guinea pigs and it is a commonly used animal in many European countries, including Poland. European rabbit as a model has many advantages. It had served as an animal model since the development of the rabies vaccine by Pasteur in 1881 and its main advantage is its intermediate size between rodents and primates and longer than rodents' life span (Esteves et al. 2018). In order to develop a good animal model for scientific use, it is worth determining the reference values for immunological factors e.g. B and T lymphocytes. Until today, there have been few papers on lymphocyte values in rabbits. There are reports on the values in rabbits of mixed breed in Poland (Deptuła et al. 1995, 1998, Nowaczyk et al. 2004, Deptuła et al. 2009) in genetic lines LP and V (Ferrian et al. 2012), in Spanish mixed breed (Jain 1994), in New Zealand (Jeklova et al. 2009) and Fauve de Bourgogne (Poljičak-Milas et al. 2009). Any comparison of these values is difficult because the units used to determine the values of B and T cells and subpopulations were different in these works.

The studies on rabbit lymphocytes showed that B lymphocytes are exceptional due to the complexity of their cell surface markers and to the surprising fact that their proportion versus T cells may be different than in other species (Sabolovič et al. 1977). Therefore, the most reliable method of evaluating this subpopulation of cells is based on CD19 marker – the most pivotal receptor on those cells. As the subpopulation of T cells is more divert, it is also recommended to evaluate the markers that are most basic and identifiable, such as $CD4^+$, $CD8^+$ and $CD25^+$.

The aim of the present work was to obtain reference values (standards) for B-cells with $CD19^+$ receptor, and T-cells with $CD5^+$ receptor, and their subpopulations, i.e. T-cells with receptors $CD4^+$, $CD8^+$ and $CD25^+$ in peripheral blood in Polish mixed breed rabbits, considering the impact of the season of the year and sex of the animals on such values.

Materials and Methods

Animals

The research was performed on 200 Polish mixed-breed rabbits (50 rabbits in each season of the year – 25 males and 25 females) originating from a licensed farm, remaining under continuous veterinary and zoo-hygienic supervision (Anon 1987), weighing 3.2-4.2 kg, aged 6-8 months, females (25 rabbits) and males (25 rabbits), in four seasons of the year: spring, summer, autumn, and winter. During the experiment, the animals remained at the vivarium, where zoo-technical parameters were in line with the recommended Polish standards developed in line with the European Union Directive as regards temperature and humidity, as well as lighting and size of cages for animals (Anon 2006). After transportation to the Department vivarium, the animals were provided with a two-week adaptation period. The animals were fed with a complete feed (Królik 16, Motycz, Poland), at a volume of 0.15-0.20 kg/day, and had unlimited access to water.

Blood sampling and analysis

The study was carried out twice (each time lasting seven days) in four seasons (spring, summer, autumn, winter) on males and females. Blood for tests was drawn via an inserted port from marginal vein of the ear, at 24-hour intervals, for three consecutive days, at 08:00 h, that it at hours 0, 24 and 48 h from commencement of the study. In rabbit blood, the percentage of B-cells with $CD19^+$ receptor (Serotec, mouse anti rabbit IgM B cell marker, catalogue no. MCA812GA), T-cells with $CD5^+$ receptor (Serotec, mouse anti rabbit CD5, catalogue no. MCA800), their subpopulations – T-cells with receptors $CD4^+$ (Serotec, mouse anti rabbit CD4, catalogue no. MCA799G), $CD8^+$ (Serotec, mouse anti rabbit CD8, catalogue no. MCA1576G) and $CD25^+$ (Serotec, mouse anti rabbit CD8, catalogue no. MCA1119GA) was determined according to the method described by Deptuła et al. (1998). It is based on a particular cell pool (cytometrically calculated cell total was 10.000, which was determined as 100%), using monoclonal antibodies (mouse anti-rabbit) (Serotec, USA). The analysed samples were incubated for 45 minutes on ice, rinsed three times with Cell Wash (BD Biosciences, USA) by centrifugation at 200 × g. To this prepared cellular sediment, 10 µl of rabbit anti-mouse IgG antibody labelled with fluorescein isothiocyanate (FITC) was added (Serotec, goat anti mouse IgG: FITC, rat adsorbed, catalogue no. STAR70). After triple repetition of the rinsing procedure in Cell Wash, 2000µl of lysing solution was added to samples to eliminate erythrocytes (BD FACS Lysing Solution, BD Biosciences, USA). After ten minutes of incubation in the dark, at room temperature, measurement was performed on FACScan flow cytometer by Becton Dickinson (USA) using FACSDiva software.

