# Reference range of naïve T and T memory lymphocyte subsets in peripheral blood of healthy adult

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**Abstract:**

**Background：**

Naïve T and T memory cell subsets can provide important information for the diagnosis and treatment of immunological and hematological disorders. Lymphocyte compartment undergo dramatic changes during adulthood; age-related reference values derived from healthy individuals are crucial. However, extensively detailed immunophenotyping reference values of peripheral blood lymphocytes in whole spectrum of adulthood performed by flow cytometry-based single-platform method are rare.

**Methods:**

309 healthy adult volunteers were recruited from Tianjin in China. The absolute counts and percentages of CD3+CD4+ T cells, CD3+CD8+ T cells, naïve T cells (Tn), T memory stem cells (Tscm), central memory T cells (Tcm), effector memory T cells (Tem), terminal effector T cells (Tte) were determined by flow cytometry with single platform technologies.

**Results:**

Reference range of absolute counts and percentage of lymphocyte subsets were formulated by different age and gender. We also find out the changing regularity of them: the cells which have stem cell properties, Tn and Tscm cells, decrease with aging; memory cell subsets, Tcm and Tem increase with aging, which increase from 18 to 64 years old and present no significant change over the 65 years old. Gender have influence on the fluctuation of lymphocyte subsets, absolute count of

CD3+CD8+, CD8+ Tcm, CD8+ Tem in male are higher than that in female.

**Conclusion:**

The reference values of percentages and absolute numbers of naïve T and T memory cell subsets can help doctors to understand the immune state of patients and to evaluate conditions of prognosis then adjust treatment for patients.

**Key words：**

naïve T cell; T memory cell subsets; reference range; changing regularity; immunity; flow Cytometry.

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## [1]1.Introduction:

[1] **Abbreviations**

Long-lived memory T cells maintained long-time immunological memory in the host[1][2]. Immune system gained a long-term ability of responding to a broad diverse spectrum of pathogens and tumor cell antigens through developing special lymphocyte differentiation programs to ensure the duration of a given antigen-specific immune response[3][4]. Upon antigen stimulation, Tn enter distinct cell programs of development and differentiation and then produce Tscm, Tcm, Tem[5]. The key mediator in this process is Tscm, a kind of multipotent progenitor that can both self-renew and replenish more differentiated subsets of memory T cells, including Tcm, Tem and Tte[6]. Tscm owing to their extreme longevity and robust potential for immune reconstitution, play a vital role in many physiological and pathological human processes[7]. Some studies indicated that CD4+CD45RA+CD95+T cells from lung cancer patients exhibiting stronger antitumor function possessed certain memory cell phenotypes which could help contribute to favorable prognostic factor of disease[8]. In infection disease, such as infected with Mycobacterium tuberculosis(M. tb), M. tb-specific Tscm would be produced in the host, which were also functional and could produce IL-2, IFN-γ, TNF-α upon antigen stimulation, and the percentages of Tscm were correlated positively with long-term Calmette-Guerin-specific CD4+T cell proliferative potential after infant vaccination[9].

Detecting the changes of the circulating lymphocyte subsets can be beneficial to monitor the onset and progression of disease and determine optimal treatment[10].

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naïve T cells (Tn); T memory stem cells (Tscm); central memory T cells (Tcm); effector memory T cells (Tem); terminal effector T cells (Tte); Mycobacterium tuberculosis(M. tb)

Therefore, establishing reference range for lymphocyte subsets is important for clinical decision-making. There is rare reference range of naïve T and T memory cell subsets provided by single platform method, the need of precision reference range of naïve T and T memory cell subsets at different age stages prompts us to carry out this work. Flow cytometric analysis is a convenient and efficient method for studying immune status and has been widely used in clinical diagnosis and administrating of immune diseases associated with phenotypic and functional perturbations of lymphocyte subsets[11].

So far, almost all absolute numbers of lymphocyte subsets in the peripheral circulation have been detected by dual-platform technique in traditional way which couples percentages of positive cell subsets determined by flow cytometry with the absolute lymphocyte count obtained by automated hematology analyzers[10,12]. It is indicated that this conventional, universal technique is responsible for substantial differences in absolute lymphocyte counts reported by different laboratories[13-14]. The more advanced method of single platform, which is performed completely on the flow cytometer, has effectively increased the assay precision and allowed for greater uniformity of results between laboratories[13-15]. So, the percentages and absolute numbers of these subsets were tested by ten color flow cytometer based on a single-platform technique.

We examined T lymphocyte subsets in 309 healthy volunteers, some regional data of lymphocyte phenotypes show variations due to the influence of gender, age, ethnicity, and lifestyle differences[16], therefore, we explored reference ranges based

on age and gender.

## 2. Materials and methods

### 2.1 Clinical data

All the subjects were given the informed consent in accordance with the Declaration of Helsinki and the clinical trial was approved by the hospital ethics committee(TYLL2017[K]002) and registered at Chinese Clinic Trial Registry(ChiCTR-IOR-17014139). A total of 309 healthy adult( 171males and 138 females ) volunteers ranging from 18 to 88 years old were recruited from Tianjin in China between September 1, 2019 and July 1, 2020. According to the aging definition by the National Health and Family Planning Commission of the People's Republic of China, the subjects were classified into three age groups as follows: 18-44 years (n=101); 45-65years (n=106) and >65years (n=102)[10].

2.1.1 Inclusion criteria

All the subjects were healthy without diseases related to abnormal of heart, brain, liver, kidney, hematology, immune system and so on. Physical examination, blood routine examination, liver functions, renal functions, blood glucose levels were normal.

2.1.2 Exclusion criteria

Except the diseases including influenza, systemic infection, autoimmune diseases, connective tissue disease, HIV, abnormal tumor marker or cancer that caused the abnormal of immune.

### 2.2. Reagents and instruments

The lymphocyte subsets were analyzed using a lyse/no-wash procedure based on a single-platform technique by ten-color flow cytometry (BD FACS Canto II:U657338000541). The main reagents were Percp-cy-labelled mouse Anti-Human CD95 (cat:561655), Bv421-labelled mouse Anti-Human CD62L (cat:563862), PE-cy7 -labelled mouse Anti-Human CD4 (cat:663493), APC-labelled mouse Anti-Human CD8 (cat:663524), APC-H7-labelled mouse Anti-Human CD3 (cat:663490), V-500-C labelled-mouse Anti-Human CD45 (cat:662912), PE-labelled mouse Anti-Human CD45RO (cat:663530), FITC-labelled mouse Anti-Human CD45RA (cat:662840), BD Multitest hemolysin (340503). The EDTA blood collecting tubes and trucount tubes (340334) were also from BD Biosciences .

**2.3. Sample collection**

Two millilitres of EDTA anticoagulated fresh peripheral blood were obtained from healthy adults, intensively mixed by turning upside down immediately.

