

Influence of sex, age, pubertal maturation and body mass index on circulating white blood cell counts in healthy European adolescents - The HELENA STUDY

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Abstract

Percentiles 10th, 25th, 50th, 75th and 90th are presented for total circulating white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils and basophils in healthy European adolescents (12.5-17.5 years, n=405, 48.9% boys), considering age, sex, puberty and body mass index (BMI). CD3⁺ (mature T cells), CD4⁺ (T helper), CD8⁺ (T cytotoxic), CD16⁺56⁺ (natural killer), CD19⁺ (B cells), CD45RA⁺ (naïve) and CD45RO⁺ (memory) lymphocytes were also analysed by immunophenotyping. Girls presented higher WBC, neutrophil, CD3⁺CD45RO⁺ and CD4⁺CD45RO⁺ cell counts and CD3⁺/CD19⁺ ratio, and lower CD3⁺CD45RA⁺ and CD4⁺CD45RA⁺ counts than boys. Age was associated with higher neutrophil counts and CD3⁺/CD19⁺ ratio and lower CD19⁺ counts; in boys, with lower CD3⁺CD45RA⁺, CD4⁺CD45RA⁺ and CD8⁺CD45RA⁺ counts as well; in girls, with higher WBC, CD3⁺CD45RO⁺, and CD4⁺CD45RO⁺ counts. Pubertal maturation in boys was associated with lower WBC and lymphocyte counts; in girls, with higher basophil, CD3⁺CD45RO⁺ and CD4⁺CD45RO⁺ values. BMI was associated with higher WBC counts; in boys, also with higher lymphocyte counts; in girls, with higher neutrophil, CD4⁺, CD3⁺CD45RO⁺ and CD4⁺CD45RO⁺ counts. *Conclusions:* Our study provides normative values for circulating immune cells in adolescents, highlighting

the importance of considering sex, age, pubertal maturation and BMI when establishing reference values for WBC in paediatric populations.

Key words: adolescents; immune cells; immunophenotyping; sex; puberty; body mass index.

Abbreviations used

BMI – body mass index

WBC – white blood cells

What is known?

Reference values for white blood cell counts and immunophenotyping of lymphocyte subsets can constitute useful clinical tools and health indicators in both adult and paediatric populations.

Like other health indicators, they can vary according to a number of factors, such as age or sex.

What is new?

Specific data from WBC in European adolescents are limited, and even less is known about changes in lymphocyte subsets during adolescence. This work provides normative values obtained from adolescents from 9 European countries, considering the influence of sex, age, pubertal maturation and BMI and finding that:

- Girls had higher WBC, neutrophil and CD45RO⁺ (memory) cell values, while boys had higher percentages of lymphocytes, monocytes and eosinophils and CD45RA⁺ (naive) cell counts.
- Age was associated with higher WBC, neutrophil, CD45RO⁺ and CD3⁺/CD19⁺ values, and lower percentage of total lymphocytes and CD45RA⁺ cell counts.
- Pubertal maturation was associated, on the contrary, with lower WBC and lymphocytes, but only in boys. In girls, pubertal maturation was linked to higher CD45RO⁺ cell counts.
- BMI was associated with higher WBC, mainly due to greater lymphocyte counts in boys, and to neutrophil and CD45RO⁺ cell counts in girls.

Introduction

Total white blood cell (WBC) counts and the evaluation of the different subtypes of white blood cells are useful clinical indicators and are frequently used as diagnostic tools for adults as well as for children. Besides providing information on acute inflammatory and infectious states, the immunological status serves as an indicator of many other physiological processes, and immune markers are becoming increasingly used to study alterations other than infections – for example, as early as in the 70s, Friedman and colleagues reported an association between WBC count and myocardial infarction [9]. Immune function is closely related to overall health and nutritional status [31], and during the last decade, WBC count has been related to different metabolic alterations, such as impaired glucose tolerance, type 2 diabetes mellitus, obesity, or the metabolic syndrome, and this has been observed in adults [10, 25] as well as in children and adolescents [5, 36]. The analysis of lymphocyte subsets by flow cytometry (known as immunophenotyping) is an ever more widely used tool not only for assessing health status but also specifically in nutritional evaluation, and helps identify subjects at risk of disease.

Adequate and reliable reference values from healthy populations become a key point in the clinical use of any biological parameter. Variations can occur as a consequence of physiological, ethnic or environmental factors; therefore, normative values should be specific for a given population group. For example, childhood and adolescence are growth periods in which all the systems in the body are developing physically and functionally. The immune system itself experiences a series of modifications from birth until adulthood; during childhood and adolescence the immune cells vary in both their number and functionality [1, 6, 8, 13, 16, 18, 28, 32, 34]. Therefore, normative values for immune cells obtained from adult populations can be misleading when applied to children and adolescents. In addition, sex-related and even ethnicity-related variations in the values of circulating immune cells have been acknowledged [1, 4, 7, 17, 28, 29, 34, 38,], highlighting the importance of providing reference values for specific

population groups and geographical areas. However, information about WBC counts and specific lymphocyte subsets on healthy adolescents is still scarce [1, 8, 16, 28, 32, 34], as most available data belong to populations with particular immune-related diseases, or the studies, although useful and informative, have usually been conducted on relatively modest sample sizes for this particular age group.

The present study aims to provide normative ranges for total and differential WBC counts and for selected lymphocyte subsets in a representative sample of healthy European adolescents, attending to variations due to sex, age, degree of pubertal maturation, and body mass index.

METHODS

Study design and sample selection

A European multicentre cross-sectional study (CSS) was performed with the objective of assessing a “healthy lifestyle in Europe by nutrition in adolescence” (HELENA). The HELENA-CSS aimed to obtain reliable and comparable data on nutrition and other health indicators such as physical activity and fitness, body composition, cardiovascular disease risk factors, vitamin and mineral status, and immunological and genetic markers in European adolescents [21]. The methodology used in this study has been published elsewhere [20]. The study was performed according to the ethical guidelines of the Edinburgh revision of the 1964 Declaration of Helsinki (2000), the International Conferences on Harmonization for Good Clinical Practice and the legislation on clinical research from each of the participating countries. The protocol was approved by the Research Ethics Committees of the participating centres. Written informed consent was obtained from the parents of the adolescents and from the adolescents themselves [2].

Briefly, subjects aged 12.5-17.5 years were recruited randomly from schools in ten cities belonging to nine countries across Europe (Athens and Heraklion in Greece, Dortmund in

Germany, Ghent in Belgium, Lille in France, Pécs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain). The total eligible HELENA-CSS population consisted of 3,528 adolescents. Blood samples were obtained in one third of participants, resulting in a representative subpopulation of 1,089 adolescents (approximately 100 boys and girls per city). The size of this subpopulation was previously calculated as sufficient to account for the expected variability in blood measurements.

