RESEARCH ARTICLE

Eosinophil Count Is a Common Factor for Complex Metabolic and Pulmonary Traits and Diseases: The LifeLines Cohort Study

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Abstract

There is ongoing debate on the association between eosinophil count and diseases, as previous studies were inconsistent. We studied the relationship of eosinophil count with 22 complex metabolic, cardiac, and pulmonary traits and diseases. From the population-based LifeLines Cohort Study (N = 167,729), 13,301 individuals were included. We focused on relationship of eosinophil count with three classes of metabolic (7 traits, 2 diseases), cardiac (6 traits, 2 diseases), and pulmonary (2 traits, 2 diseases) outcomes. Regression analyses were applied in overall, women and men, while adjusted for age, sex, BMI and smoking. A p-value of <0.00076 was considered statistically significant. 58.2% of population were women (mean±SD 51.3±11.1 years old). In overall, one-SD higher of ln-eosinophil count was associated with a 0.04 (±SE 0.002; p = 6.0×10⁻⁶) SD higher levels in ln-BMI, 0.06 (±0.007; p = 3.1×10⁻¹²) SD in ln-TG, 0.04 (±0.003; p = 7.0×10⁻⁶) SD in ln-BMI, 0.04 (±0.004;p = 6.3×10⁻⁷) SD in LDL, 0.04 (±0.006; p = 6.0×10⁻⁵) SD in HbA1c; and with a 0.05 (±0.004;p = 1.7×10⁻⁶) SD lower levels in HDL, 0.05 (±0.007; p = 3.4×10⁻²⁵) SD in FEV1, and 0.09 (±0.001; p = 6.6×10⁻²⁶) SD in FEV1/FVC. A higher ln-eosinophil count was associated with 1.18 (95%CI 1.09–1.28; p = 2.0×10⁻⁵) odds ratio of obesity, 1.29 (1.19–1.39; p = 1.1×10⁻¹⁰) of metabolic syndrome, 1.40 (1.25–1.56; p = 2.7×10⁻⁹) of COPD and 1.81 (1.61–2.03;p = 1.0×10⁻²³) of asthma. Similar results were found in women. We found no association between ln-eosinophil count either with blood pressure indices in overall, women and men; or with BMI, LDL, HbA1c and obesity in men. In a large population based cohort, we
confirmed eosinophil count as a potential factor implicated in metabolic and pulmonary outcomes.

Background

Eosinophils are specialized multifunctional leukocytes which constitute up to 4% of peripheral white blood cells [1–2]. They are involved in the regulation of immune response by acting as antigen-presenting cells and augmenting of the expression of pro-inflammatory or inhibitory cytokines [3–5]. As summarized in Table A in S1 File, a number of clinical and epidemiological studies have reported differential findings on the association of eosinophil count with complex traits and diseases in human. On the one hand, higher eosinophil count has been associated with increased coagulation factors level of serum fibrinogen and platelet count [4, 6], an increase in the risk and severity of coronary atherosclerosis [3, 4, 6], exacerbation of asthma [7–11], pulmonary hypertension [12], metabolic syndrome (MetS) [13], and inflammatory bowel diseases [14, 15]. On the other hand, a protective effect of higher eosinophil count has been reported on glucose homeostasis [16], risk of type 2 diabetes (T2D) [16–18], and hypertension (HTN) [18], as summarized in Table A in S1 File. Nevertheless, the eosinophil counts and its association with complex diseases are likely to be biased by disease modifying factors, such as age and gender [19–23]. Furthermore, the reproducibility of these associations is questionable as the corresponding studies, with a few exceptions, had included a relatively small number of subjects, and were performed across heterogeneous populations. This argument is endorsed as no assessment of the association between eosinophil count and different complex outcomes has been performed in the same homogenous population. Hence, the role of eosinophil count in complex diseases remains inconclusive (Table A in S1 File). Although the analyses of disease intermediate traits are instructive and powerful to unravel disease pathogenesis; little attention has been given to the relationship between eosinophil count and disease associated intermediate traits. One may therefore propose a population based cohort study offers an ideal setting to unearth the role of eosinophil count as an emerging universal risk factor for multifactorial disorders [24]. We aimed therefore to investigate whether eosinophil count is consistently associated with common complex intermediate traits and diseases in the baseline measurements of a randomly selected subpopulation of the LifeLines cohort study, in men as well as in women. LifeLines is a prospective population-based cohort study examining 167,729 residents of the Netherlands and aims to unravel how lifetime exposure to environmental and genetic factors influences individual susceptibility to multifactorial diseases. In the present study, we examined the association of eosinophil count with 22 complex metabolic, cardiac, and pulmonary traits and disorders in 13,301 of total population (overall), in men and in women from the LifeLines study.