**2.4. Cellular staining and analysis**

The procedure was performed by the flow cytometer with single platform technique. The manipulation was done according to BD operating instruction. CD3+CD4+T cells, CD3+CD8+T cells, Tn, Tscm, Tcm, Tem and Tte were identified according to published protocols,such as Tn(CD45RA+CD45RO-CD62L+CD95-), Tscm(CD45RA+CD45RO-CD62L+CD95+),Tcm(CD45RA-CD45RO+CD62L+CD95 +),Tem(CD45RA-CD45RO+CD62L-CD95+),Tte(CD45RA+CD45RO-CD62L-CD95 +).[7,17-18,20].

(1) For each sample, 20μl of PE-labelled mouse Anti-Human CD45RO and FITC

mouse Anti-Human CD45RA reagents were respectively pipetted into the bottom of trucount tubes; then 5μl of Percp-cy-labelled mouse Anti-Human CD95, Bv421-labelled mouse Anti-Human CD62L, PE-cy7-labelled mouse Anti-Human CD4, APC-labelled mouse Anti-Human CD8, APC-H7-labelled mouse Anti-Hum-Human CD3 and V-500-C-labelled mouse Anti-Human CD45 were added into the the bottom of trucount tubes respectively ,too.

(2) Next, 50μl of well-mixed and anticoagulated whole blood was pipetted into the bottom of every tube.

(3) Then vortexed gently to mix, incubated for 15 min in dark at room temperature.

(4) Finally, 400μl of 1×BD Multitest IMK kit lysing solution was pipetted into every tube. The solution was vortexed gently to mix and incubated for 15 min in dark at room temperature.

(5) Samples were analyzed on the flow cytometer.

**2.5. Statistical analysis**

Statistical analysis was performed by SPSS software 25.0. Kolmogorov-Smirnov was used for the distribution test. Reference ranges were calculated 2.5% and 97.5% percentiles for non-parametric data. Comparisons among three variables were performed using Kruskal-Wallis test. Variables were grouped by gender comparing with Mann–Whitney U test for non-parametric data. Use a non-parametric Spearman's rank correlation test to analyse the association between variables and age. Probability value was obtained from 2-sided tests and $P<0.05$ was considered statistically significant.

## 3. Result

### 3.1. Gating strategy

The gating strategy for T cells ranging from naïve and memory T cell subsets was shown in Fig. 1. Firstly, we gated lymphocyte identified by CD45 from leukocyte, then gated CD3+ T cells from lymphocyte. T cell subsets populations Tcm (CD95+ CD62L-) and Tem (CD95+CD62L+) were gated from CD3+CD4+ (CD45RO+ CD45RA-) and CD3+CD8+ (CD45RO+ CD45RA-)T subsets; Tscm (CD95+ CD62L+), Tn (CD95-CD62L+) and Tte (CD95+CD62L-) were gated from CD3+CD4+ (CD45RO-CD45RA+) and CD3+CD8+ (CD45RO-CD45RA+)T subsets.

### 3.2. Reference range of T lymphocyte subsets in different age groups

The healthy volunteers were recruited for assessment of human lymphocyte subsets including 171 males ( 55.33 %) and 138 females ( 44.67 %). 101( 32.7%) were in 18-44 years group (56 males, 45 females, mean age 30.73 years), 106 (34.3%) belonged to 45-65 years group (56 males, 50 females, mean age 54.63 years) and 102 (33.0%) belonged to the over 65 years old group (59 males, 43 females, mean age 73.59 years). The Kolmogorov-Smirnov test demonstrated that absolute counts and percentages of each lymphocyte subsets were abnormal distribution among the three cohorts ($p<0.001$). So we use the percentile method to determine the reference range of each parameter. The median and reference range of lymphocytes absolute counts and percentage for each group were shown in Table 1,2.

### 3.3 The difference of absolute counts of T lymphocyte subsets in each age group

In order to analyze the absolute counts further, the data in table 1 were performed

by statistics. The absolute counts of CD4+ Tn and Tscm decreased gradually with aging among the three groups, CD4+Tn was 34.38 cells/μl, 23.06 cells/μl and 14.9 cells/μl in each group with aging respectively ($P$<0.05, Fig.2A), and CD4+Tscm was 140.9 cells/μl, 85.49 cells/μl and 69.2 cells/μl in every group respectively ($P$<0.05, Fig.2B). The same changes can be seen in CD8+Tn and CD8+ Tscm cell populations ($P$<0.05, Fig.2F,G).

The absolute counts of CD4+Tcm, CD4+Tem were increased with aging($P$<0.05, Fig.2C,D), but there was no obviously difference between the group of 45-64 years old and over 65 years old($P$>0.05, Fig.2C,D). The absolute count of CD8+Tcm showed increase with aging which was also similar to CD4+Tcm ($P$<0.05, Fig.2H). The absolute counts of CD4+Tte, CD8+Tem, CD8+Tte did not change with age($P$>0.05, Fig.2E, I,J).

**3.4 Age-related T cell changes in distribution**

There was a weak negative correlation between age and the counts of CD4+Tn(r=-0.379, $P$<0.01, Fig.3A) and CD4+Tscm (r=−0.335, $P$<0.01, Fig.3B) which stated that the numbers of CD4+Tn and CD4+Tscm did not fluctuate much. A strong negative correlation between age and the counts of CD8+Tn(r=-0.718, $P$<0.01, Fig.3E) and CD8+Tscm r=-0.656, $P$<0.01, Fig.3F) suggested that the numbers of CD8+Tn and CD8+Tscm fluctuated significantly with aging. The weak positive correlation between age and the counts of CD4+Tcm (r=0.261, $P$<0.01, Fig.3C), CD4+Tem (r=0.280, $P$<0.01, Fig.3D), CD8+Tcm (r=0.171, $P$<0.01, Fig.3G) was showed in our study. The cells which have stem cell properties, such as Tn, Tscm cells

decrease with age. However, memory cell subsets, which have no stem cell properties such as Tcm, Tem cells increase with age(Fig.3I,H).

### 3.5 Reference range of T lymphocyte subsets in different gender

Gender has influence on the fluctuation of lymphocyte subsets[10]. So we established the reference range of lymphocyte subgroups according to gender(Table 3, 4). Compared parameters between genders, absolute counts of CD3+CD8+, CD8+Tcm, CD8+Tem in male group were higher than those in female group($P$<0.05, Fig.4 A,B,C).

### 3.6 The difference of Tte in CD4+ and CD8+ cells

The analysis showed that the absolute counts of CD4+Tte and CD8+Tte were different, CD8+Tte cells were higher than CD4+Tte ($P$<0.05).