Figs. 1-4. show data on B- and T-cells and their subpopulations in percentage, as obtained from three blood draws from each rabbit (at 0, 24, 48 h), performed twice at the interval of seven days in each season of the year. Data were processed with statistical analysis using Student's t- test at $p<0.05$.

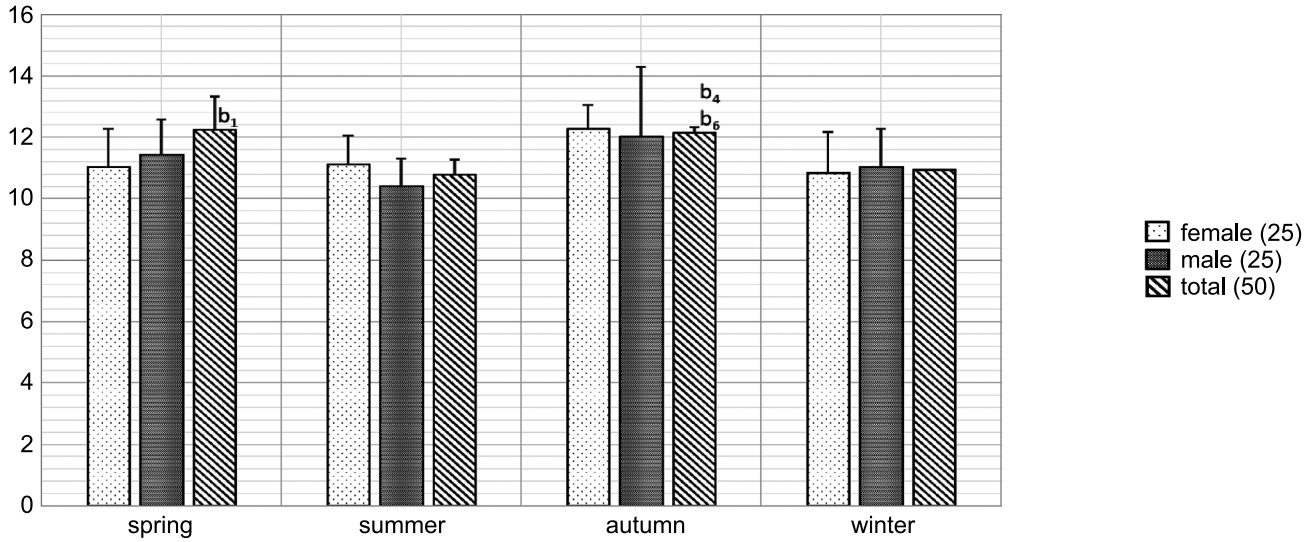

Fig. 1. Lymphocytes B with CD19+ receptor in rabbit males, females and total throughout the year.
Legend: [a] – statistically significant difference between male and female, [b] – statistically significant difference between seasons (total), where [b1] – statistically significant difference between spring and summer; [b2] – statistically significant difference between spring and autumn; [b3] – statistically significant difference between spring and winter; [b4] – statistically significant difference between summer and autumn; [b5] – statistically significant difference summer and winter; [b6] – statistically significant difference between autumn and winter.