Data exclusion

Those subjects with conditions that might interfere with or imply stimulation of normal immune function were excluded from the analysis. Exclusion criteria included: suffering from allergies, suffering from fever on the 24 hours prior to blood sampling, having had a cold or any infection during the week prior to the day of blood sampling, having taken any medication in the previous 24 hours or for more than 7 days in the previous 30 days, having taken any vitamin or mineral supplement during the previous month, and having been vaccinated in the two weeks prior to the day of blood sampling. After applying these exclusion criteria, the final study population consisted of 405 subjects (48.9% boys).

Measurements of pubertal maturation and body mass index

Evaluation of the degree of pubertal maturation was assessed by a medical doctor, according to the Tanner and Whitehouse classification [33]. Anthropometric data were also collected following harmonized protocol procedures previously described [23]. Body mass index (BMI) was calculated as: $\text{body weight (kg)} / [\text{height (m)}]^2$. Standardized BMI values (z-scores) were calculated and the sample was classified according to quartiles of standardized BMI values.

Blood sampling, white blood cell profiling and immunophenotyping

Venous blood samples were collected in EDTA Monovette (Sarstedt, Germany) tubes between 8.00 and 10.00 a.m. after a 12-hour overnight fast. WBC counts and percentages were determined in each participating city with automated blood cell counters. For immunophenotyping of lymphocyte subsets, blood samples were collected in EDTA-K3E Vacutainer (BD Biosciences) tubes. Blood aliquots were taken into 1.5 ml plastic tubes and diluted 1:1 with Cytochex™ Reagent (Streck Laboratories, Omaha, NE, USA). The samples were all sent to the CSIC group laboratory (Madrid, Spain) within 7 days from collection. The methodology for WBC determination and for collection, preparation and shipping of the blood samples to Madrid was standardized amongst all participating cities [11].

Immunophenotyping

Blood aliquots were incubated for 30 minutes at room temperature in the dark with fluorochrome-conjugated monoclonal antibodies (BD Biosciences, San José, CA, USA), to differentially label those cells positive for the surface markers CD45 (the pan-leukocyte marker), CD3 (T mature cells), CD4 (helper T cells), CD8 (cytotoxic T cells), CD19 (B cells), CD16⁺56 (natural killer cells), CD45RO (memory cells) and CD45RA (naïve cells). A quadruple immunostaining procedure was performed as follows: CD3/CD8/CD45/CD4, CD45RA/CD45RO/CD8/CD3, CD45RA/CD45RO/CD4/CD3 and CD3/CD16⁺56/CD45/CD19. After lysis of red blood cells, lymphocytes were analysed by flow cytometry (FACScan Plus Dual Laser, Becton Dickinson Sunnyvale, CA). The lympho-gate was defined on the forward and side scatter patterns of lymphocytes. The analysis protocol gated on lymphocytes stained with PerCP and/or APC and the selected population was then analysed with the two remaining colours (FITC and PE) to obtain percentages of cell expressing the specific antigens.

Percentages of the following lymphocyte subsets were obtained: total mature T cells (CD45⁺CD3⁺, or CD3⁺ for simplicity), helper T cells (CD45⁺CD3⁺CD4⁺, or CD4⁺), cytotoxic T

cells ($CD45^+CD3^+CD8^+$, or $CD8^+$), natural killer (NK) cells ($CD45^+CD3^-16^+56^+$, or $CD16^+56^+$), B cells ($CD45^+CD3^-CD19^+$, or $CD19^+$), naïve T cells ($CD3^+CD45RA^+$, $CD4^+CD45RA^+$ and $CD8^+CD45RA^+$, or $CD3RA^+$, $CD4RA^+$ and $CD8RA^+$), and memory T cells ($CD3^+CD45RO^+$, $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$, or $CD3RO^+$, $CD4RO^+$ and $CD8RO^+$). Absolute cell counts were calculated from total lymphocyte numbers, which were determined with automated blood cell counters in each participating city. The $CD4^+/CD8^+$ and the $CD3^+/CD19^+$ ratios were also calculated.

Statistical analysis

All the analyses conducted on the HELENA-CSS data were adjusted by a weighing factor to balance the studied population according to the age and sex distribution of the theoretical sample. Adolescents were grouped into four age categories: 12.5-13.9 years (from 12.5 years to the day before the 14th birthday), 14-14.9 years (from the 14th birthday to the day before the 15th birthday), 15-15.9 years (from the 15th birthday to the day before the 16th birthday), and 16-17.5 years (from the 16th birthday to 17.5 years). The *Chi-squared* (X^2) test was used to compare frequencies of sex, age categories, and Tanner stages between the studied and the excluded groups, as well as to compare frequencies of age categories and Tanner stages between sexes.

Absolute counts and percentages of cells are presented as percentiles 10th, 25th, 50th (median), 75th and 90th for description of the population classified by sex and age. Data were then studied according to sex, age, Tanner stage and standardized BMI categories. Normality of variables was checked by the Kolmogorov-Smirnov test, and those not normally distributed were appropriately transformed when necessary.

Differences between sexes were analysed with the Mann-Whitney U test. Subsequent tests to assess the influence of age, pubertal maturation and BMI were conducted separately in boys and girls. Associations between cell values and age and between cell values and BMI were

assessed with Pearson partial correlation test, controlling for city of origin (centre). Differences in cell values between Tanner stages and BMI categories were assessed by analysis of covariance (ANCOVA), adjusting for centre (and age when comparing Tanner stages). Due to the small number of individuals found within Tanner stages I and II, these two groups were combined to allow statistical analysis.

Statistical significance was set at $P < 0.05$. All statistical analyses were performed with IBM® SPSS® Statistics v.19 for Windows.

RESULTS

Characteristics of the population

There were no differences in the proportions of sexes between included and excluded adolescents (48.9 vs. 45.5% boys; $\chi^2 = 1.196$, $P = 0.274$). Similarly, no significant differences were found in the distributions of age categories ($\chi^2 = 1.146$, $P = 0.766$), Tanner stages ($\chi^2 = 3.851$, $P = 0.278$) or BMI categories ($\chi^2 = 7.164$, $P = 0.067$) between the adolescents included in the analysis and their excluded peers.