## 4. Discussion

Human T cells subsets were classified based on the expression of the surface receptor molecules: naïve and memory T cells can be identified by the expression of the CD45RA+ and CD45RO+ isoforms respectively; Tcm and Tem are discriminated by lymphoid-homing molecules CCR7 and CD62L (L-selectin); Tscm cells have been described as a long-lived memory T cell population which expressed CD45RO-, CCR7+, CD45RA+, CD62L+,CD27+, CD28+, and IL-7Rα+, CD95+. CD95+ indicates that there are some cells with naïve markers that have memory properties and can be used to distinguished Tn from Tscm ; CD45RA- is used to identify memory T cell population; CD62L+ states that cells have limited effector functions, CD62L- represents mediating rapid effector functions[19-24]. So we identify the

memory cells by phenotype of major clusters including Tn (CD45RA+CD45RO-CD62L+CD95-),Tscm(CD45RA+CD45RO-CD62L+CD95+),Tcm(CD45RA-CD45RO+CD62L+CD95+),Tem(CD45RA-CD45RO+CD62L-CD95+), Tte(CD45RA+CD45RO-CD62L-CD95+)[25].

In this study, we explored the percentages and absolute counts of T lymphoid subgroups by single platform technique. There are two reasons: firstly, the precision and difference between laboratories of detection of percentages and absolute numbers of lymphocyte subsets are different. The single-platform was of more accuracy and consistency which performed by using the known total number of fluorescent microbeads as the standard internal parameters and added fluorescent labeled antibodies into the trucount tubes, then applied acquisition and analysis software in the flow cytometry to gain accurate data according to the formula as follow.

$$(\text{cells/ul}=\frac{acquired\ \ cells\times total\ \ beads}{acquired\ \ beads\times voulme\ \ of\ \ sample}\times 100\%)$$

Secondly, the clinical significance are different. The percentages of T lymphocyte subsets represent the proportion or composition of each subsets, indicating the development and differentiation of lymphocyte, while absolute counts suggest the proliferation capacity of lymphocyte characterized by precise amount[26]. The common reports pointing up cell percentages are misleading in clinic because they do not consider total white blood cell count, which might constantly change in these patients, especially those who are receiving anticancer therapies[26]. In our previous study we have proved that comparing healthy controls to patients, absolute numbers

of CD3+, CD3+CD4+, CD3+CD8+, B and NK cells decreased in the Non-small cell lung cancer patients obviously, but the percentages of them were normal[27]. Therefore, it's crucial and urgent to detect both percentages and absolute numbers of lymphocyte subsets in clinic, it will help us to know the changes in the patient's immunologic function comprehensively, analyze clinical condition and predict curative effect of patients for clinicians[28].

From result, we can see stem-like cells, such as Tn, Tscm cells decrease with aging. With increasing age, thymus occurs changes which begin during childhood, including a reduction in thymic volume, loss of epithelial cells, increase in perivascular space, and replacement of thymic tissue by fat[29]. Thymus output declines with aging[30], which probably leads to the gradual decline in CD3+CD4+ and CD3+CD8+ naïve T cell numbers, although naïve T cell numbers decline less dramatically than thymocyte numbers[31]. In vitro, researchers have demonstrated that Tscm originated from Tn[24], so it's easier to understand why Tscm decrease with aging, the production of naïve T cells diminish with the involution of the thymus[32-34]. These cells have the ability not only to self-renew but also to differentiate into all subsets of memory and effector T cells[35-36]. Combined with their longevity, the preservation of Tscm plasticity may play a central role in maintaining immunologic competence with aging[37]. The exhaustion of Tn and Tscm reservoir suggests that T cell pool is a major target of the aging process and may define a parameter possibly related to the life span of humans[31].

Contrast to Tn and Tscm, the absolute counts of CD4+Tcm, CD4+Tem increase

with aging, the counts of 45-64 years old and over 65 years old are higher than those in 18-45 years old ($P<0.05$, Fig.2.C,D), but there was no difference between the group of 45-64 years old and over 65 years old($P>0.05$). The absolute count of CD8+Tcm in over 65 years old group is more than that in 18-45 years old($P<0.05$, Fig.2.G). Tcm and Tem increase with aging and keep less fluctuation over 65 years old[38]. With advancing age, the major goal of T cells shifts to mounting appropriate responses against novel infections and protecting the host against reinfection with common pathogens[39]. When a first stimulus triggers a first response[1], Tn encounter cognate antigen and expand clonally to generate effector cells that migrate to peripheral tissues and eliminate virus and malignant cells[40]. During this effector response, most effector cells become terminally differentiated, termed short-lived effector cells, while a fraction of effector cells, termed as memory precursor effector cells, acquirie the ability to survive under the contraction stage of the immune response[41], and further differentiate into a heterogeneous pool of memory cells under optimal developmental conditions, then a second stimulus triggers a second response, more stronger, speedy, durative and specificity occurs[41].

The majority of effector T cells contract rapidly and are not present in significant proportions at steady state, a population of Tte exhibiting CD45RA+CD62L− phenotypes can persist in circulation[42]. Tte cells are mostly present within the CD8+ T cell lineage, exhibiting high capacity for IFNγ production and low proliferative capacity[43], CD4+ Tte cells are rarely detected, some researchers found that the expansion of CD4+Tte cells with cytotoxic function occurs

in individuals infected with Dengue virus and is associated with protection[44].

All in all, we should pay more attention to the percentages and absolute counts' changes of T lymphocyte subsets in clinic simultaneously, for it may give us more important references on treatment.

**Conclusion:**

The reference values of percentages and absolute numbers of naïve T and T memory cell subsets can help doctors to understand the immune state of patients and to evaluate conditions of prognosis then adjust treatment for patients.

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**Author Contributions**

Jianchun Yu :Conceptualization,Methodology. Ying Xia, Aqing Liu, Wentao Li:Writing - Original Draft, Formal analysis,Writing-Review & Editing. Yunhe Liu, Songshan Ye, Guan Zhang, Zhijieruo Zhao: Data Curation, Validation . Juan Shi, Yingjie Jia, Xu Liu, Yongtie Guo, Huayu Chen: Resources.

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**Availability of data and materials**

All data generated and analyzed during this study are available from the corresponding author in response to reasonable requests.

**Ethics approval and consent to participate**

All clinical and ethical regulations were given the informed consent in accordance with Declaration of Helsinki and the clinical trial was approved by the First teaching hospital of Tianjin University of TCM ethics committee review(TYLL2017[K]002) and registered at Chinese Clinic Trial Registry(ChiCTR-IOR-17014139).

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no potential conflicts of interest.

