Immunosenescence of Lymphocytes T and Implications for Age-Related Disorders

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Research

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Abstract

Background. The decrease in immunity with age is still a major health concern as elderly people are more susceptible to infections and increased incidence of autoimmunity. Consequently, there is an increasing interest in immunosenescence and changes in immunology cells like T cells. The aim of our study was to find a disproportion in subpopulation of T cells as well as CD4/CD8 ratio depending on the age, gender or comorbidities.

Results. In the present study, a flow cytometry was used to indicate the differences between age, sex, disorders and fat content in the CD4+ and CD8+ T cells population divided into naïve and memory cells as well as CD4/CD8 ratio in people aged 71.9± 5.8 years (females n=83, males n=16) compared to young people aged 20.6 ± 1.1 years (females n=12, males n=19). The percentage of naïve CD4+ and CD8+ cells was found to be statistically significantly lower in the elderly compared to the young. In addition, gender was observed to play an important role in the outcomes in the analysed subpopulations and in female group, who live statistically longer than males, our older group of Polish women demonstrated a significantly higher percentage of naïve lymphocytes in both the CD4+ and CD8+ populations compared to men. The CD4/CD8 ratio increases with age, which can be considered one of the markers determining longevity. Elderly people with age-related diseases (hypertension) also show an increased level of CD4/CD8 ratio as well as CD4+.

Conclusion. We demonstrated that changes in the T cells population, including naïve cell population as well as CD4/CD8 ratio, are important markers which can be predictive of healthy status. In order to accurately determine longevity, gender or age-associated diseases should be taken into account.

Background

Ageing is a highly complex process which affects various aspects of tissue functions such as immunity. Age-associated changes in immune functions, generally termed immunosenescence, are characterised by diminished adaptive and innate immune competence leading to reduced infection resistance, increased risk of autoimmunity, which may underlie chronic inflammatory diseases in the elderly [1]. However, many differences between the younger and older generation are in fact only assumed to be detrimental without unequivocal evidence. It is clear that ageing process does not always lead to predictable decline in immune functions but it does lead to their alterations [2].

The most dramatic change in the adaptive immune system is the involution of the thymus, the sole organ devoted to generation of T cells, and the most important change involves the output of new naïve T cells (CD45RA+CD45RO−CD62L+CCR7+) diminishing with age. The T cell pool is part of subpopulations of antigen-inexperienced naïve cells and antigen-experienced memory cells. The human immune compartment is composed of ~ 10^{12} T cells in total, ~10^{11} of which are naïve [3]. During the process of ageing, the population of naïve T cells decreases, while the population of memory T cells undergoes intensively proliferation, thereby reversing the balance of naïve and memory T cells that persisted at
younger ages [4,5]. The loss of naïve T lymphocytes of approx. 30% in older individuals is compensated by the expansion of T cells with CD8⁺CD45RO⁺CD25⁺ phenotype. Increase of memory T cells enhances immunological memory of previously encountered antigens, thereby increasing the existent immune protection. The remaining naïve pool of T cells experiences a loss of T cell receptor (TCR) ‘structural diversity’: the number of distinct TCR complexes present across the entire naïve pool [6,7]. The diversity of T cell lymphocyte clones, associated with the different number of distinct TCR complexes among the cell population, ensure the suitable range of antigen specificity [3].

The number of naïve CD4⁺ T cells is reduced at about 70 years of age whereas a decline in naïve CD8⁺ cells occurs much earlier due to their sensitivity to apoptosis thereby increasing a risk of infectious diseases and contributing to the cardiovascular, metabolic, autoimmune, and neurodegenerative diseases [8].

Males and females due to differences in the mechanism of acquired and innate immunity differ in the immune response to both new and self-antigens. Some of these immunological changes are present throughout life, while some are revealed after puberty. This suggests that both genes and hormones as well as exposure to the microenvironment affect immune function in both sexes. Importantly, immunological differences in sex (men vs. women) contribute to differences in the incidence of many autoimmune diseases, cancers, infectious diseases and response to vaccination [9]. It has been proved that women are much less susceptible to infections, but definitely more often than men are exposed to the development of and autoimmune diseases such as hypothyroidism or rheumatoid arthritis. Both androgens and estrogens contribute to thymic involution reducing the number of naïve T cells. Moreover, androgens polarise naïve CD4 T cells towards the Th1 subset, contrary to estrogens that stimulate the Th2 response and activate the production of antibodies [10].