Methods

Population

The present study was conducted within the framework of the LifeLines Cohort Study. Details on study design and objectives of LifeLines have been described elsewhere [25, 26]. In brief, LifeLines is a prospective population-based cohort study, covering three generations, including 167,729 residents of three Northern provinces of the Netherlands. It employs a broad range of procedures to assess the biomedical, socio-demographic, behavioural, physical and psychological factors, which contribute to health and disease of the general population, with a special focus on multi-
morbidity and complex genetics. LifeLines is conducted according to the guidelines of the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethics Committee of the University Medical Center Groningen (UMCG). A written informed consent was obtained from all participants. For the present study, we included the third data release of LifeLines belonging to 13,301 unrelated individuals for whom dedicated detailed data on a wide variety of phenotypes were in the third release of LifeLines, and included blood measurements and genome-wide genotyping data.

Outcomes
We focused on three classes of metabolic, cardiac, and pulmonary disorders and underlying intermediate traits.

The class of metabolic outcomes included the intermediate traits of body mass index (BMI, kg/m\(^2\)), lipid profile (triglycerides [TG, mmol/L], total cholesterol [TC, mmol/L], high density lipoprotein [HDL, mmol/L], low density lipoprotein [LDL, mmol/L]), hemoglobin A1c (HbA1c, %), and fasting glucose (FG, mmol/L) as well as obesity, MetS and T2D. To calculate BMI, height and weight were measured without shoes and in light clothing to the nearest 0.1 cm and 0.1 kg, respectively, and BMI was calculated as weight/height squared (kg/m\(^2\)) according to the National Heart, Lung and Blood Institute guidelines [27] and TG, TC, HDL and LDL were measured using enzymatic colorimetric assay (Modular Roche) [27]. FG was measured with a hexokinase method (Integra Roche) and the HbA1c level was measured using a turbidimetric inhibition immunoassay [27]. More details have been explained previously [28, 29]. Obesity was defined based on BMI \(\geq 30\) (kg/m\(^2\)) [30]. The presence of MetS was defined as the presence of three or more of the following four risk factors: a) abdominal obesity defined as waist circumference in men \(>102\) cm and in women \(>88\) cm; b) dyslipidemia defined as serum triglycerides \(\geq 1.7\) mmol/L or pharmacologic treatment for elevated triglycerides and serum HDL cholesterol \(<1.03\) mmol/L in men and \(<1.29\) mmol/L in women or pharmacologic treatment for low HDL cholesterol; c) HTN defined as SBP \(\geq 130\) mmHg or DBP \(\geq 85\) mmHg or pharmacologic treatment for elevated blood pressure; d) hyperglycemia defined as FG \(\geq 5.6\) mmol/L or pharmacologic treatment for elevated plasma glucose [31]. A diagnosis of T2D was assigned to any participant who had either self-reported T2D or use of anti-T2D medication(s) or a FG \(\geq 7.0\) mmol/L or an HbA1c \(\geq 6.5\%\) [32–35]. This algorithm is explained in detail in the Methods I in S1 File and Figs A and B in S1 File.

The exclusion criterion for the analyses of TG, TC, HDL, and LDL was the use of lipid lowering medication. For the analyses on fasting glucose, exclusion criteria were doctor’s diagnosed T2D, use of anti-T2D medications and people who a FG \(\geq 7\) mmol/L or an HbA1c \(\geq 6.5\%\).