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Table 1: Reference range of absolute counts of T lymphocyte subsets in different age groups

| parameters | | | all n=309 | (18-44) years n=101 | (45-65) years n=106 | >65years n=102 |
|---|---|---|---|---|---|---|
| age | mean±SD | | 50.52±18.00 | 30.73±6.52 | 54.63±6.37 | 73.59±6.60 |
| sex | male:female | | 171:138 | 56:45 | 56:50 | 59:43 |
| Lymphocyte counts(cells/μl) | median | | 1766.39 | 1692.59 | 1791.59 | 1668.16 |
| | Reference *CI 95%* | range | (1074.22~2816.88) | (978.29~2287.2) | (1213.34~2832.61) | (897.93~2831.32) |
| CD3+(cells/μl) | median | | 1139.49 | 1137.67 | 1203.23 | 1045.59 |
| | Reference *CI 95%* | range | (702.12~1791.98) | (702.19~1758.76) | (743.94~1783.47) | (594.32~1736.18) |
| CD3+CD4+(cell/μl) | median | | 585.91 | 538.20 | 647.07 | 562.21 |
| | Reference *CI 95%* | range | (356.5~1085.28) | (361.15~984.04) | (350.01~1088.99) | (347.64~968.83) |
| CD4+Tn(cells/μl) | median | | 25.11 | 34.38 | 23.06 | 14.9 |
| | Reference *CI 95%* | range | (3.72~90.15) | (5.10~89.87) | (3.8~98.99) | (1.1~70.79) |
| CD4+Tscm(cells/μl) | median | | 102.90 | 140.9 | 85.49 | 69.2 |
| | Reference *CI 95%* | range | (11.55~336.22) | (35.14~461.11) | (11.71~330.49) | (4.23~307.18) |
| CD4+Tcm(cells/μl) | median | | 154.85 | 141.74 | 183.84 | 155.81 |
| | Reference *CI 95%* | range | (65.34~374.33) | (63.94~239.74) | (66.63~371.22) | (65.5~396.65) |
| CD4+Tem(cells/μl) | median | | 116.59 | 100.20 | 132.72 | 123.77 |
| | Reference *CI 95%* | range | (48.49~339.63) | (44.73~172.16) | (63.43~354.88) | (47.69~371.07) |
| CD4+Tte(cells/μl) | median | | 2.41 | 2.24 | 2.58 | 2.94 |
| | Reference *CI 95%* | range | (0.15~75.34) | (0.24~108.97) | (0.14~73.61) | (0.02~98.55) |
| CD3+CD8+(cell/μl) | median | | 423.88 | 449.64 | 404.97 | 323.01 |
| | Reference *CI 95%* | range | (191.41~784.26) | (193.79~759.35) | (195.83~743.84) | (148.77~852.93) |
| CD8+Tn(cells/μl) | median | | 12.42 | 27.5 | 10.49 | 3.01 |
| | Reference *CI 95%* | range | (0.88~52.07) | (4.48~53.21) | (1.6~56.19) | (0.18~22.87) |
| CD8+Tscm(cells/μl) | median | | 57.24 | 110.47 | 48.63 | 18.67 |
| | Reference *CI 95%* | range | (3.54~246.89) | (17.74~301.66) | (3.24~161.99) | (0.53~101.14) |
| CD8+Tcm(cells/μl) | median | | 36.15 | 31.18 | 36.70 | 37.61 |
| | Reference *CI 95%* | range | (10.73~124.41) | (9.95~75.08) | (12.53~142.58) | (8.14~102.33) |
| CD8+Tem(cells/μl) | median | | 85.75 | 94.33 | 83.44 | 75.19 |
| | Reference *CI 95%* | range | (15.1~289.76) | (12.86~237.43) | (9.7~312.97) | (9.91~280.52) |

| | | | | | | |
|---|---|---|---|---|---|---|
| CD8+Tte(cells/μl) | median | | 53.23 | 47.93 | 57.55 | 38.05 |
| | Reference | range | (3.98~287.27) | (5.9~159.83) | (2.76~335.3) | (3.69~399.06) |
| | *Cl 95%* | | | | | |

Table 2: Reference range of percentages of T lymphocyte subsets in different age groups

| parameters | | | all n=309 | (18-44) years n=101 | (45-65) years n=106 | >65years n=102 |
|---|---|---|---|---|---|---|
| age | mean±SD | | 50.52±18.00 | 30.73±6.52 | 54.63±6.37 | 73.59±6.60 |
| sex | male:female | | 171:138 | 56:45 | 56:50 | 59:43 |
| CD3+%Lymphocyte cells | median | | 67.90% | 68.5% | 66.55% | 64.2% |
| | Reference | range | (46.86%~80.24%) | (48.01%~80.11%) | (44.99%~79.74%) | (38.56%~81.65%) |
| | *Cl 95%* | | | | | |
| CD3+CD4+%CD3+ | median | | 52.8% | 50.4% | 53.95% | 57.05% |
| | Reference | range | (33.68%~71.56%) | (33.01%~66.77%) | (39.72%~73%) | (30.18%~77.29%) |
| | *Cl 95%* | | | | | |
| CD3+CD4+%Lymphocyte cells | median | | 34.1% | 32.35% | 35.05% | 35.1% |
| | Reference | range | (20.70%~51.71%) | (20.41%~52.43%) | (23.62%~53.54%) | (19.01%~54.19%) |
| | *Cl 95%* | | | | | |
| CD4+Tn%CD3+CD4+ | median | | 4.3% | 6% | 3.45% | 2.6% |
| | Reference | range | (0.7%~12.23%) | (1.24%~13.77%) | (0.92%~12.38%) | (0.2%~10.39%) |
| | *Cl 95%* | | | | | |
| CD4+Tscm%CD3+CD4+ | median | | 17.6% | 26.35% | 13.55% | 11.4% |
| | Reference | range | (2.29%~40.89%) | (7.11%~47.43%) | (2.54%~39.24%) | (0.58%~35.73%) |
| | *Cl 95%* | | | | | |
| CD4+Tcm%CD3+CD4+ | median | | 27.00% | 26.1% | 28.1% | 26.45% |
| | Reference | range | (12.08%~46.87%) | (9.34%~41.65%) | (14.92%~44.48%) | (13.3%~54.69%) |
| | *Cl 95%* | | | | | |
| CD4+Tem%CD3+CD4+ | median | | 20.3% | 17.6% | 22.1% | 20.85% |
| | Reference | range | (7.89%~42.55%) | (6.02%~36.43%) | (10.42%~48.31%) | (8.61%~46.04%) |
| | *Cl 95%* | | | | | |
| CD4+Tte%CD3+CD4+ | median | | 0.4% | 0.5% | 0.4% | 0.7% |
| | Reference | range | (0.1%~12.82%) | (0.1%~17.06%) | (0.1%~12.21%) | (0.1%~20.76%) |
| | *Cl 95%* | | | | | |
| CD3+CD8+%CD3+ | median | | 36.8% | 38% | 35.3% | 34.8% |
| | Reference | range | (20.49%~56.82%) | 23.32%~58.1%) | (21.66%~50.9%) | (15.63%~61.13%) |
| | *Cl 95%* | | | | | |
| CD3+CD8+%Lymphocyte cells | median | | 24.9% | 26.15% | 22.85% | 20.85% |
| | Reference | range | (11.47%~41.41%) | (15.67%~41.73%) | (11.73%~36.29%) | (9.05%~42.66%) |
| | *Cl 95%* | | | | | |
| CD8+Tn%CD3+CD8+ | median | | 3.2% | 6.45% | 2.5% | 0.8% |
| | Reference | range | (0.29%~10.63%) | (1.14%~11.73%) | (0.32%~10.69%) | (0.1%~6.25%) |
| | *Cl 95%* | | | | | |
| CD8+Tscm%CD | median | | 13.7% | 28.05% | 11.6% | 6.1% |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3+CD8+ | Reference range *Cl 95%* | | (1.39%~46.2 %) | (6.09%~53.0 3%) | (1.42%~35.97 %) | (0.35%~25. 74%) |
| CD8+Tcm%CD3 +CD8+ | median | | 8.9% | 7.4% | 8.8% | 12.15% |
| | Reference range *Cl 95%* | | (2.5%~27.88 %) | (2.09%~22.0 7%) | (2.62%~38%) | (2.61%~31. 01%) |
| CD8+Tem%CD3 +CD8+ | median | | 22.8% | 22.3% | 21.35% | 27.45% |
| | Reference range *Cl 95%* | | (2.89%~52.39 %) | (2.99%~42.7 1%) | (2.5%~53.75 %) | (2.24%~59. 08%) |
| CD8+Tte%CD3 +CD8+ | median | | 12.2% | 10.5% | 13.85% | 10.6% |
| | Reference range *Cl 95%* | | (1.39%~46.2 %) | (1.64%~35.2 2%) | (0.92%~56.71 %) | (1.34%~49. 48%) |