The average age of the studied population was 14.9 ± 1.2 years (range 12.5-17.4 y). There were significantly fewer adolescents between 16 and 17.5 years (22%) than in the other age groups ($\chi^2 = 16.392$, $P < 0.01$). In relation to pubertal maturation, most of the adolescents (78.2%) were found at either the IV or V Tanner stages, as could be expected according to the age range. Boys and girls were similar in age, Tanner stage distributions and mean BMI values in each quartile of BMI z-scores (boys: 17.7 ± 1.1 (Q1), 19.8 ± 0.8 (Q2), 21.8 ± 1.2 (Q3), and 27.0 ± 3.2 (Q4) kg/m²; girls: 17.9 ± 1.1 (Q1), 20.0 ± 0.8 (Q2), 22.0 ± 1.1 (Q3), and 26.3 ± 3.0 (Q4) kg/m²).

Influence of sex on immune cell counts and percentages

Statistical comparison between sexes showed that girls in general presented higher values for total WBC and neutrophil counts (**table 1**). Percentages of neutrophils were also higher in girls, while those of lymphocytes, monocytes and eosinophils were greater in boys (**supplementary table 1**).

Table 2 shows the percentiles 10th, 25th, 50th (median), 75th and 90th of the absolute counts of the selected lymphocyte subsets, separately by sex and group of age. Percentiles of the lymphocyte subsets percentages can be found in **supplementary table 2**. Boys showed a tendency to higher counts of CD3⁺CD45RA⁺ and CD4⁺CD45RA⁺ naïve cells (differences significant at 14-14.9 y), while girls presented higher counts of CD3⁺CD45RO⁺ (differences being significant at 14-14.9 and 16-17.5 y) and CD4⁺CD45RO⁺ memory cells (significant at 16-17.5 y). The ratio CD3⁺/CD19⁺ was also higher in girls (difference being significant at 15-15.9 years) (**table 2**). Similarly, boys had higher percentages of CD3⁺CD45RA⁺ (significant at 14-14.9 y and 16-17.5 y), CD4⁺CD45RA⁺ and CD8⁺CD45RA⁺ (differences significant at 14-14.9 y); girls in turn had higher percentages of CD3⁺ and CD4⁺ (differences significant at 15-15.9 y), CD3⁺CD45RO⁺ (differences significant at 14-14.9 and 16-17.5 y), CD4⁺CD45RO⁺ and CD8⁺CD45RO⁺ (differences significant at 14-14.9 y) (**supplementary table 2**).

Influence of age on immune cell counts and percentages

Total WBC and neutrophil counts increased with age in the total population ($r = 0.121$, $P = 0.015$ and $r = 0.153$, $P = 0.003$, respectively; and **table 1**). Neutrophil percentages also increased with age, while the percentage of lymphocytes decreased, both in the total population ($r = 0.167$ and $r = -0.173$, respectively, $P = 0.001$) and in boys (**supplementary table 1**).

Age was also associated with increased counts of CD4⁺CD45RO⁺ ($r = 0.179$, $P = 0.001$) and the ratio CD3⁺/CD19⁺ ($r = 0.240$, $P < 0.001$), and decreased counts of CD19⁺ ($r = -0.250$,

$P < 0.001$), $CD3^+CD45RA^+$ ($r = -0.118$, $P = 0.036$), and $CD4^+CD45RA^+$ ($r = -0.124$,
 $P = 0.027$). The percentages of these subsets showed the same relationships with age ($\%CD19^+$:
 $r = -0.255$; $\%CD3^+CD45RA^+$: $r = -0.184$; $\%CD4^+CD45RA^+$: $r = -0.238$; $\%CD3^+CD45RO^+$:
 $r = 0.206$; $\%CD4^+CD45RO^+$: $r = 0.235$; all $P < 0.01$). In boys alone, age was inversely
correlated with counts of $CD19^+$, $CD8^+$ and naïve cells ($CD3^+CD45RA^+$, $CD4^+CD45RA^+$ and
 $CD8^+CD45RA^+$), and positively with the ratio $CD3^+/CD19^+$ (**table 2**). In girls alone, older age
was associated as well with lower $CD19^+$ and higher $CD3^+/CD19^+$, but also with increased
counts of $CD3^+CD45RO^+$ and $CD4^+CD45RO^+$ (**table 2**). As a result, in both boys and girls there
was a trend to decreased percentages of naïve cells and increased of memory cells with age
(**supplementary table 2**).

Influence of pubertal maturation on immune cell counts and percentages

In boys, pubertal maturation (Tanner stage) was associated with lower WBC and lymphocyte
counts, independently of age (**table 3**); lymphocyte percentages were also lower in more
developed Tanner stages (**supplementary table 3**). This decrease was reflected in most
lymphocyte subsets: $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD3^+CD45RA^+$, $CD3^+CD45RO^+$,
 $CD4^+CD45RO^+$, $CD8^+CD45RA^+$ and $CD8^+CD45RO^+$ cell counts were all lower in more
advanced Tanner stages (**table 4**).

In girls, higher basophil counts (**table 3**) and percentages (**supplementary table 3**), and
 $CD3^+CD45RO^+$ and $CD4^+CD45RO^+$ cell counts were higher in more advanced pubertal
maturation stages (**table 4**).

Influence of BMI on immune cell counts and percentages

In boys, counts of total WBC, neutrophils and lymphocytes were higher with increasing BMI
(**table 5**). The increase in lymphocyte counts was reflected in most subsets ($CD3^+$, $CD4^+$, $CD8^+$,

natural killer, CD19⁺, CD3⁺CD45RO⁺, CD4⁺CD45RO⁺ and CD8⁺CD45RO⁺), whereas the ratio CD4⁺/CD8⁺ (**table 6**) and the percentages of naïve cells (CD3⁺CD45RA⁺ and CD4⁺CD45RA⁺) decreased (**supplementary table 6**).

In girls, BMI showed a positive relationship with total WBC and neutrophil counts (**table 5**), neutrophil percentages (**supplementary table 5**), and memory cell counts (CD3⁺CD45RO⁺ and CD4⁺CD45RO⁺) (**table 6**), and a negative one with percentages of CD8⁺CD45RA⁺ cells (**supplementary table 6**).

DISCUSSION

The current study describes the immune cell profile of a representative sample of healthy European adolescents. Our results show that sex, age, pubertal maturation and BMI are factors that influence normal circulating counts and percentages of immune cells in adolescents.

There were clear sex-related differences in the percentages and absolute counts of immune cells in our population. In general, girls had higher total WBC, neutrophil and memory T cells values (in particular, CD3⁺CD45RO⁺ and CD4⁺CD45RO⁺), while boys had higher percentages of lymphocytes and eosinophils, and higher counts of naïve T cells (all three subsets, CD3⁺CD45RA⁺, CD4⁺CD45RA⁺ and CD8⁺CD45RA⁺). Similar trends for differences in WBC between sexes were observed in a previous study in Spanish adolescents, although the authors did not find statistical significance [27]. With regards to lymphocyte subsets, our observations are also in agreement with previous studies [1, 28, 34]. On the contrary, other authors have found no effect of sex on WBC counts [38] or lymphocyte subsets [32] in adolescents. Sex dimorphism in WBC counts and percentages can be explained by differences in sex hormones, as suggested by Rudy and colleagues [28]. Sex hormones have been shown to modulate immune function at various levels, and a greater immune responsiveness has been observed in girls in general [12, 35].