During the past decade, prospective cohort studies were performed to assess the immune risk profile (IRP) in the elderly, mainly defined by an inverted CD4/CD8 ratio [11, 12,13]. The results revealed some common features of immunosenescence but also many differences between the studies [14]. Therefore, this study was designed to explain whether changes in the numbers of naïve CD4⁺ and CD8⁺ T cells as well as CD4/CD8 ratio are associated with gender, selected concomitant diseases and body composition in the elderly and to determine a potential cellular profile which could affect longevity and health status.

Results

Body composition (Table 1). Among the elderly, Body Mass Index (BMI) ranged from 19.4 to 35.3 kg/m². 29% of investigated seniors had normal body mass, 50% were classified as overweight and 20% as obese. In younger group, 90% of the subjects had a normal BMI. Fat Mass Index (FMI) values ranged from 3.8 to 15.2 kg/m² in the elderly, and from 1.0 to 6.8 kg/m² in younger subjects in the normal BMI ranges (<25 kg/m²). FMI was 2.5-fold higher in the elderly than young individuals and significantly related to the numbers of CD4⁺ (r=0.339, P<0.001) and CD4⁺CD45R0⁺ (r=0.468, P<0.001). This indicates that an elevated fat content may indirectly impact on immune cells population.
**Flow cytometry analysis.** CD4⁺ and CD8⁺ T cells were analysed within naïve and memory subset in University of the Third Age (U3A) students and young individuals. The percentage of CD4⁺ was significantly higher in elderly individuals compared to young subjects (Fig. 1a), a higher percentage of CD8⁺ was also shown but the difference was not statistically significant (Fig. 1b). As a result, the CD4/CD8 ratio was significantly higher in the elderly individuals compared to the young ones (Fig. 1c). The CD4/CD8 ratio ≥1 was identified in the majority of the young individuals (77%) while 64% of elderly individuals were recorded to have CD4/CD8 ratio ≥1 or ≤2.5; the CD4/CD8 ratio ≥2.5 was found in 27% of the elderly individuals and only 14% of them had the CD4/CD8 ratio ≤1. Furthermore, an increased percentage of naïve CD4⁺ (Fig. 1d) and CD8⁺ (Fig. 1f) cells was shown in young individuals compared to elderly ones, and the percentage of memory CD4⁺ T cells (Fig. 1e) and CD8⁺ (Fig. 1g) was significantly higher in elderly individuals compared to young subjects.

Figure 1. Percentages of CD4⁺ and CD8⁺ naïve and memory cells in young (gray bars) and elderly (black bars) and average CD4/CD8 ratios.

Analyses of changes to T cells in youngest-old vs. middle-old/oldest-old showed statistically significant differences in the CD8 cytotoxic T cells population (Fig. 2b) in the CD8 naïve T cells population (Fig. 2f) and the CD4/CD8 ratio was found to be significantly higher in 75-89 yrs. age group (Fig. 2c). The ratio CD4/CD8 ≥1 or 2.5 was noted in 81% of the elderly falling in 60-74 years age range and only in 33% of the subjects aged 75-89 years. An increased ratio of CD4/CD8 ≥2.5 was found in 5% of subjects aged 60-74 years and in 67% of individuals aged 75-89 years. An inverted CD/CD8 ratio was observed only in 14% of subjects aged 60-74 years.

Figure 2. Percentages of CD4⁺ and CD8⁺ naïve and memory cells in 60-74 years of age (black bars) and 75-90 years of age (gray bars) and average CD4/CD8 ratios.

A statistically significant difference was observed between women vs. men in the naïve CD4⁺ (Fig. 3d) and CD8⁺ (Fig. 3f) population of T cells. There was a trend towards an increased CD4/CD8 ratio in women but the value was not statistically significant. The CD4/CD8 ≤1 was observed in 25% men and 6% women. The ratio CD4/CD8 ≥1 or 2.5 was recorded in 44% men and 67% women, and CD4/CD8 ≥2.5 was found in 31% men and 26% women.