The class of cardiac traits and diseases included systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), pulse pressure (PP, mmHg), estimating glomerular filtration rate (eGFR, mL/min/1.73 m\(^2\)), urine albumin-to-creatinine ratio (UACR, mg/mmol) as well as HTN and myocardial infarction (MI). SBP and DBP of participants were recorded by an automatic blood pressure monitor (DinaMap, PRO 100V2) every minute during 10 minutes. This resulted in 10 blood pressure measures per participant. The mean of the last three measures was used for calculating blood pressure per participant. For all individuals taking antihypertensive or blood pressure lowering medication, imputed SBP and DBP were calculated based on the guidelines of the international consortium on blood pressure (ICBP) [36] by adding 15mmHg to the measured SBP level and 10mmHg to the measured DBP level. Using the imputed SBP and DBP values, MAP and PP were calculated based on following formulas: MAP = (2DBP+SBP)/3 and PP = SBP-DBP [36]. CKD-EPI
equation was used for estimating GFR which expressed for specified race, sex and serum creatinine in mg/dl based on the study by Levey et al. [37, 38]. UACR is a ratio between the urine albumin (BCG, colorimetric assay; Modular Roche) and the urine creatinine (enzymatic, IDMS traceable; Modular Roche) [38]. HTN was defined as a SBP ≥ 140 mmHg and/or a DBP ≥ 90 mmHg or used anti-hypertensive medication [39]. MI was defined as a positive answer to the question “Have you ever had a myocardial infarction?”. Exclusion criteria for the analyses of blood pressure indices were the presence of self-reported MI or doctor’s diagnosed heart failure or coronary artery disease.

The class of pulmonary traits and diseases included forced expiratory volume in one second (FEV$_1$, L), ratio of FEV$_1$ and forced vital capacity (FEV$_1$/FVC) as well as chronic obstructive pulmonary disease (COPD) and asthma. FEV$_1$ and FVC were measured by a spirometer (Welch Allyn version 1.6.0.489, PC-Based SpiroPerfect with CardioPerfect Workstation software) following the American Thoracic Society criteria [40]. According to the test criteria the difference between the best and the next best FEV$_1$ and FVC should not exceed 150 ml. If the difference was greater than 150 ml, the test was repeated. The presence of COPD was defined as a FEV$_1$/FVC ratio < 70% in ever (ex- or current) smokers with age ≥ 40 years old; Asthma was defined as a clinical diagnosis of asthma, or at least two asthma symptoms (wheeze, attack at rest, woken by an attack) in addition to asthma medication use (Methods II in S1 File and Fig C in S1 File) [41, 42].

### Determinants

**Eosinophil.** Eosinophil count ($\times 10^3$ cells/µl) were measured using Automated Hematology Blood Analyzer (XE2100--system; Sysmex, Japan). In the main analyses, absolute eosinophil count was utilized. Absolute eosinophil count was calculated by multiplying the relative eosinophil count by the total leukocyte count.

**Modifiers.** Age and sex were recorded according to the community registry database. Smoking status was reported as nonsmoker, former smoker, or current smoker. The number of pack-years was calculated based on self-reported information on the number of cigarettes smoked per day and the number of years smoked. Pack-years is the number of years with 20 cigarettes per day.

### Data analysis

Eosinophil count, BMI, TG, SBP, DBP, MAP, and PP were not normally distributed. To test for normality distribution, skewness test were used. If skewness was between -0.5 and 0.5, the distribution is approximately normally distributed; otherwise, a natural logarithmic transformation (ln) was carried out to achieve approximate normality and fulfill regression assumptions for these variables. These variables were presented as median with inter-quartile range in the descriptive table. TC, HDL, LDL, HbA1c, FG, FEV$_1$, and FEV$_1$/FVC ratio were normally distributed and were presented as mean±standard deviation (SD). Because of previous reports on gender differences in eosinophil count and complex diseases, all analysis models were stratified by gender. Variables were compared between men and women using independent t tests or Mann-Whitney U tests when appropriate. Categorical variables were shown as frequency with percentage and were compared by $\chi^2$ test.