A trend was observed towards higher WBC counts with age, in agreement with the findings reported by Bartlett and colleagues [1]. In particular, neutrophil counts were elevated in older boys and girls in our study; this led to increasing percentages of this cell type and decreasing percentages of lymphocytes in boys, while no significant changes in cell percentages with age were observed in girls. In contrast, age was associated with decreasing values for B cells (CD19⁺) leading to a higher CD3⁺/CD19⁺ ratio, and with a significant shift towards lower naïve/memory cells ratios. Lower B cell numbers with age were also observed by Bartlett [1]. Likewise, the changes in naïve and memory T cells are in agreement with previous studies [16, 28], and coherent with an age-related maturation process of the immune system [32]. Age-sex interactions were observed: boys presented higher counts of naïve cells than girls, but the difference was ameliorated by age; in contrast, the counts of memory cells were higher in girls, and the gap increased with age. As a consequence, the changes in percentages of naïve and memory T cells, which were similar in both boys and girls, were the result of decreased naïve cell counts in boys, and of increased memory cell counts in girls. This difference between boys and girls could suggest a more mature or experienced immune system in the girls. As our adolescents were age-matched, the cause must be related to other parameters of growth rather than age, like pubertal maturation.

For that reason, pubertal maturation was considered for the analysis of immune cell profiles in our study. Similarly to age, the effect of pubertal maturation on WBC counts and percentages showed a distinct sex-related pattern. In boys, pubertal maturation was associated with lower WBC counts, lymphocyte counts and percentages; the decrease in lymphocyte counts was reflected in most subsets (CD3⁺, CD4⁺, CD8⁺, CD19⁺, CD3⁺CD45RA⁺, CD3⁺CD45RO⁺, CD8⁺CD45RA⁺ and surprisingly, also CD4⁺CD45RO⁺ and CD8⁺CD45RO⁺ cells). This could be explained by the physiological increase in androgen levels, mainly testosterone, since studies in men have reported negative associations between testosterone levels and WBC counts [3, 30]. In

girls, pubertal maturation was associated with increases in basophil counts and percentages and higher memory T cell counts. The lack of other significant relationships between pubertal maturation and immune cell counts could be related to the fact that the degree of pubertal maturation of the female sample in this study was high, with 82% of them being in the IV and V Tanner stages (in boys this proportion was lower, 73.7%).

Finally, BMI z-scores were also significantly associated with values of circulating immune cells in the studied population, and again the relationship was sex-specific. Higher BMI was in general associated with higher counts of total WBC and memory T cells (all three subsets in boys and $CD3^+CD45RO^+$ and $CD4^+CD45RO^+$ in girls). In boys, both neutrophils and lymphocytes were elevated, but the increase in lymphocytes was proportionally greater. In this line, in addition to memory cells, higher BMI z-scores in boys were also associated to elevated cell counts in most subsets ($CD3^+$, $CD4^+$, $CD8^+$, NK, $CD19^+$, and $CD8^+CD45RA^+$) and with a lower $CD4^+/CD8^+$ ratio. In girls, BMI was mainly linked to counts of neutrophils and memory cells. Alterations in immune parameters have been associated with both insufficient and excessive body weight [reviewed in 19 and 26]. In adults, positive associations were reported between BMI and circulating WBC, neutrophils, lymphocytes and monocytes [15]. Later, other authors have found similar age-independent, positive associations between WBC counts and BMI in children and adolescents [14, 36], in line with our observations. Furthermore, obesity in children and adolescents has been linked to elevated counts of neutrophils, monocytes, total T cells and helper T cells [37]. Our results, like those by Hsieh [14] and Wu [36], show that this increase is not merely the result of obesity, but it takes place in a linear manner with increasing BMI. It is worth highlighting this correlation, as WBC count has been related to features of the metabolic syndrome in both adult [10, 22] and paediatric populations [5, 14, 36], and a follow-up study in Japanese adults concluded that higher WBC counts were associated with higher risk of developing metabolic syndrome in the future [24]. The explanation for the relationship between

BMI and memory T cells or its potential implications is less straightforward. On the one hand, it could suggest that increasing BMI provides a more favorable environment for immune system development, similarly to age and pubertal maturation. On the other hand, we could instead be facing similar outcomes with different origins, and BMI-related changes could indicate abnormal or excessive activation of the immune system. This hypothesis would be supported by the relationships between circulating WBC and the metabolic syndrome mentioned above. However, the underlying processes linking nutritional status, BMI and immune function in children and adolescents clearly requires further research.

In clinical practice to date, sex and age have been routinely taken into account for the establishment of normative ranges, since they constitute easy-to-obtain information. Other physiological features like pubertal maturation or BMI, however, are not frequently available or assessed when analysing blood variables. In light of ours and other authors' results, we recommend considering these characteristics when setting reference values for or performing blood measurements in paediatric populations.

Finally, two considerations should be made in relation to the present study. On the one hand, the number of subjects suitable for statistical analysis was not balanced in relation to the actual population size of each city. On the other, the sample studied included adolescents from different ethnic origins, and as it was mentioned above, ethnicity can be an influential factor for differences in WBC counts [1, 17, 38]. Despite these caveats, our work contributes to the development of a database of haematological reference values in healthy European adolescents. This is particularly strengthened by the use of standardized protocols and methods across all centres participating in the study.

In conclusion, the present work provides data on normal values for white blood cell counts and percentages from healthy European adolescents, and highlights the importance of taking into account the influence of sex, age, the degree of pubertal maturation and BMI when

comparing or using white blood cell counts for clinical and research purposes in paediatric populations.

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Conflict of interest

None of the authors had a personal or financial conflict of interest.

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513 **APPENDIX: HELENA Study Group**

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Table 1. White blood cell (WBC) counts (cell/ μ l) in European adolescents, according to age categories and stratified for sex.