Figure 3. Percentages of CD4⁺ and CD8⁺ naïve and memory cells in women (black bars) and man (gray bars) and average CD4/CD8 ratios in U3A students.

The analyses showed no statistically significant differences in the number of helper T lymphocytes compared to cytotoxic ones and between naïve vs. memory subsets in the elderly with regard to diabetes, thyroid disorder or rheumatoid arthritis (Table 2). Statistically significant differences were observed in the CD4 helper lymphocyte population (Fig. 4a) and in CD4⁺ memory T cells (Fig. 4e) only in individuals diagnosed with hypertension when compared to other diseases.
Figure 4. Percentages of CD4$^{+}$ and CD8$^{+}$ naïve and memory cells in patients diagnosed with hypertension (black bars) and in patients with other diseases (gray bars) and average CD4/CD8 ratios.

**Haematological variable** (Table 3). Both in the elderly and younger subjects all the parameters of the white blood cell count were found to fall within the referential value range. No statistically significant differences were observed in white blood cell count (WBC), lymphocytes (LYM), mid absolute count (MID), granulocytes (GRA), LYM%, MID%, GRA% between the elderly and the young subjects.

**Biochemical markers** (Table 4). Triglycerides (TG) concentration was found to range from 88.43 mg/dL to 443.19 mg/dL in elderly and from 60.1 mg/dL to 270.1 mg/dL in young. High density lipoprotein (HDL) level ranged from 40.23 mg/dL to 102.68 mg/dL in elderly and 36.1 mg/dL to 122.9 mg/dL in young individuals. However, non-HDL was higher in the elderly than in young individuals. The high levels of total cholesterol (TC) and low density lipoproteins (LDL) were observed in 30% (>200 mg/dL) and 35% (>130 mg/dL) of the elderly and 22% (>200 mg/dL) and 22% (>130 mg/dL) of the younger subjects, respectively. Finally, non-HDL exceeded the level of 145 mg/dL in 48% of the elderly. C reactive protein (CRP) concentration was significantly higher in elderly than younger subjects. The highest values of CRP were observed in our subgroup with rheumatoid arthritis. According to the health questionnaire, approx. 10% of the subjects took lipid-lowering and/or anti-inflammatory agents.

**Discussion**

Since the immune function is a marker of health and longevity and a positive relation has been shown between a good function of several immune cells and health status, we considered several immune function parameters associated with gender and body composition to be adequate biomarkers of the biological age. As mentioned in the introduction, changes in the elderly regarding acquired immunity associated with the T cells pool mainly involve a reduction in the number of naïve T cells, an increase in the number of memory T cells [15,16] and a diminished response to vaccines [17]. One of the major findings of the current research is that ageing-related adaptation of the immune system seems to result from a reduced repertoire of naïve CD4$^{+}$ and CD8$^{+}$ T cells due to the thyme involution as well as from prolonged exposure to different antigens throughout life and the accumulation of memory CD4$^{+}$ and CD8$^{+}$ which compensate for a number of naïve T cells. In our research a statistically significantly larger population of CD4$^{+}$ helper lymphocytes was found in the elderly than in the younger subjects contrary to Alam et al. [18], who showed a high level of CD4$^{+}$ in the elderly subjects. It is not entirely clear how age influences changes in the CD8$^{+}$ T cells population. The present study found a tendency towards the presence of high percentage of CD8$^{+}$ in the elderly but this difference did not reach the significance level. Nevertheless, Hirokawa et al. [19] observed a decrease in the number of CD8$^{+}$ T cells with age. Changes in the T cell system affect the ability to mediate effective immune responses against new antigens and the ability has been proven to be reduced in the elderly in comparison to the young. An increase in the number of senescent T cells and a decrease in the number of naïve cells diminish the protection range and capacity against pathogens. Therefore, the issue of ageing populations prompts researches
worldwide to keep trying for years to design an immune risk profile that determines a potential rate of ageing of the immune system, and thus the whole body. The assessment of the immune risks profile including analyses of changes in the number of specific subpopulation of immune cells may be relevant when predicting the occurrence of neoplastic, autoimmune and neurodegenerative diseases as well as metabolic disorders, such as type 2 diabetes, or cardiovascular diseases that are more common in the elderly [20].