Table 3: Reference range of absolute counts of T lymphocyte subsets in different gender

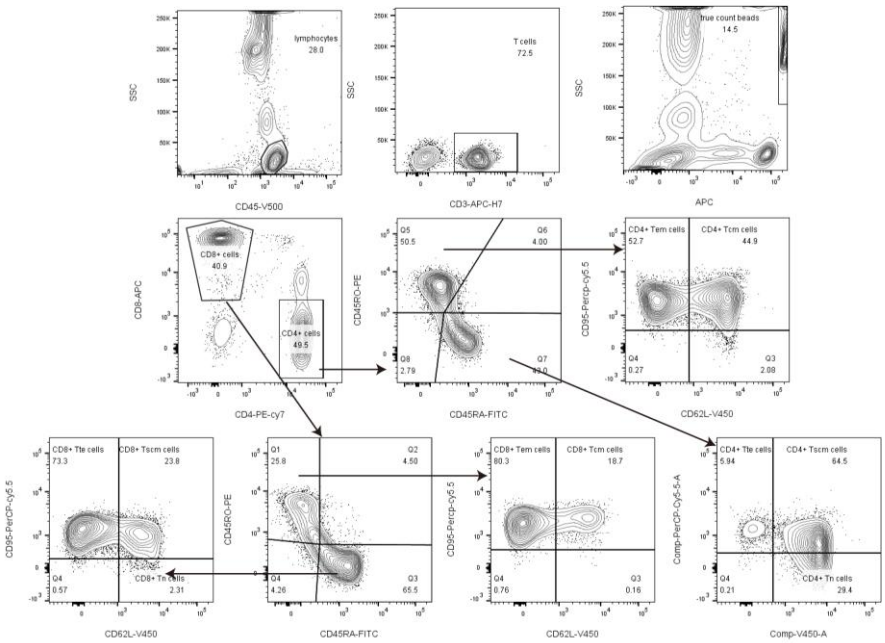
| parameters | | male | female |
|---|---|---|---|
| CD3+(cells/μl) | median | 1179.20 | 1097.76 |
| | Reference range *Cl 95%* | (662.33~1820.19) | (706.46~1780.6) |
| CD3+CD4+(cells/μl) | median | 592.17 | 579.8 |
| | Reference range *Cl 95%* | (360.64~1093.51) | (351.58~1077.91) |
| CD4+Tn(cells/μl) | median | 24.93 | 25.46 |
| | Reference range *Cl 95%* | (3.62~89.75) | (2.52~98.38) |
| CD4+Tscm(cells/μl) | median | 107.31 | 98.84 |
| | Reference range *Cl 95%* | (16.25~450.36) | (9.18~318.99) |
| CD4+Tcm(cells/μl) | median | 156.55 | 145.2 |
| | Reference range *Cl 95%* | (62.06~369.8) | (67.87~395.86) |
| CD4+Tem(cells/μl) | median | 117.46 | 114.84 |
| | Reference range *Cl 95%* | (48.15~304.55) | (40.65~344.7) |
| CD4+Tte(cells/μl) | median | 2.46 | 2.19 |
| | Reference range *Cl 95%* | (0.16~88.25) | (0.11~51.18) |
| CD3+CD8+(cells/μl) | median | 447.28 | 378.38 |
| | Reference range *Cl 95%* | (187.28~866.56) | (196.56~708.8) |
| CD8+Tn(cell/μl) | median | 12.59 | 12.01 |
| | Reference range *Cl 95%* | (1.02~51.96) | (0.62~58.58) |
| CD8+Tscm(cells/μl) | median | 67.60 | 43.24 |
| | Reference range *Cl 95%* | (3.95~249.4) | (3.11~254.32) |
| CD8+Tcm(cells/μl) | median | 40.10 | 31.37 |
| | Reference range *Cl 95%* | (11.1~126.44) | (9.31~130.73) |
| CD8+Tem(cells/μl) | median | 91.12 | 72.71 |
| | Reference range *Cl 95%* | (14.38~293.56) | (9.87~287.21) |
| CD8+Tte(cells/μl) | median | 54.32 | 43.63 |
| | Reference range *Cl 95%* | (2.88~299.7) | (6.6~290.91) |

1

Table 4: Reference range of percentages of T lymphocyte subsets in different gender