Boys						Girls					
Age range (years)	12.5-13.9	14-14.9	15-15.9	16-17.5		12.5-13.9	14-14.9	15-15.9	16-17.5		
N	48	55	46	49	R	56	48	63	40	R	
WBC	10 th	4,106	4,382	3,787 ^{§§}	4,630	0.107	4,493	4,368	4,934^{§§}	5,230	0.154*
	25 th	4,854	5,011	5,214 ^{§§}	5,055	5,151	5,202	5,670^{§§}	5,682		
	50 th	5,721	5,706	6,100 ^{§§}	6,105	6,010	6,404	6,741^{§§}	6,520		
	75 th	6,572	6,579	6,804 ^{§§}	6,810	6,929	7,823	8,011^{§§}	7,702		
	90 th	7,605	8,000	7,568 ^{§§}	7,490	8,457	9,573	8,753^{§§}	8,350		
Neutrophils	10 th	1,786[§]	1,906^{§§}	1,787[§]	2,197^{§§}	0.184*	2,310[§]	1,870^{§§}	2,138[§]	2,700^{§§}	0.162*
	25 th	2,117[§]	2,328^{§§}	2,371[§]	2,576^{§§}		2,834[§]	2,587^{§§}	2,860[§]	3,153^{§§}	
	50 th	2,940[§]	2,681^{§§}	3,310[§]	3,190^{§§}		3,227[§]	3,555^{§§}	3,990[§]	3,835^{§§}	
	75 th	3,448[§]	3,641^{§§}	3,984[§]	3,635^{§§}		3,898[§]	4,873^{§§}	4,900[§]	4,370^{§§}	
	90 th	4,771[§]	4,458^{§§}	4,737[§]	4,700^{§§}		5,158[§]	6,357^{§§}	5,591[§]	4,930^{§§}	
Lymphocytes	10 th	1,688	1,539	1,450	1,460	−0.130	1,483	1,418	1,570	1,700	0.054
	25 th	1,895	1,757	1,700	1,918		1,800	1,691	1,870	1,846	
	50 th	2,200	2,241	2,040	2,120		2,100	2,141	2,140	1,960	
	75 th	2,411	2,561	2,334	2,283		2,408	2,535	2,500	2,488	
	90 th	3,106	2,887	2,768	2,960		2,663	3,088	3,003	2,940	
Monocytes	10 th	295	300	330	300	0.074	324	280	350	280	0.092
	25 th	360	370	376	399		395	340	400	376	
	50 th	420	461	445	460		440	430	500	510	
	75 th	522	600	543	510		514	551	570	578	
	90 th	700	769	648	620		700	743	714	610	
Eosinophils	10 th	80	80	40	60	−0.056	57	49	64	60	-0.074
	25 th	100	100	100	100		100	80	100	70	
	50 th	119	160	140	135		137	130	125	110	
	75 th	200	224	232	191		230	200	200	186	
	90 th	300	400	455	310		424	255	334	280	
Basophils	10 th	0	0	0	0	0.029	0	1	0	0	0.090
	25 th	0	10	8	20		0	10	10	20	
	50 th	20	20	20	30		20	30	30	20	
	75 th	40	45	30	31		33	40	50	40	
	90 th	79	92	64	60		58	76	100	50	

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. [§]Significant differences between boys and girls for a given age category, as assessed by the Mann-Whitney U test, [§] $P < 0.05$, ^{§§} $P < 0.01$. R is the partial correlation coefficient between cell counts and age, controlling for centre; bold rows indicate significant correlations, * $P < 0.05$, ** $P < 0.01$.

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Table 2. Estimated cell counts (cell/ μ l) of selected lymphocyte subsets in adolescents, according to age categories and stratified for sex.

		Boys					Girls				
Age range (years)		12.5-13.9	14-14.9	15-15.9	16-17.5		12.5-13.9	14-14.9	15-15.9	16-17.5	
N		36	49	41	45	R	39	42	50	26	R
CD3 ⁺	10 th	1,147	1,005	952	943	-0.149	1,041	922	1,117	1,151	0.081
	25 th	1,239	1,208	1,139	1,145		1,278	1,138	1,291	1,275	
	50 th	1,477	1,524	1,355	1,464		1,384	1,498	1,536	1,374	
	75 th	1,758	1,737	1,695	1,598		1,573	1,772	1,951	1,819	
	90 th	2,330	1,974	2,002	2,127		1,889	2,124	2,180	1,930	
CD4 ⁺	10 th	580	535	491	487	-0.139	573	459	616	567	0.109
	25 th	652	660	628	581		651	612	706	687	
	50 th	806	824	771	791		779	789	842	785	
	75 th	1,055	962	902	972		888	950	1,054	1,095	
	90 th	1,154	1,150	1,044	1,070		1,057	1,181	1,292	1,212	
CD8 ⁺	10 th	372	349	343	309	-0.154*	393	333	392	383	0.009
	25 th	490	443	441	358		442	425	463	472	
	50 th	541	601	531	539		581	558	584	515	
	75 th	770	724	704	683		672	735	725	633	
	90 th	1,016	915	850	888		757	970	983	725	
CD3-CD16 ⁺ 56 ⁺	10 th	164	119	145	165	-0.030	148	172	168	138	0.022
	25 th	248	201	222	217		191	242	238	180	
	50 th	333	298	350	376		275	312	312	322	
	75 th	410	417	447	447		434	418	388	400	
	90 th	754	647	562	542		579	531	507	784	
CD3-CD19 ⁺	10 th	160	157	153	131	-0.263**	181	140	150	99	-0.266**
	25 th	221	198	195	174		231	196	174	127	
	50 th	286	283	251	219		267	244	238	241	
	75 th	333	377	311	279		385	304	299	285	
	90 th	514	514	439	375		501	409	354	321	
CD3+CD45RA ⁺	10 th	637	643[§]	485	461	-0.199*	570	510 [§]	609	535	-0.016
	25 th	770	735[§]	711	612		718	660 [§]	678	673	
	50 th	958	1,000[§]	809	860		870	815 [§]	887	824	
	75 th	1,113	1,138[§]	1,016	1,001		1,024	1,015 [§]	1,149	911	
	90 th	1,448	1,218[§]	1,213	1,195		1,236	1,386 [§]	1,408	1,193	
CD3+CD45RO ⁺	10 th	346	332 [§]	317	385 [§]	-0.016	347	322[§]	437	517[§]	0.237**
	25 th	451	437 [§]	392	463 [§]		440	475[§]	530	572[§]	
	50 th	526	482 [§]	564	575 [§]		520	630[§]	625	660[§]	
	75 th	735	674 [§]	705	672 [§]		619	778[§]	749	791[§]	