The immunological risk profile, which, among others, included the analysis of prevalence of inverted CD4/CD8 ratio, was compiled with the participation of Swedes, Dutch and Belgians. Some of the observations are comparable, there are, however, some differences due to the lifestyle and environmental factors. In the OCTO/NONA project, the inverted CD4/CD8 ratio was found to be associated with increased mortality and a higher risk of infection. The BELFRAIL project in women reported the accumulation of CD4+naive T cells and the CD4/CD8 ratio > 5 to be negatively correlated with increased survival as opposed to CD4/CD8 ratio < 1, which showed a positive correlation with 3-year survival [14]. The CD4/CD8 ratio in healthy individuals is poorly defined but a value between 1.0-1.5 to 2.5 is used as the norm. Research shows that many factors like: age, ethnicity, genetics, gender, exposures and infections may all impact the ratio. The inverted ratio may result from isolated apoptotic or targeted cell death of circulating CD4 cells, expansion of CD8 cells, or a combination of both. Some of the researchers cl

aim the incidence of inverted CD4/CD8 ratio increases with age. An inverted ratio has already been recorded in 8% of individuals aged 20–59 and in 16% of individuals aged 60–94 [21]. In older adults with confirmed inverted CD4/CD8 ratio remodelling enumerative and functional immune changes have been observed and these included: reduced number of B cells (CD19+) and an expansion of late-differentiated or senescent T cells (CD8+CD28−), which are important T cells against cytomegalovirus (CMV). Some research suggests that inverted CD4/CD8 is an immune risk factor which could be associated with various pathologies and it is frequently associated with premature immunosenescence and repeated viral infections [22].

On the other hand, researchers like Strindhall et al. [20] showed that in surviving OCTO/NONA participants older than 100 years, no inverted CD4/CD8 ratio had been recorded from the age of 85 on and the mean CD4/CD8 ratios were found to increase with age. The increase of CD4/CD8 with age was also confirmed in the Japanese population [19]. Interestingly, in Polish population we showed that in elderly individuals the inverted CD4/CD8 ratio occurred only in 60–74 age group and in the group of 75-90-year-olds the CD4/CD8 ratio ≥ 2.5 was identified in 67% of the individuals. In turn, in the Leiden 85+ study, only 2% of individuals aged 89 years who participated in the study showed an CD4/CD8 ratio < 1 compared with 20% in those between 70 and 81 years of age. The observed increase in the CD4/CD8 ratio with age was similar to the results obtained by Vasson et al. [23] in the Austrian elderly but opposed to the results from Spanish or French population where a trend towards a decreasing CD4/CD8 ratio was observed. The differences
between countries/regions can be related to lifestyle factors such as habits, nutrition, physical and mental activity.