| parameters | | male | female |
|---|---|---|---|
| CD3+ %Lymphocyte | median | 67.70% | 68.00% |
| cells | Reference range *Cl 95%* | (45.95%~81.06%) | (47.05%~79.8%) |
| CD3+CD4+ % | median | 52.80% | 52.80% |
| CD3+ | Reference range *Cl 95%* | (33.31%~70.9%) | (35.63%~72.6%) |
| CD4+Tn %CD4+ | median | 4.10% | 4.50% |
| | Reference range *Cl 95%* | (0.7%~10.83%) | (0.45%~14.73%) |
| CD4+Tscm %CD4+ | median | 18.30% | 16.90% |
| | Reference range *Cl 95%* | (3.04%~41.42%) | (1.4%~41.38%) |
| CD4+Tcm %CD4+ | median | 27.15% | 26.10% |
| | Reference range *Cl 95%* | (11.61%~43.5%) | (13.08%~54.93%) |
| CD4+Tem %CD4+ | median | 20.35% | 20.00% |
| | Reference range *Cl 95%* | (7.7%~42.23%) | (9.9%~45.98%) |
| CD4+Tte %CD4+ | median | 0.50% | 0.40% |
| | Reference range *Cl 95%* | (0%~13.39%) | (0%~9.23%) |
| CD3+CD8+ %Lymph | median | 37.80% | 35.20% |
| ocyte cells | Reference range *Cl 95%* | (21.95%~58.6%) | (19.83%~55.1%) |
| CD8+Tn %CD8+ | median | 3.25% | 3.10% |
| | Reference range *Cl 95%* | (0.3%~10.2%) | (0.13%~10.98%) |
| CD8+Tscm %CD8+ | median | 15.15% | 12.70% |
| | Reference range *Cl 95%* | (1.47%~46.2%) | (1.23%~49.1%) |
| CD8+Tcm %CD8+ | median | 9.10% | 8.40% |
| | Reference range *Cl 95%* | (2.57%~30.64%) | (2.43%~27.08%) |
| CD8+Tem %CD8+ | median | 23.10% | 21.30% |
| | Reference range *Cl 95%* | (2.87%~52.82%) | (2.65%~55.13%) |
| CD8+Tte %CD8+ | median | 12.40% | 10.90% |
| | Reference range *Cl 95%* | (1.2%~44.08%) | (2.18%~51.15%) |

Figure 1


lymphocytes
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SSC
CD45-V500
T cells
72.5
CD3-APC-H7
true count beads
14.5
APC
CD8+ cells
40.9
CD8-APC
CD4+ cells
49.5
CD4-PE-cy7
Q5
50.5
Q6
4.00
Q8
2.79
Q7
CD45RO-PE
CD45RA-FITC
CD4+ Tem cells
52.7
CD4+ Tcm cells
44.9
CD95-Percp-cy5.5
Q4
0.27
Q3
2.08
CD62L-V450
CD8+ Tte cells
73.3
CD8+ Tscm cells
23.8
Q4
0.57
CD8+ Tn cells
2.31
Q1
25.8
Q2
4.50
Q4
4.26
Q3
65.5
CD8+ Tem cells
80.3
CD8+ Tcm cells
18.7
Q4
0.76
Q3
0.16
CD4+ Tte cells
5.94
CD4+ Tscm cells
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Q4
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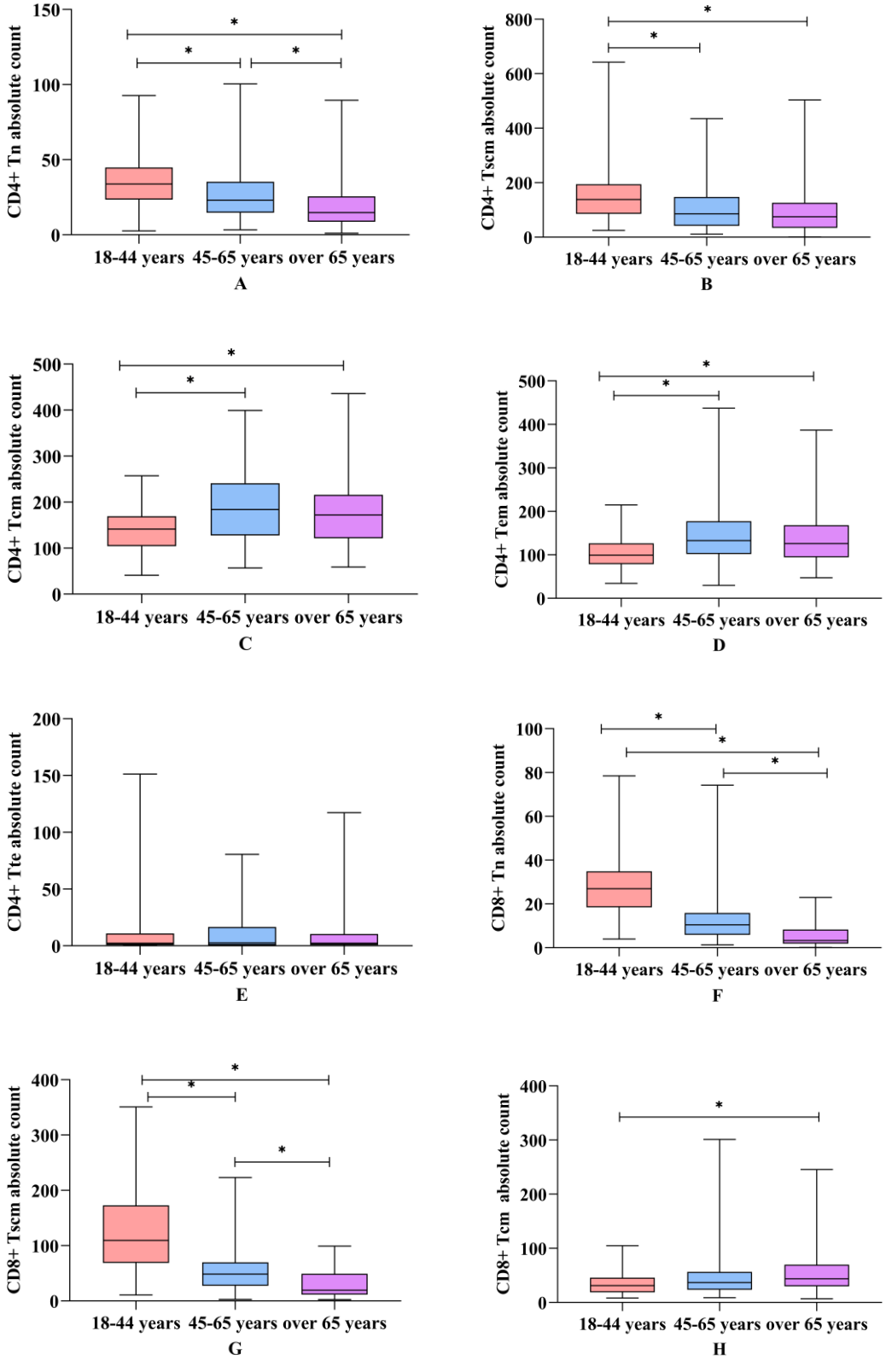
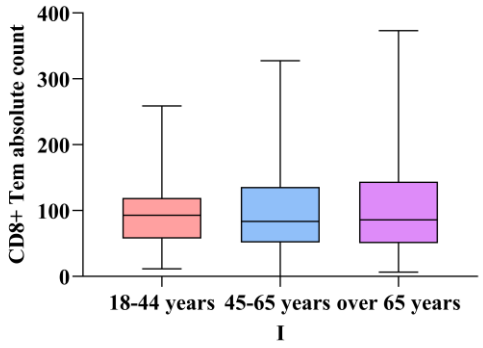
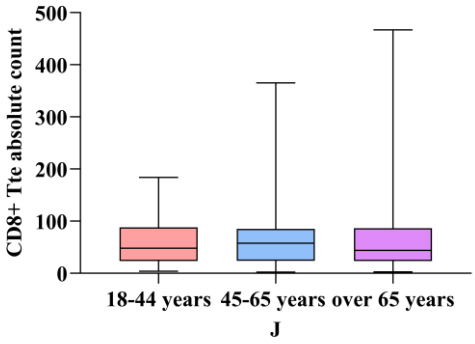
CD4+ Tn cells
29.4
Comp-PerCP-Cy5-5-A
Comp-V450-A