CD4+CD45RA+	90 th	867	807 [§]	847	832 [§]	-0.196*	818	897[§]	928	942[§]	-0.042
	10 th	306	311[§]	248	201		306	232 [§]	287	197	
	25 th	382	381[§]	322	304		364	288 [§]	343	347	
	50 th	497	524[§]	416	418		479	422 [§]	448	451	
	75 th	626	629[§]	482	596		570	590 [§]	643	512	
CD4+CD45RO+	90 th	752	709[§]	640	708	0.006	716	694 [§]	744	590	0.344**
	10 th	227	194	194	256 [§]		205	187	279	261[§]	
	25 th	245	251	234	292 [§]		252	269	313	338[§]	
	50 th	313	304	338	337 [§]		291	358	371	389[§]	
	75 th	414	430	442	406 [§]		343	428	483	566[§]	
CD8+CD45RA+	90 th	487	556	508	466 [§]	-0.186*	401	522	540	644[§]	0.021
	10 th	268	249	221	206		200	203	255	230	
	25 th	311	298	285	231		282	271	299	301	
	50 th	384	414	362	367		380	342	391	334	
	75 th	489	523	505	446		471	450	543	441	
CD8+CD45RO+	90 th	645	663	619	603	-0.036	574	558	678	497	0.032
	10 th	90	65	79	78		84	85	92	94	
	25 th	107	98	102	110		118	130	128	139	
	50 th	164	159	152	155		146	182	180	173	
	75 th	224	225	199	224		217	290	231	213	
CD4+/CD8+	90 th	264	290	249	328	0.051	313	354	300	240	0.104
	10 th	1.04	0.85	0.98	0.87		1.03	0.89	1.00	1.14	
	25 th	1.16	1.21	1.16	1.13		1.22	1.11	1.19	1.24	
	50 th	1.29	1.49	1.36	1.62		1.44	1.46	1.55	1.56	
	75 th	1.58	1.86	1.78	1.97		1.64	1.64	1.78	1.87	
CD3+/CD19+	90 th	2.18	2.23	2.22	2.36	0.189*	1.98	2.02	2.11	2.86	0.311**
	10 th	3.08	3.10	3.44[§]	3.90		3.04	3.62	3.99[§]	4.74	
	25 th	4.32	3.75	4.02[§]	5.24		3.87	4.06	5.27[§]	6.10	
	50 th	5.95	5.25	5.82[§]	6.11		4.75	5.69	6.61[§]	6.89	
	75 th	7.10	7.14	6.90[§]	7.74		6.68	7.14	8.31[§]	9.93	
	90 th	8.71	9.24	8.58[§]	10.76		7.87	11.00	12.04[§]	13.79	

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Lymphocyte populations are designated by their cell markers. [§]Significant differences between boys and girls for a given age category, as assessed by the Mann-Whitney U test; [§]*P*<0.05, ^{§§}*P*<0.01. R is the partial correlation coefficient between cell counts and age, controlling for centre; bold rows indicate significant correlations, **P*<0.05, ***P*<0.01.

603 **Table 3.** White blood cell (WBC) counts (cell/ μ l) in European adolescents, according to Tanner stages and stratified for sex.

		Boys					Girls				
Tanner stage		I+II	III	IV	V		I+II	III	IV	V	
N		12	34	77	55	P	7	26	97	60	P
WBC	10 th	4,073	4,803	4,160	4,357	0.037	3,920	4,353	4,696	4,995	0.404
	25 th	4,751	5,469	4,940	4,946		4,423	5,030	5,481	5,357	
	50 th	6,050	5,965	5,706	5,938		5,091	6,065	6,541	6,500	
	75 th	7,271	6,769	6,581	6,811		6,878	8,179	7,612	8,003	
	90 th	9,129	8,273	7,454	7,300		-	9,581	8,693	8,400	
Neutrophils	10 th	1,243	2,129	1,766	2,021	0.068	1,290	1,908	2,153	2,444	0.089
	25 th	2,158	2,833	2,169	2,447		1,543	2,361	2,864	2,975	
	50 th	3,095	3,206	2,891	3,210		2,259	3,515	3,599	3,662	
	75 th	4,057	3,636	3,504	4,000		3,601	5,056	4,486	4,699	
	90 th	4,794	5,097	4,197	4,710		-	7,111	5,335	5,476	
Lymphocytes	10 th	1,803	1,650	1,537	1,298	0.002	1,550	1,385	1,566	1,500	0.981
	25 th	1,898	1,880	1,903	1,575		1,917	1,658	1,850	1,828	
	50 th	2,247	2,138	2,155	1,935		2,122	2,097	2,120	2,140	
	75 th	2,984	2,567	2,397	2,310		2,339	2,468	2,510	2,500	
	90 th	3,845	3,275	2,870	2,728		-	2,790	2,991	2,893	
Monocytes	10 th	350	299	290	316	0.825	340	279	331	291	0.873
	25 th	367	360	350	382		346	362	400	370	
	50 th	420	408	420	469		392	452	494	439	
	75 th	540	540	521	510		702	570	570	526	
	90 th	661	720	618	632		-	735	671	627	
Eosinophils	10 th	85	54	50	69	0.922	51	61	60	74	0.206
	25 th	111	100	100	100		98	94	80	100	
	50 th	164	150	130	130		130	158	110	140	
	75 th	219	220	223	203		165	263	200	200	
	90 th	428	426	311	358		-	513	280	354	
Basophils	10 th	5	0	4	0	0.061	10	1	0	0	0.011
	25 th	22	16	10	9		15	10	10	20	
	50 th	38	20	20	20		30	29	20	30	
	75 th	41	40	30	40		40	32	40	60	
	90 th	60	74	46	60		-	72	50	100	

604 Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Bold rows indicate significant differences between Tanner stages, as
605 assessed by analysis of covariance (ANCOVA), controlling for centre and age, $P < 0.05$.

Table 4. Estimated cell counts (cell/ μ l) of selected lymphocyte subsets in European adolescents, according to Tanner stages and stratified for sex.