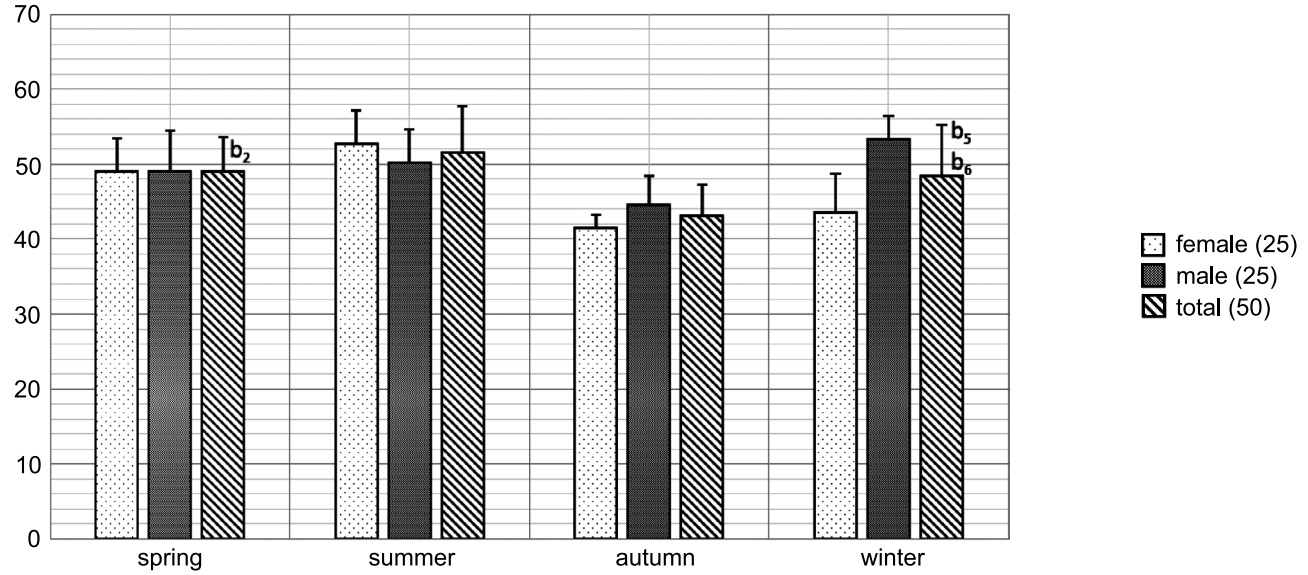

Fig. 2. Lymphocytes T with CD5+ receptor in rabbit males, females and total throughout the year.
Legend: [a] – statistically significant difference between male and female, [b] – statistically significant difference between seasons (total), where [b1] – statistically significant difference between spring and summer; [b2] – statistically significant difference between spring and autumn; [b3] – statistically significant difference between spring and winter; [b4] – statistically significant difference between summer and autumn; [b5] – statistically significant difference summer and winter; [b6] – statistically significant difference between autumn and winter.

Results

Values of B- and T-cells and their subpopulations

The values of CD19+ B-cells in Polish mixed-breed rabbits remain within the range of 10.76 – 12.23%, for CD5+ T-cells: 43.06 – 51.46%, CD4+ T-cells: 30.33 – 40.01%, CD8+ T-cells: 13.56 – 15.77%, and for CD25+ T-cells: 12.15 – 12.85% .

The analysis of the impact of the seasons on the analysed elements of blood in rabbits (Fig. 1) revealed that the highest values for CD19+ B-cells were obtained in spring, while the lowest in summer; for CD5+ T-cells (Fig. 2) the highest values were recorded in summer, while the lowest in autumn, while for CD4+ T-cells (Fig. 3) the highest values were observed in summer, while the lowest in spring. The highest values for CD8+ T-cells (Fig. 4) were obtained in winter, and