The finding of an increasing prevalence of the CD4/CD8 ratio and the increased or decreased mortality should be confirmed in additional populations in other countries. The nature and cause of this immunosuppressed status is certainly an important topic for future research, but there is no doubt that whether the CD4/CD8 ratio in older people decreases or increases with age also depends on gender. Previous studies by Wikby et al. [24] and Hirokawa et al. [19] reported that the inverted CD4/CD8 ratio concerned mainly men, while the number of naïve cells CD8+ and the CD4/CD8 ratio were significantly higher in women. We also observed a significantly higher percentage of naïve CD4+ and CD8+ T cells in women compared to men. The inverted CD4/CD8 ratio was detected in 5 out of 83 examined women while the ratio ≤ 1 was found in 4 out of 16 examined men. This is not only the consequence of the thymus atrophy but also hormonal changes. The effect of the hormones on the CD4/CD8 ratio was confirmed by the correlation between low plasma estradiol levels, high CD8+ values and low CD4/CD8 ratio [21]. In our study, the majority of the study subjects (84%) were females with only 16% being males and yet, approx. 25% of the male group showed CD4/CD8 ratio < 1. To provide more substantial evidence, however, the next stage of the study should include a larger male sample. An impaired immune status may increase a risk of cardiovascular diseases including hypertension. The prevalence of hypertension in our subjects was similar in women and men and it amounted to approx. 56–58%. A large body of evidence has suggested that innate and adaptive immune responses are involved in hypertension-mediated low-grade inflammation. The recent study on mouse models has revealed that lymphocytes may be involved in the regulation of T lymphocyte proliferation and the production of pro-inflammatory cytokines, which contribute to the hypertensive inflammatory response [25]. In humans with hypertension, T lymphocytes polarise towards Th1-type CD4+ T helper cells, which initiates inflammatory response to neoantigens resulting from hypertensive factors activity and the activation of the immune system [26]. When determining the T cells phenotype in newly diagnosed hypertensive patients, Youn et al. [27] showed a significantly higher number of circulating immunosenescent pro-inflammatory CD8+ T cells in individuals with hypertension compared to healthy people. Itani et al. [28] showed higher values of circulating CD4+ CD8+ memory T cells in individuals with elevated blood pressure. In our study, we also observed higher numbers of the CD4+ and CD4+ memory cells in individuals with hypertension compared to the others.

According to Alonso-Fernandez and De la Fuente [29], the CD4/CD8 ratio could be useful as a marker of immune function in the elderly and also as a predictor of health status. In our study, a CD4/CD8 ratio > 2.5 was observed in approx. 52% of the subjects with hypertension. The other individuals suffered from additional diseases such as rheumatoid arthritis, diabetes, thyroid disorders. Therefore, it is difficult to determine whether the high values of memory CD4+ cells in this group were exclusively the effect of hypertension or the consequence of other disease entities interference. The explanation of this effect requires further investigation.
Adipose tissue lies at the crossroads of nutrition, physical exercise and immunity. An increase of fat content is accompanied by dynamic changes in immune cell populations. Nearly all immune cell studied to date are quantitatively altered by obesity with most immune cells increasing in adipose tissue. Obesity-induced changes in their number and activity result in the activation of local and later systemic inflammatory response, marking the transition from simple adiposity to diseases such as type 2 diabetes mellitus, arterial hypertension and ischemic heart disease. In our study, 50% of investigated seniors were classified as overweight and 29% as obese and FMI was found to be correlated with the numbers of $\text{CD4}^+$ and $\text{CD4}^+\text{CD45RO}^+$. Both $\text{CD4}^+$ and $\text{CD8}^+$ T cells have been shown to increase with obesity and preceded macrophage infiltration [30,31]. Further evidence demonstrated that it is the T-cells rather than the macrophage that initiate adipose tissue inflammation and dysfunction [32].

There are some limitations of the present study with the first one being a relatively small sample size, especially in the male group, which made it difficult to reach definitive conclusions. Secondly, the study is limited by a lack of information on the lifestyle and environmental factors, nutrition, physical activity and exposure to pathogens during lifetime. Therefore, in order to address these limitations, the study will be designed to increase the sample size and to explain the effect of adequate nutrition (dietary inflammatory index) and appropriate mental and physical activity as well as sleep quality.

**Conclusions**

In conclusion, the results of the present study show that the CD4/CD8 ratio and disproportion in naïve T cells in elderly people can be important predictors of morbidity and mortality in the elderly thereby affecting healthy longevity. However, in the light of recent reports, data needs to be expanded to include information on the lifestyle and environmental factors.