		Boys					Girls				
Tanner stage		I+II	III	IV	V		I+II	III	IV	V	
N		9	26	67	52	<i>P</i>	7	19	65	55	<i>P</i>
CD3 ⁺	10 th	1,157	1,039	1,066	876	0.002	1,135	819	1,112	1,089	0.362
	25 th	1,259	1,401	1,237	1,007		1,295	990	1,236	1,202	
	50 th	1,694	1,517	1,467	1,316		1,366	1,364	1,470	1,549	
	75 th	2,095	1,903	1,672	1,526		1,466	1,575	1,835	1,858	
	90 th	-	2,254	1,991	1,909		-	1,938	2,205	2,051	
CD4 ⁺	10 th	584	542	551	479	0.010	632	477	585	572	0.308
	25 th	644	735	681	575		727	593	684	652	
	50 th	780	894	835	737		815	689	827	834	
	75 th	1,084	960	994	882		881	862	1,080	1,015	
	90 th	1,457	1,168	1,143	1,014		-	1,069	1,252	1,223	
CD8 ⁺	10 th	492	383	347	279	0.012	330	291	382	392	0.325
	25 th	510	454	430	358		412	388	447	455	
	50 th	671	602	554	464		528	478	559	590	
	75 th	822	843	692	653		606	632	676	733	
	90 th	1,026	1,038	780	846		-	815	883	938	
CD3-CD16 ⁺ 56 ⁺	10 th	247	161	136	149	0.067	119	127	168	170	0.536
	25 th	276	249	210	210		185	183	241	221	
	50 th	319	357	342	323		326	304	339	286	
	75 th	506	430	446	455		496	360	440	375	
	90 th	-	687	540	590		-	540	554	571	
CD3-CD19 ⁺	10 th	162	182	159	116	0.049	144	152	137	128	0.078
	25 th	210	209	200	172		169	185	217	171	
	50 th	282	276	256	225		342	240	273	232	
	75 th	337	325	359	287		418	293	321	289	
	90 th	-	586	460	398		-	466	417	355	
CD3 ⁺ CD45RA ⁺	10 th	737	649	591	476	0.024	745	515	528	533	0.698
	25 th	814	754	732	582		832	631	666	674	
	50 th	1,005	980	939	777		901	727	846	901	
	75 th	1,405	1,189	1,094	975		1,016	1,039	1,056	1,076	
	90 th	-	1,294	1,193	1,129		-	1,329	1,427	1,297	
CD3 ⁺ CD45RO ⁺	10 th	354	394	356	297	<0.001	304	284	422	465	0.021
	25 th	477	486	459	373		357	354	474	528	
	50 th	549	592	559	467		454	479	634	636	
	75 th	722	775	682	585		536	684	784	753	

	90 th	-	907	777	776		-	813	918	942	
CD4 ⁺ CD45RA ⁺	10 th	318	331	227	238	0.102	388	263	245	235	0.824
	25 th	408	355	370	296		472	332	363	332	
	50 th	487	449	491	384		538	390	475	426	
	75 th	639	667	629	506		565	608	598	580	
	90 th	783	769	701	646		-	720	740	729	
CD4 ⁺ CD45RO ⁺	10 th	213	198	227	191	0.005	186	182	241	270	0.025
	25 th	235	293	280	233		239	203	289	314	
	50 th	303	400	327	303		281	283	363	387	
	75 th	352	457	419	361		306	312	445	493	
	90 th	851	487	505	485		-	398	558	619	
CD8 ⁺ CD45RA ⁺	10 th	336	282	222	219	0.032	193	201	228	257	0.473
	25 th	367	316	297	245		247	256	301	288	
	50 th	458	413	383	325		407	325	363	412	
	75 th	622	540	451	446		509	440	469	499	
	90 th	-	684	593	624		-	560	631	649	
CD8 ⁺ CD45RO ⁺	10 th	107	88	79	49	0.008	61	60	105	94	0.069
	25 th	120	117	109	89		82	103	128	138	
	50 th	159	160	164	130		109	150	171	186	
	75 th	211	242	199	197		166	236	229	268	
	90 th	-	366	261	246		-	320	303	332	
CD4 ⁺ /CD8 ⁺	10 th	1.09	0.98	0.99	0.92	0.478	1.04	0.90	1.16	0.94	0.349
	25 th	1.13	1.12	1.22	1.14		1.26	1.21	1.27	1.14	
	50 th	1.20	1.28	1.55	1.43		1.78	1.52	1.50	1.48	
	75 th	1.39	1.72	1.93	1.91		2.14	1.79	1.76	1.77	
	90 th	1.48	2.07	2.15	2.47		-	2.03	2.11	1.99	
CD3 ⁺ /CD19 ⁺	10 th	3.09	3.40	3.28	3.54	0.417	2.99	3.71	3.64	3.86	0.257
	25 th	5.34	4.81	4.37	4.13		3.22	4.04	4.73	4.97	
	50 th	6.38	5.38	5.79	5.79		4.47	5.12	5.56	6.77	
	75 th	7.28	7.28	6.97	7.68		7.09	7.35	7.32	9.68	
	90 th	-	8.08	8.11	10.96		-	10.56	10.54	11.78	

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Lymphocyte populations are designated by their cell markers. Bold rows indicate significant differences between Tanner stages, as assessed by analysis of covariance (ANCOVA), controlling for centre and age, $P < 0.05$.

610 **Table 5.** White blood cell (WBC) counts (cell/ μ l) in European adolescents, according to BMI z-scores and stratified for sex.

Boys							Girls					
BMI z-scores		Q1	Q2	Q3	Q4			Q1	Q2	Q3	Q4	
N		43	42	42	43	P	R	41	41	38	39	P
WBC	10 th	4,629	4,180	4,160	5,175	0.001	0.195**	4,392	4,484	5,208	5,221	0.005
	25 th	5,223	4,700	4,909	5,775			5,054	5,101	5,716	5,772	
	50 th	6,079	5,463	5,711	6,403			6,260	6,300	6,804	6,574	
	75 th	6,512	6,504	6,781	7,200			7,161	7,751	7,938	8,321	
	90 th	7,709	7,150	7,487	8,825			8,221	8,480	9,068	9,836	
Neutrophils	10 th	2,032	1,769	1,893	2,211	0.020	0.115	1,799	2,225	2,529	2,644	0.003
	25 th	2,381	2,275	2,111	2,800			2,693	2,636	3,000	3,170	
	50 th	3,084	2,799	2,910	3,269			3,190	3,512	3,806	4,146	
	75 th	3,791	3,539	3,537	4,009			4,200	4,384	4,902	5,070	
	90 th	4,658	4,387	4,308	5,042			4,666	5,646	6,021	6,287	
Lymphocytes	10 th	1,373	1,456	1,551	1,879	<0.001	0.331**	1,590	1,497	1,488	1,367	0.806
	25 th	1,703	1,671	1,729	2,170			1,829	1,694	1,856	1,870	0.029
	50 th	2,060	1,992	2,089	2,380			2,093	2,035	2,195	2,122	
	75 th	2,378	2,190	2,331	2,817			2,482	2,456	2,439	2,552	
	90 th	2,676	2,553	2,862	3,157			2,834	2,940	2,802	3,092	
Monocytes	10 th	300	300	319	300	0.261	0.115	294	284	349	300	0.224
	25 th	363	388	370	380			390	363	413	370	0.071
	50 th	420	420	460	500			476	432	500	463	
	75 th	511	500	583	580			559	553	555	600	
	90 th	672	613	660	709			665	654	707	733	
Eosinophils	10 th	80	90	50	50	0.066	-0.095	70	60	50	50	0.742
	25 th	100	100	100	100			90	95	81	100	0.034
	50 th	139	130	190	120			120	105	143	100	
	75 th	209	190	277	180			200	200	220	200	
	90 th	307	320	516	246			316	272	300	398	
Basophils	10 th	0	0	0	0	0.506	-0.126	0	0	0	0	0.527
	25 th	10	10	10	10			11	10	10	0	-0.045
	50 th	30	20	20	20			30	21	21	20	
	75 th	50	30	40	30			47	35	50	40	
	90 th	70	45	60	60			100	50	80	100	

611 Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Bold rows indicate significant differences between quartiles of standardized body mass index values
612 (BMI z-scores), as assessed by analysis of covariance (ANCOVA), controlling for centre, $P < 0.05$. R is the partial correlation coefficient between cell counts and BMI z-scores,
613 controlling for centre, * $P < 0.05$, ** $P < 0.01$.