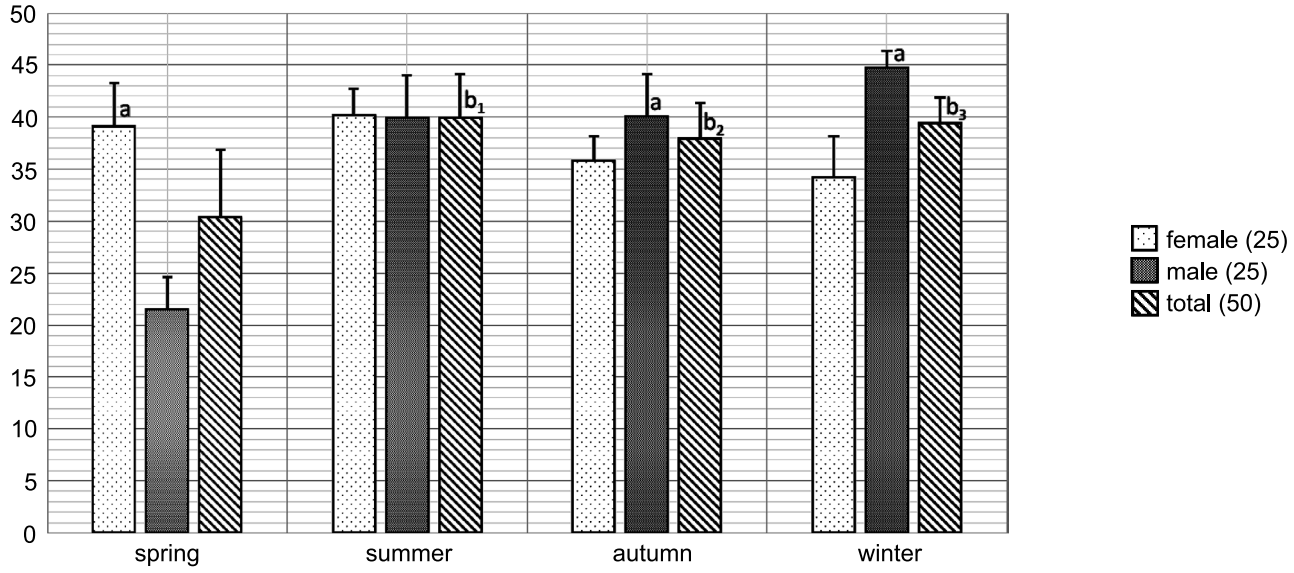

Fig. 3. Lymphocytes T with $CD4^+$ receptor in rabbit males, females and total throughout the year.
Legend: [a] – statistically significant difference between male and female, [b] – statistically significant difference between seasons (total), where [b1] – statistically significant difference between spring and summer; [b2] – statistically significant difference between spring and autumn; [b3] – statistically significant difference between spring and winter; [b4] – statistically significant difference between summer and autumn; [b5] – statistically significant difference summer and winter; [b6] – statistically significant difference between autumn and winter.