Multivariate linear regression analyses were performed to evaluate the relationship between blood eosinophil count and each of the studied intermediate traits. The analyses were adjusted for the potential confounding effect of major baseline characteristics including age and sex. To correct for a potential non-linear relationship of age with outcomes, we added also square of age in models. Next for each classes, additional covariables were added when appropriate,
including BMI and smoking habit in metabolic and cardiac classes. Additionally, SBP, DBP, height, overweight and obesity were adjusted in eGFR and UACR models. Besides, smoking habit and height were added in pulmonary class. The association of eosinophil count and intermediate traits was reported by standardized coefficient ($\beta$) which means a one-standard deviation higher eosinophil count would result in a $\beta \times \text{SD}$ change in the outcome variables.

Multivariate logistic regression analyses were performed to obtain the association of ln-transformed eosinophil count as independent variable and diseases outcomes. Beside age, age and sex as potential confounders, we added BMI as a confounder in T2D, HTN and MI diseases as well as smoking habit in all diseases outcomes and height in COPD and asthma diseases. The relation of eosinophil count and diseases outcomes was reported as odds ratio (OR) with the corresponding 95% confidence intervals (95% CIs). Since the study was nested within a population-based cohort study, OR was considered as approximation for fold increase risk of studies outcomes [43].

The Bonferroni correction for multiple testing was employed yielding a significance threshold of $p$-value $< 0.00076$ (conservatively calculated as 0.05/66, which 66 being the total number of tests for 22 outcomes tested in three groups [overall, men and women]). Data analyses were performed by using SPSS statistical software (version 22; SPSS Inc., Chicago, USA).

**Results**

The baseline characteristics of the total population and stratified by gender are presented in Table 1. Out of 13,301 (mean±SD age 51.3±11.1 years old) participants, 58.2% were women (51.1±10.9 years old) and men were slightly older (51.6±11.3 years old). Overall, 6.4% and 22.8% of participants reported using lipid lowering and antihypertensive medication, respectively, and 22.3% were current smokers with a mean of 13.3 (±SD 11.7) pack-years. The median eosinophil count was 0.16 ($\times 10^3$ cells/µl) in overall and was significantly ($p<0.001$) higher in men (0.17; range 0.11–0.25) compared to women (0.15; 0.10–0.22). The level of the majority of the intermediate traits (with the exception of HDL and FEV1/FVC) was significantly higher in men than women (Table 1). Overall, the prevalence of HTN was 29.5%, followed by MetS (17.6%), COPD (9.5%), asthma (7.3%), T2D (3.8%) and MI (1.4%). The prevalence of the major disease outcomes (Table 1) in men was higher than women for MetS (19.4% vs. 16.2%;$p<0.001$), T2D (4.6% vs. 3.3%;$p<0.001$), HTN (36.3% vs. 24.6%;$p<0.001$), MI (2.6% vs. 0.6%;$p<0.001$), and COPD (11.0% vs. 8.4%;$p<0.001$). Obesity and asthma were more prevalent in women than men (17.2% vs. 15.3% and 7.6% vs. 6.8%; respectively; Table 1).

Table 2 shows the multivariate regression results of eosinophil count with metabolic traits and diseases in overall, in men and in women. A one-SD higher ln-transformed eosinophil count was significantly associated with 0.04 ($\pm 0.002; p = 6.0\times 10^{-6}$) SD higher ln-transformed BMI, and also with a higher levels of ln-transformed TG, TC, LDL, and HbA1C. In contrast, it was significantly associated with 0.05 ($\pm 0.004; p = 1.7\times 10^{-8}$) SD lower levels of HDL. When we adjusted our full model for total leukocytes count, only TC ($\beta\pm SE 0.04\pm 0.003; p = 1.7\times 10^{-5}$) and LDL ($\beta\pm SE 0.04\pm 0.004; p = 3.3\times 10^{-5}$) remained significant (Table B in S1 File, Model III). Stratified analysis showed that ln-transformed eosinophil count was significantly associated with higher levels of ln-transformed TG, TC, LDL and with lower levels of HDL in both men and women; while it was significantly associated with higher ln-transformed BMI levels only in men, and with higher HbA1c only in women (Table 2). Logistic regression analyses showed that a one-unit higher ln-transformed eosinophil count was significantly associated with an (OR) 1.18 (95% CI 1.09–1.28;$p = 2.0\times 10^{-5}$) fold increase risk of obesity and 1.29 (1.19–1.39; $p = 1.1\times 10^{-10}$) fold increase risk of MetS. In men, a one-unit higher ln-transformed eosinophil count was significantly associated with the increased risk of MetS (OR 1.29,
# Characteristics of the study population (N = 13,301).