**Material And Methods**

*Subjects.* Ninety nine seniors aged 60–89 years (females n = 83, males n = 16) from the University of the Third Age (U3A) and thirty one younger persons aged 20–24 years (females n = 12, males n = 19) participated in the study (Table 1). Inclusion criteria were as follows: 60–90 years of age for older group and 20–30 years of age for younger group, signed informed consent. Exclusion criteria included acute infectious diseases, oncological diseases, neurodegeneration diseases and implanted pacemakers, confirmed by the assessment of the responsible doctor of medicine. The group of U3A students who qualified for the study included 50 individuals with hypertension, 44 with thyroid diseases, 12 individuals with type 2 diabetes, 18 with rheumatoid arthritis (Fig. 5). Based on the WHO protocol the elderly participants were divided into two categories: the youngest-old aged 60–74 years (n = 63) and the middle-old/oldest-old aged 75–89 years (n = 36). The current health status and lifestyle of the subjects were estimated by a doctor of medicine by means of the health history questionnaire [33]. All subjects were informed of the aim of the study and gave their written consent for participation in the project. The protocol of the study was approved by The Bioethics Commission at Regional Medical Chamber Zielona Gora, Poland (N°21/103/2018) in accordance with the Helsinki Declaration.
Body composition. Body mass and body composition fat-free mass and fat mass were estimated by a bioelectrical impedance method using Tanita Body Composition Analyser MC-980 (Japan) calibrated prior to each test session in accordance with the manufacturer's guidelines. Duplicate measures were taken with the participant in a standing position; the average value was used for the final analysis. The recurrence of measurement was 98%. The measurements were taken between 7:00 and 9:00 a.m., before blood sampling. FFM and FM indices were calculated according to the definition by Vantallie et al. [34]:

\[
\text{FFMI} = \frac{\text{FFM (kg)}}{\text{height(m}^2)} \quad \text{and} \quad \text{FMI} = \frac{\text{FM (kg)}}{\text{height(m}^2)}.
\]

Mathematically, \(\text{BMI (kg/m}^2) = \text{FFMI} + \text{FMI}\). Thus, measured FFMI, FMI and %FM values falling below the values of BMI of 18.5 kg/m\(^2\) were defined as low; measured FFMI, FMI and %FM values falling in the range of BMI between 18.5 and 25.0 kg/m\(^2\) were considered normal, and values above that range were considered high.

Blood sampling. Blood samples were taken from the median cubital vein between 7.00 and 9.00 a.m. using S-Monovette-EDTA K\(^2\) tubes (Sarstedt, Austria) for flow cytometry analysis and S-Monovette tubes for other biochemical markers. Within 20 min, they were centrifuged at 3000 g and +8° C for 10 min. Aliquots of serum were stored at -80 °C.

Flow cytometry analysis. Cytometric analysis was performed using an 8 parameter CyFlow Space Sorter flow cytometer by Sysmex Partec (Germany). For the analysis of immune cells, CyLyse kit by Sysmex (Germany) was used. 100 µL venous blood was mixed with fluorochrome labeled monoclonal antibodies (CD8 APC, CD4 FITC, CD45 RA Pacific Blue™ CD45RO PE) and incubated 15 minutes in the dark at room temperature. After the incubation 100 µL of Leukocyte Fixation Reagent A was added and incubated again in the dark for 10 minutes. In the last step, 2.5 mL Erythrocytes Lysing Reagent B was added, mixed and incubated in the dark for 20 minutes and further measurements were made. T helper and cytotoxic lymphocytes were gated by positive surface staining for CD4 and CD8 and were expressed as a percentage of gated lymphocytes. Memory and naïve subpopulations were gated by positive surface staining for CD45RO and CD45RA, respectively.

Haematological variables. The white blood cell counts (WBC, LYM, GRA, MID, LYM %, MID%, GRA%) were determined using 3 diff BM HEM3 Biomaxima (Poland).

Biochemical markers. Total cholesterol, high-density lipoproteins and as well as triglycerides were determined using BM200 Biomaxima (Poland). The non-HDL cholesterol was calculated by subtracting HDL from total cholesterol concentration, and LDL was calculated by using the Friedewald formula: TC (mg/dL) - HDL-c (mg/dL) - TG (mg/dL)/5. The serum C-reactive protein concentration was determined in duplicate by DRG ELISA kit (Poland). Detection limit was 0.001 mg/L, and CV for the CRP kit was < 3%. The serum glucose was determined by using Diaglobal spectrophotometer (Germany).