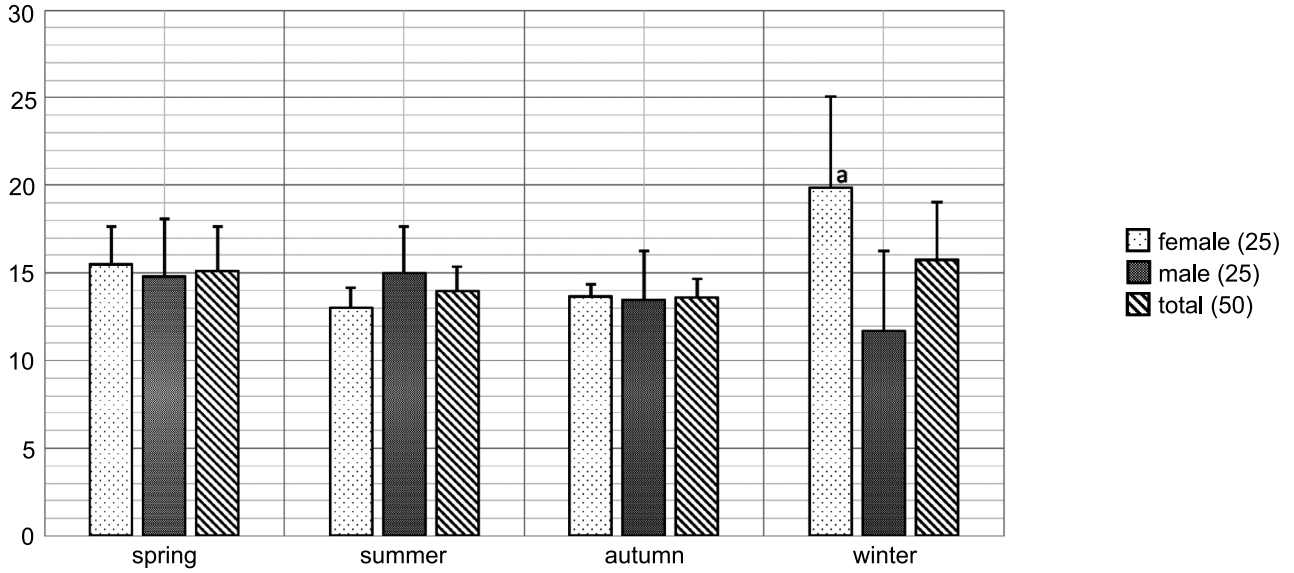

Fig. 4. Lymphocytes T with $CD8^+$ receptor in rabbit males, females and total throughout the year.
Legend: [a] – statistically significant difference between male and female, [b] – statistically significant difference between seasons (total), where [b1] – statistically significant difference between spring and summer; [b2] – statistically significant difference between spring and autumn; [b3] – statistically significant difference between spring and winter; [b4] – statistically significant difference between summer and autumn; [b5] – statistically significant difference summer and winter; [b6] – statistically significant difference between autumn and winter.

lowest in autumn, while for $CD25^+$ T-cells (Fig. 5) the values remained at a similar level throughout the year.

Summing up, that higher values of B and T cells were recorded in warm seasons (spring and summer), with lower values in colder periods of the year (mainly autumn).

Furthermore, statistical analysis revealed significant differences between values obtained in spring and summer for $CD19^+$ B-cells and $CD4^+$ T-cells; between spring and autumn – for $CD5^+$ T-cells and $CD4^+$ T-cells, and between spring and winter – for $CD4^+$ T-cells. The differences were also found between summer and autumn in the percentage of $CD19^+$ B and $CD5^+$ T-cells, differences between summer and winter – exclusively in case of $CD5^+$ T-cells, whereas differences between