**Figure 2**


CD4+ Tn absolute count
150
100
50
0
*
*
*
18-44 years
45-65 years
over 65 years
A
CD4+ Tscm absolute count
800
600
400
200
0
*
*
18-44 years
45-65 years
over 65 years
B
CD4+ Tcm absolute count
500
400
300
200
100
0
*
*
18-44 years
45-65 years
over 65 years
C
CD4+ Tem absolute count
500
400
300
200
100
0
*
*
18-44 years
45-65 years
over 65 years
D
CD4+ Tte absolute count
200
150
100
50
0
18-44 years
45-65 years
over 65 years
E
CD8+ Tn absolute count
100
80
60
40
20
0
*
*
*
18-44 years
45-65 years
over 65 years
F
CD8+ Tscm absolute count
400
300
200
100
0
*
*
*
18-44 years
45-65 years
over 65 years
G
CD8+ Tcm absolute count
400
300
200
100
0
*
18-44 years
45-65 years
over 65 years
H


CD8+ Tem absolute count
400
300
200
100
0
18-44 years
45-65 years
over 65 years

I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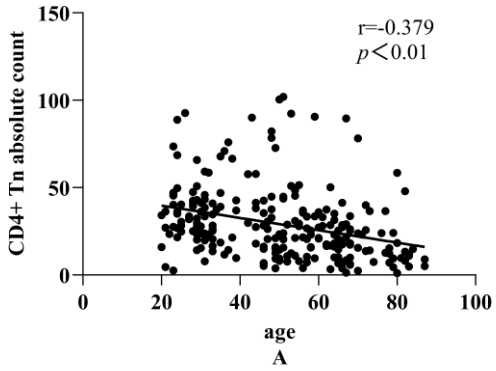
400
300
200
100
0
18-44 years
45-65 years
over 65 years

J

Figure 3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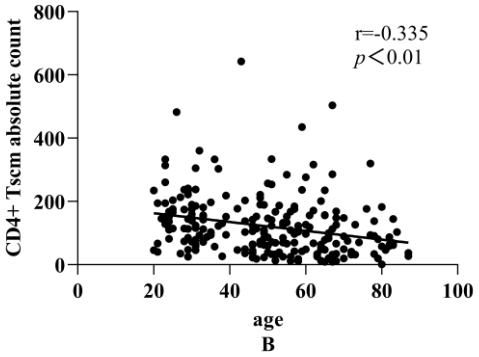
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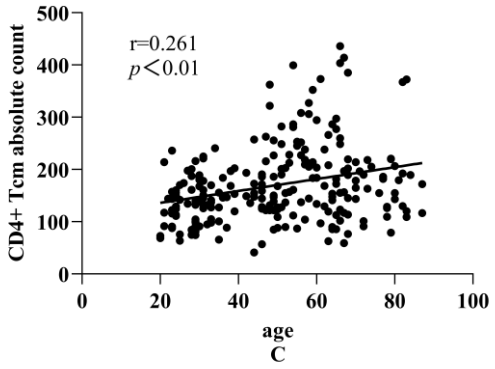
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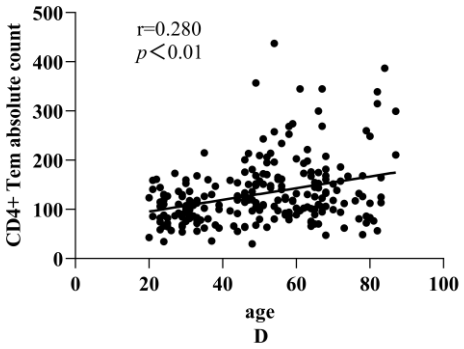
C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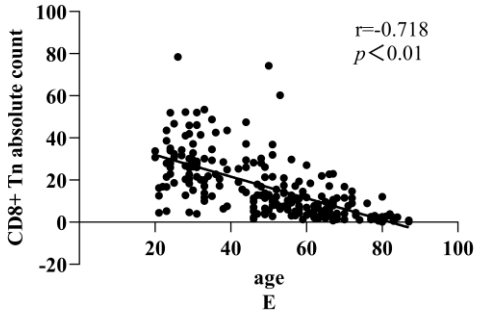
D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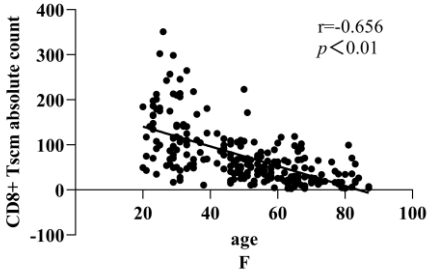
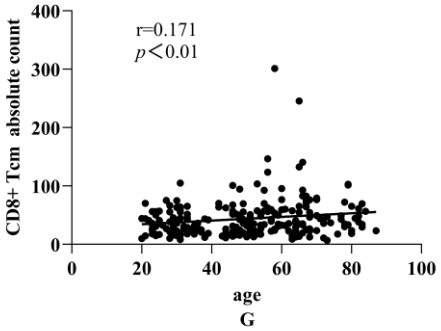
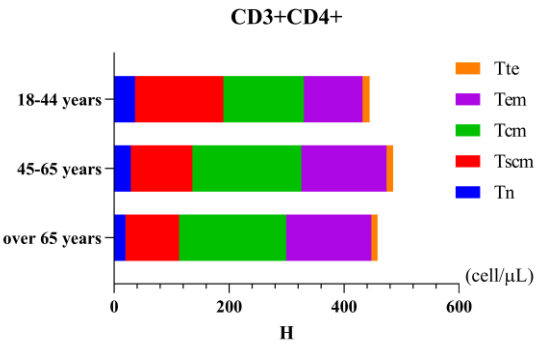
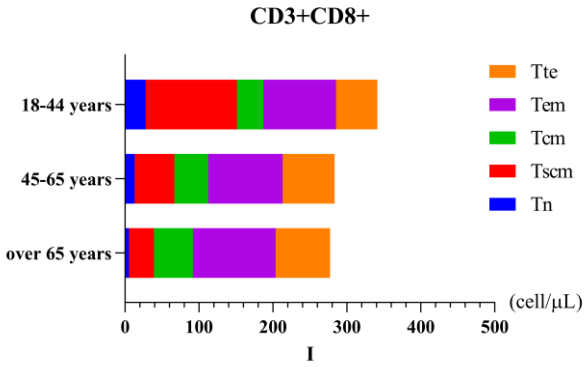
CD8+ Tn absolute count
age
E


r=-0.656
p<0.01
CD8+ Tscm absolute count
age
F


r=0.171
p<0.01
CD8+ Tcm absolute count
age
G


CD3+CD4+
18-44 years
45-65 years
over 65 years
Tte
Tem
Tcm
Tscm
Tn
(cell/μL)
H


CD3+CD8+
18-44 years
45-65 years
over 65 years
Tte
Tem
Tcm
Tscm
Tn
0
100
200
300
400
500
(cell/μL)