Statistical analysis. All statistical analyses were performed using the R system (https://www.r-project.org/). Analysis of variance (ANOVA) was used to analyse the differences among group means. In order for ANOVA results to be reliable, certain assumptions must be checked, i.e. a) each group sample is drawn from a normally distributed population, b) all group samples have a common variance and c) all
samples are drawn independently of each other. The first condition was checked using the Shapiro-Wilk test. The second condition was checked using the Levene's test and the Bartlett's test. The third condition is fulfilled by the fact that each patient was examined completely independently of the others. When ANOVA results were not sufficiently clear, additional analysis i.e. the nonparametric Kruskal-Wallis rank sum test was performed. An alpha of 0.05 was used as the cut off for significance.

**Abbreviations**

BMI: Body Mass Index; CMV: cytomegalovirus; CRP: C-reactive protein; DBP: diastolic blood pressure; FFM: Fat Free Mass; FFMI: Fat Free Mass Index; FM: Fat Mass; FMI: Fat Mass Index; GRA: granulocytes; HDL: high-density lipoproteins; IRP: immune risk profile; LDL: low-density lipoproteins; LYM: lymphocytes; MID: mid absolute count; SBP: systolic blood pressure; TC: total cholesterol; TCR: T cell receptor; TG: triglycerides; WBC: white blood cell count.

**Declarations**

*Ethics approval and consent to participate.* All subjects were informed of the aim of the study and gave their written consent for participation in the project. The protocol of the study was approved by The Bioethics Commission at Regional Medical Chamber Zielona Gora, Poland (N°21/103/2018) in accordance with the Helsinki Declaration.

*Consent for publication.* Not applicable.

*Availability of data and materials.* Data will be made available on request.

*Competing interests.* The authors declare that they have no competing interests.

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*Authors’ contribution.* AT, BM and AZL contributed to the research concept and design, data acquisition. AT analysed and interpreted the data and coordinated the preparation of the manuscript. AZL raised funds for research and revised the manuscript for its substantive correctness. AG performed the statistical analyses. All authors read and approved the final manuscript.

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Tables

Table 1. Anthropometrics and body composition of study subjects (mean ± SD).

|                      | Elders | Young | Elders vs. Young |
|----------------------|--------|-------|------------------|
|                      | $n = 99$ | $n = 31$ |                  |
| **Age [yr]**         | 71.9 ± 5.8 | 20.6 ± 1.1 | $P<0.001$       |
| **Weight [kg]**      | 70.2 ± 9.9 | 70.7 ± 13.0 | $P>0.05$        |
| **Height [cm]**      | 161.0 ± 5.9 | 177.4 ± 9.4 | $P<0.001$       |
| **BMI [kg/m²]**      | 27.1 ± 3.3 | 22.4 ± 2.8  | $P<0.001$       |
| **FFM [kg]**         | 46.0 ± 7.3 | 59.2 ± 11.7 | $P<0.001$       |
| **FFMI [kg/m²]**     | 17.7 ± 2.1 | 18.7 ± 2.4  | $P<0.01$        |
| **FM [kg]**          | 24 ± 5.6  | 11.7 ± 4.3  | $P<0.001$       |
| **FM%**              | 34.1 ± 5.3 | 16.5 ± 5.5  | $P<0.001$       |
| **FMI [kg/m²]**      | 9.3 ± 2.2  | 3.7 ± 1.4  | $P<0.001$       |
| **SBP mmHg**         | 140.7 ± 19.4 | 146 ± 10.0 | $P>0.05$        |
| **DBP mmHg**         | 79.4 ± 11.9 | 80 ± 7.0  | $P>0.05$        |

Abbreviations: BMI Body Mass Index; FFM Fat-Free Mass; FFMI Fat-Free Mass Index; FM Fat Mass; FMI Fat Mass Index; SBP systolic blood pressure; DBP diastolic blood pressure

Table 2. The comparison of naïve and memory T cells population in U3A students depending on concomitant diseases (mean ± SD).