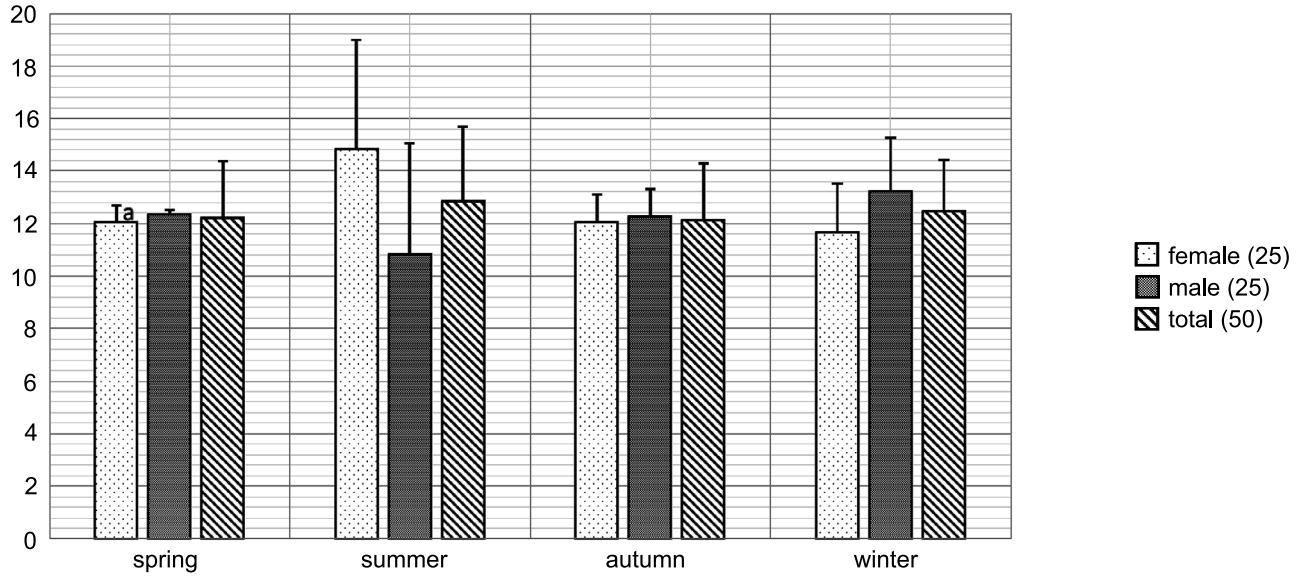

Fig. 5. Lymphocytes T with CD25$^+$ receptor in rabbit males, females and total throughout the year.
Legend: [a] – statistically significant difference between male and female, [b] – statistically significant difference between seasons (total), where [b1] – statistically significant difference between spring and summer; [b2] – statistically significant difference between spring and autumn; [b3] – statistically significant difference between spring and winter; [b4] – statistically significant difference between summer and autumn; [b5] – statistically significant difference summer and winter; [b6] – statistically significant difference between autumn and winter.

autumn and winter – as regards CD19$^+$ B-cells and CD5$^+$ T-cells.

Analysis of the impact of sex of the animals on the percentage of B- and T-cells and their subpopulations in peripheral blood of Polish mixed-breed rabbits in seasons of the year (spring, summer, autumn, winter) showed higher values in females than in males.

Discussion

B- and T-cell values and their subpopulations change throughout the year, the highest values in all the analysed peripheral blood factors were most frequently recorded in summer, while the lowest in autumn. The season most significantly affected the values of CD5$^+$ T-cells. The impact of the season on such peripheral blood factors has not been confirmed in any study so far, and the present study is the first in the literature. Moreover, our study on haematological factors in Polish mixed-breed rabbits, determined that the season of the year to a small degree affected the analysed peripheral blood factors, as it only affected the number of monocytes, lymphocytes and haemoglobin concentration (Tokarz-Deptuła et al. 2014).

Analysis of the impact of sex of the animals on the percentage of B- and T-cells and their subpopulations in peripheral blood evidenced that more significant values were recorded in females rather than males. When referring such facts to haematological studies in Polish mixed-breed rabbits, it was evidenced (Tokarz-Deptuła et al. 2014) that sex is of importance principally in autumn and winter for the number of lymphocytes and thrombocytes in males, and monocytes in females.

When analysing season-dependent changes B- and T-cell values and their subpopulations, the highest values in all the analysed peripheral blood factors were most frequently recorded in spring and winter, while the lowest in autumn.

One of the most interesting observations as regards T and B cells in the two groups of studied rabbits is the fact, that there was a change of B and T cells number in different seasons depending on the subpopulation of B and T cells. The highest values of the parameters were registered in warm part of the year (spring and summer), and the lowest in autumn. The only exception is the T CD5+ subpopulation, which has the same pattern of changes throughout the year regardless of the breed or sex of rabbits. The study on the impact of the season on haematological factors in Polish mixed-breed rabbits evidenced that the season of the year profoundly affected the parameters. The study also measured the volume of basophils, eosinophils, monocytes, and to a lesser extent erythrocytes, lymphocytes, haemoglobin concentration and the volume of neutrophils (Tokarz-Deptuła et al. 2014).

The sex impact was different in females and males, as in females more significant values were recorded in summer and winter, mainly CD25$^+$ T-cells, while in males mainly CD19 and B cells were found and that

was only in winter. Previous studies on Polish mixed-breed rabbits evidenced (Tokarz-Deptuła et al. 2005, 2014) that the sex changes the values in summer, autumn and winter affecting the volume of eosinophils and neutrophils in females, and neutrophils and thrombocytes in males.

Conclusions

The season of the year strongly impacted the distribution of B and T cells, giving the highest values in spring and summer and lowest in autumn and winter. Exchanges of B and T cells throughout the year were observed, with one exception – $CD5^+$ cells had the same pattern of distribution throughout the year, regardless of the sex.

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