|                      | Overall (n = 13,301) | Men (n = 5,557) | Women (n = 7,744) |
|----------------------|----------------------|-----------------|-------------------|
| Gender: n (Female %) | 7,744 (58.22)        | -               | -                 |
| Age (yrs.)           | 51.32 ± 11.10        | 51.65 ± 11.34   | 51.09 ± 10.92*    |
| Lipid lowering medication: n (%) | 851 (6.40) | 448 (8.06) | 403 (5.20)** |
| Antihypertensive medication: n (%) | 3,029 (22.78) | 1,294 (23.28) | 1,735 (22.40) |
| Current smoking: n (%) | 2,973 (22.35) | 1,247 (22.44) | 1,726 (22.28) |
| Pack-years           | 13.29 ± 11.67        | 13.64 ± 11.90   | 13.03 ± 11.48*    |
| Eosinophils (×10^6 cells/μL) | 0.16 (0.10–0.23) | 0.17 (0.11–0.25) | 0.15 (0.10–0.22)** |
| BMI (kg/m²)          | 25.77 (13.88–28.60)  | 26.25 (24.28–28.54) | 25.30 (22.86–28.40)** |
| Normal (<25, %)      | 5,500 (41.37)        | 1,863 (33.53)   | 3,637 (47.00)**   |
| Overweight (25:<BMI<30, %) | 5,610 (42.20) | 2,842 (51.15) | 2,768 (35.77) |
| Obese (≥30, %)       | 2,185 (16.43)        | 851 (15.32)     | 1,334 (17.24)     |
| Triglycerides (mmol/L) | 1.04 (0.05–1.47) | 1.23 (0.89–1.78) | 0.94 (0.71–1.32)** |
| Total Cholesterol (mmol/L) | 5.13 ± 0.99 | 5.18 ± 0.98 | 5.07 ± 1.00*** |
| Low-density lipoprotein (mmol/L) | 1.45 ± 0.39 | 1.27 ± 0.31 | 1.57 ± 0.38** |
| Low-density lipoprotein (mmol/L) | 3.32 ± 0.89 | 3.42 ± 0.87 | 3.18 ± 0.90** |
| HbA1c (%)            | 5.51 ± 0.34         | 5.52 ± 0.33     | 5.50 ± 0.34**     |
| Fasting glucose (mmol/L) | 4.99 ± 0.56 | 5.12 ± 0.56 | 4.89 ± 0.54** |
| Imputed SBP (mmHg)   | 130.00 (86.00–237.00) | 135.00 (125.00–146.00) | 125.00 (115.00–139.00)** |
| Imputed DBP (mmHg)   | 76.00 (35.00–150.00) | 79.00 (73.00–87.00) | 74.00 (68.00–82.00)** |
| Mean Arterial Pressure (mmHg) | 94.33 (59.00–178.00) | 97.67 (91.00–106.33) | 91.00 (84.33–100.66)** |
| Pulse Pressure (mmHg) | 53.00 (22.00–124.00) | 55.00 (48.00–63.00) | 51.00 (43.00–60.00)** |
| eGFR (mL/min/1.73 m²) | 93.43 ± 14.75        | 94.49 ± 14.83   | 92.67 ± 14.65***  |
| UACR (mg/mmol)       | 0.20 (0.11–0.34)     | 0.18 (0.10–0.31) | 0.22 (0.13–0.37)** |
| FEV₁ (L)             | 3.38 ± 0.83          | 4.01 ± 0.76     | 2.94 ± 0.55***    |
| FEV₁/FVC              | 0.76 ± 0.07          | 0.75 ± 0.08     | 0.77 ± 0.07***    |
| Metabolic syndrome: n (%)| 2,337 (17.57) | 1,079 (19.41) | 1,258 (22.44)** |
| T2D: n (%)           | 507 (3.81)           | 257 (4.62)      | 250 (3.28)**      |
| Hypertension: n (%)   | 3,921 (29.47)        | 2,019 (36.33)   | 1,902 (24.56)**   |
| Myocardial infarction: n (%)| 190 (1.43) | 143 (2.57) | 47 (0.61)** |
| COPD: n (%)          | 1,265 (9.51)         | 611 (11.00)     | 654 (8.44)**      |
| Asthma: n (%)        | 967 (7.27)           | 379 (6.82)      | 588 (7.60)**      |