| Percentage of cells [%] | Thyroid disease | Type 2 diabetes | Rheumatoid arthritis |
|-------------------------|-----------------|-----------------|---------------------|
|                         | $n = 44$        | $n = 12$        | $n = 18$            |
| **CD4⁺**               | 37.23 ± 11.30   | 32.22 ± 11.73   | 36.57 ± 11.80       |
| **CD8⁺**               | 19.16 ± 8.92    | 20.88 ± 8.71    | 20.65 ± 6.70        |
| Naive CD4⁺             | 6.29 ± 4.55     | 4.62 ± 3.81     | 5.80 ± 3.83         |
| Memory CD4⁺            | 21.96 ± 8.52    | 19.92 ± 7.33    | 19.60 ± 7.96        |
| Naive CD8⁺             | 10.02 ± 6.24    | 10.45 ± 6.75    | 10.20 ± 4.77        |
| Memory CD8⁺            | 8.75 ± 6.31     | 9.86 ± 6.07     | 8.38 ± 5.11         |
| CD4⁺/CD8               | 2.24 ± 0.95     | 1.70 ± 0.67     | 1.95 ± 0.84         |
Table 3. Haematological variables; white blood cell count (mean ± SD).

| Variable | Reference values | Elders, n = 99 | Young, n = 31 | Elders vs. Young |
|----------|-----------------|---------------|---------------|------------------|
| WBC [10^3/µl] | 5.0 - 11.6 | 6.5 ± 1.6 | 6.7 ± 1.3 | P>0.05 |
| LYM [10^3/µl] | 1.3 - 4.0 | 2.0 ± 0.6 | 2.2 ± 0.8 | P>0.05 |
| MID [10^3/µl] | 0.3 - 1.0 | 0.5 ± 0.2 | 0.5 ± 0.3 | P>0.05 |
| GRA [10^3/µl] | 2.4 - 7.6 | 4.0 ± 1.4 | 4.0 ± 1.2 | P>0.05 |
| LYM % | 19.1 - 48.5 | 32.1 ± 9.1 | 32.8 ± 10.3 | P>0.05 |
| MID % | 4.5 - 12.1 | 7.6± 3.5 | 7.8 ± 4.5 | P>0.05 |
| GRA % | 43.6 - 73.4 | 60.3 ± 10.1 | 59.4 ± 9.7 | P>0.05 |

Abbreviations: WBC white blood cells; MID mid absolute count, LYM lymphocytes; GRA granulocytes

Table 4. Lipoprotein-lipid profile, glucose and C-reactive protein (mean ± SD).

| Variable | Reference values | Elders, n = 99 | Young, n = 31 | Elders vs. Young |
|----------|-----------------|---------------|---------------|------------------|
| Glucose [mg/dL] | 60 - 115 | 95.8 ± 16.1 | 83.5 ± 13.0 | P<0.05 |
| TC [mg/dL] | <200 | 222.9 ± 41.0 | 204.2 ± 44.3 | P<0.01 |
| TG [mg/dL] | <150 | 146.7 ± 47.2 | 130.7 ± 48.9 | P<0.01 |
| HDL [mg/dL] | desirable >60 | 78.8 ± 12.1 | 70.9 ± 19.1 | P<0.05 |
| LDL [mg/dL] | <130 | 115.0 ± 39.2 | 116.4 ± 23.6 | P>0.05 |
| non-HDL [mg/dL] | <130 | 144.0 ± 40.2 | 133.3 ± 33.3 | P>0.05 |
| CRP [mg/L] | 0.068 - 8.2 | 2.3 ± 2.2 | 1.5 ± 1.9 | P<0.05 |

Abbreviations: TC total cholesterol, TG triglycerides, HDL high density lipoprotein, LDL low density lipoprotein; CRP C-reactive protein

Figures
Figure 1

Percentages of CD4+ and CD8+ naïve and memory cells in young (gray bars) and elderly (black bars) and average CD4/CD8 ratios.
Figure 2

Percentages of CD4+ and CD8+ naïve and memory cells in 60-74 years of age (black bars) and 75-90 years of age (gray bars) and average CD4/CD8 ratios.

Figure 3

Percentages of CD4+ and CD8+ naïve and memory cells in women (black bars) and man (gray bars) and average CD4/CD8 ratios in U3A students.
Figure 4

Percentages of CD4+ and CD8+ naïve and memory cells in patients diagnosed with hypertension (black bars) and in patients with other diseases (gray bars) and average CD4/CD8 ratios.
Figure 5

Characteristics of the study subjects; division of the elderly in relation to the age-accompanying diseases.