Table 6. Estimated cell counts (cell/ μ l) of lymphocyte subsets in European adolescents, according to BMI z-scores and stratified for sex.

Boys							Girls						
BMI z-scores		Q1	Q2	Q3	Q4			Q1	Q2	Q3	Q4		
N		43	42	42	43	P	R	41	41	38	39	P	R
CD3 ⁺	10 th	850	994	958	1,244	0.014	0.238**	1,084	1,017	946	1,126	0.301	0.122
	25 th	1,083	1,088	1,162	1,411			1,158	1,183	1,269	1,314		
	50 th	1,457	1,238	1,476	1,696			1,382	1,364	1,523	1,517		
	75 th	1,657	1,590	1,622	1,978			1,578	1,756	1,741	1,881		
	90 th	2,038	1,740	1,979	2,269			1,986	2,165	1,930	2,301		
CD4 ⁺	10 th	443	529	551	591	0.085	0.174*	579	522	572	621	0.265	0.164*
	25 th	632	600	617	749			620	649	671	723		
	50 th	808	748	788	942			711	783	871	845		
	75 th	957	858	895	1,086			903	960	1,020	1,075		
	90 th	1,139	1,053	982	1,178			1,248	1,224	1,145	1,237		
CD8 ⁺	10 th	280	300	346	425	0.009	0.266**	385	359	381	373	0.634	0.067
	25 th	386	380	413	537			413	455	434	463		
	50 th	507	492	554	659			526	552	557	578		
	75 th	649	643	684	864			700	681	711	713		
	90 th	852	758	809	1,013			863	787	782	1,046		
CD3-CD16 ⁺ 56 ⁺	10 th	149	149	137	192	0.042	0.192*	119	150	154	181	0.766	0.064
	25 th	222	204	198	303			191	216	199	240		
	50 th	307	323	282	389			320	275	307	320		
	75 th	374	422	437	558			464	361	400	403		
	90 th	463	491	579	704			565	496	531	570		
CD3-CD19 ⁺	10 th	131	153	152	186	0.013	0.221**	145	105	153	132	0.700	-0.001
	25 th	185	169	202	224			185	167	202	188		
	50 th	239	219	286	287			257	239	266	241		
	75 th	291	281	352	433			316	288	327	310		
	90 th	417	336	482	502			430	431	397	422		
CD3 ⁺ CD45RA ⁺	10 th	469	505	513	642	0.413	0.133	577	513	522	556	0.962	0.036
	25 th	699	649	700	789			664	633	717	695		
	50 th	928	808	905	1,015			805	880	878	857		
	75 th	1,113	976	1,040	1,215			1,003	1,097	1,031	1,073		
	90 th	1,192	1,112	1,193	1,365			1,282	1,455	1,267	1,429		
CD3 ⁺ CD45RO ⁺	10 th	292	356	339	479	0.003	0.266**	367	426	393	456	0.036	0.171*
	25 th	375	401	441	572			454	486	456	556		
	50 th	475	499	514	690			556	569	576	656		
	75 th	636	589	649	780			696	731	745	775		

CD4 ⁺ CD45RA ⁺	90 th	819	654	874	914	0.932	0.045	880	903	861	940	0.917	0.034
	10 th	216	263	259	234			223	247	244	266		
	25 th	357	314	328	368			336	330	362	348		
	50 th	483	391	453	475			428	449	488	424		
	75 th	613	501	543	637			567	596	577	598		
CD4 ⁺ CD45RO ⁺	90 th	718	705	667	719	0.002	0.261**	655	828	709	690	0.012	0.254**
	10 th	191	204	196	278			225	225	219	261		
	25 th	227	256	254	308			274	286	289	306		
	50 th	291	302	328	408			313	362	341	398		
	75 th	410	373	399	487			387	406	438	493		
CD8 ⁺ CD45RA ⁺	90 th	487	436	466	556	0.131	0.191*	533	485	612	620	0.854	0.033
	10 th	220	227	222	291			248	199	230	199		
	25 th	246	252	287	348			279	292	298	297		
	50 th	382	343	370	431			341	373	380	373		
	75 th	471	446	450	609			474	462	486	472		
CD8 ⁺ CD45RO ⁺	90 th	549	489	638	713	0.019	0.248**	570	568	635	697	0.060	0.095
	10 th	51	74	89	110			84	113	88	92		
	25 th	85	97	107	150			127	135	110	155		
	50 th	144	133	150	206			158	172	155	209		
	75 th	190	196	197	236			229	233	226	266		
CD4 ⁺ /CD8 ⁺	90 th	242	244	312	300	0.286	-0.161*	290	334	290	336	0.713	0.100
	10 th	0.91	1.07	1.08	0.91			0.89	1.03	1.15	0.99		
	25 th	1.16	1.20	1.21	1.05			1.09	1.23	1.24	1.20		
	50 th	1.54	1.41	1.36	1.30			1.43	1.43	1.55	1.51		
	75 th	2.03	1.91	1.70	1.71			1.84	1.72	1.75	1.83		
CD3 ⁺ /CD19 ⁺	90 th	2.48	2.36	2.04	2.19	0.250	-0.057	2.05	2.10	1.95	2.15	0.336	0.094
	10 th	3.58	3.74	3.12	3.44			3.74	3.71	3.71	3.70		
	25 th	4.02	5.34	3.74	4.56			4.03	4.60	4.75	5.44		
	50 th	5.89	6.07	4.73	5.46			5.33	6.31	5.57	6.77		
	75 th	7.85	7.12	6.74	7.11			7.87	8.54	6.68	7.63		
	90 th	9.90	7.67	10.24	8.61			10.90	13.48	9.29	11.50		

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Lymphocyte populations are designated by their cell membrane markers. Bold rows indicate significant differences between quartiles of standardized body mass index (BMI z-scores), as assessed by analysis of covariance (ANCOVA), controlling for centre, $P < 0.05$. R is the partial correlation coefficient between cell counts and BMI z-scores, controlling for centre; * $P < 0.05$, ** $P < 0.01$.