I

Figure 4

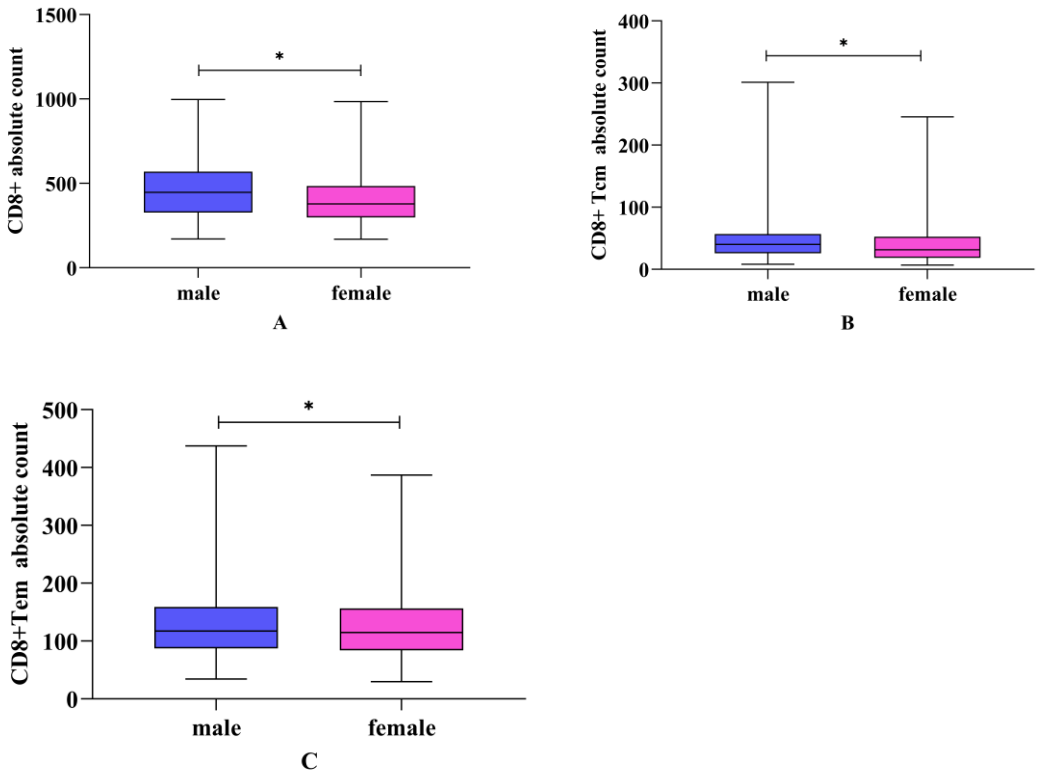

CD8+ absolute count
1500
1000
500
0
*
male
female
A
CD8+ Tcm absolute count
400
300
200
100
0
*
male
female
B
CD8+Tem absolute count
500
400
300
200
100
0
*
male
female
C

Figure 5

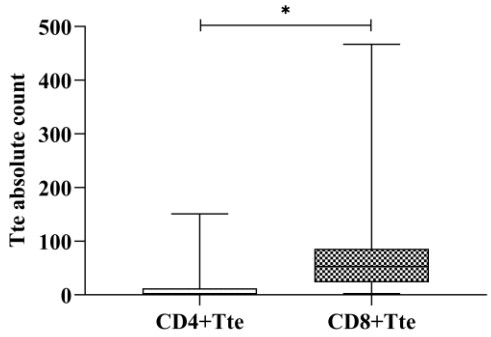


**Figure captions**

Fig 1. Gating strategies. Firstly, we gated lymphocyte identified by CD45 from leukocyte, then gated CD3+ T cells from lymphocyte. T cell subsets populations Tcm (CD95+ CD62L-) and Tem (CD95+ CD62L+) were gated from CD3+CD4+ (CD45RO+ CD45RA- ) and CD3+CD8+ (CD45RO+ CD45RA- )T subsets; Tscm (CD95+CD62L+), Tn (CD95-CD62L+) and Tte (CD95+CD62L-) were gated from CD3+CD4+ (CD45RO- CD45RA+ ) and CD3+CD8+ (CD45RO- CD45RA+ )T subsets.

Fig 2. Comparison of absolute counts of T lymphocyte subsets in different age groups. (**A, B, F, G)** showed the absolute count of CD4+Tn, CD4+Tscm, CD8+Tn, CD8+Tscm in group of 18-45 years were higher than those in group of 45-65years and over 65years ($P$<0.05); (**C, D)** stated that the absolute counts of CD4+Tcm, CD4+Tem in groups of 45-64 years old and over 65 years old are higher than those in 18-45 years old ($P$<0.05), but there no difference between the group of 45-64 years old and over 65 years old ($P$>0.05); (**H)** indicated that the absolute count of CD8+Tcm in over 65 years old group is more than those in 18-45 years old ($P$<0.05); (**E, I, J)** demonstrated that the absolute counts of CD4+Tte, CD8+Tem, CD8+Tte showed not changes with age. (* represents significant differences)

Fig 3. Relationship between age and T lymphocyte subsets and changes in distribution. (**A, B, E, F)** showed a trend of decrease in CD4+ Tn cell counts (r=-0.379，$P$<0.01),

CD4+ Tscm cell counts (r=-0.335, $P$<0.01), CD8+ Tn cell counts (r=-0.718, $P$<0.01), and CD8+ Tscm cell counts (r=-0.656, $P$<0.01)with increased age. (**C,D,G)** indicated an increase trend with aging in CD4+Tcm (r=0.261, $P$<0.01), CD4+ Tem (r=0.280, $P$<0.01), CD8+ Tcm (r=0.171, $P$<0.01). (**I, H)** represented that the Tn, Tscm cells which had stem cell properties increased with age, however, memory cell subsets, such as Tcm, Tem, decreased with age.

Fig 4. Comparison of the absolute counts of T lymphocyte subsets in different gender. (**A,B**,**C)** showed that the absolute counts of CD3+CD8+, CD8+ Tcm, CD8+Tem in male group are higher than those in female group($P$<0.05).

Fig 5.The absolute count are different in CD3+CD4+Tte and CD3+CD8+Tte.