**Abbreviations: BMI: body mass index, HbA1c: Hemoglobin A1c, SBP: systolic blood pressure, DBP: diastolic blood pressure, eGFR: estimated glomerular filtration rate, UACR: Urine Albumin-to-Creatinine Ratio, FEV₁: Forced expiratory volume in one second, FVC: Forced vital capacity, COPD: Chronic Obstructive Pulmonary Disease.**

† Imputed SBP and DBP were calculated as a following: For all individuals who taking antihypertensive or blood pressure lowering medication, were added 15mmHg to the measured SBP level, and 10mmHg to the measured DBP level. For individuals not taking such medication, the imputed values were left equal to the measured level.

* Metabolic syndrome was defined as the presence of three or more of the following four traits: 1) abdominal obesity defined as waist circumference in men >102 cm and in women >88 cm; 2) dyslipidemia determined as serum triglycerides >1.7 mmol/L or pharmacologic treatment for elevated triglycerides and serum HDL cholesterol <1.03 mmol/L in men and <1.29 mmol/L in women or pharmacologic treatment for low HDL cholesterol; 3) hypertension defined as either SBP ≥130 mmHg or DBP ≥85 mmHg or pharmacologic treatment for elevated blood pressure; 4) hyperglycemia determined as fasting glucose ≥5.6 mmol/L or pharmacologic treatment for elevated plasma glucose.

* Type 2 diabetes was defined by either clinical diagnosis, self–reported type 2 diabetes, type 2 diabetes pharmacologic treatment or undiagnosed type 2 diabetes defined by FG ≥7.0 mmol/L or HbA1c ≥6.5%.

* Hypertension was defined as SBP ≥140 mmHg and/or DBP ≥90 mmHg or anti-hypertension medication use.

* Myocardial infarction was based on self-reported.

* COPD was based on FEV₁/FVC ratio <70% and being an ever smoker (ex- or current smoker).

* Asthma was based on a clinical diagnosis of asthma or two or more of the symptoms wheeze, attack at rest, woken by an attack and asthma medication use.

* Women compared to men:

* p<0.05,

*** p<0.001.

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Table 2. Multivariate regression results of ln-transformed eosinophil count with studied intermediate traits and diseases in metabolic class.

|                          | Overall #1       | Men                   | Women                  |
|--------------------------|------------------|-----------------------|------------------------|
|                          | $\beta \pm SE$ 2 | $p$-value | N | $\beta \pm SE$ 2 | $p$-value | N | $\beta \pm SE$ 2 | $p$-value | N |
| In-transformed BMI       | 0.04 ± 0.002     | 6.0 × 10^{-6}        | 11,789                 | 0.01 ± 0.003           | 5.0 × 10^{-6} | 4,908 | 0.06 ± 0.003 | 1.0 × 10^{-6} | 6,881 |
| In-transformed Triglycerides | 0.06 ± 0.007     | 3.1 × 10^{-12}       | 10,661                 | 0.06 ± 0.012           | 4.376 | 0.07 ± 0.009 | 1.6 × 10^{-8} | 6,285 |
| Total Cholesterol        | 0.04 ± 0.003     | 7.0 × 10^{-6}        | 10,661                 | 0.05 ± 0.005           | 3.8 × 10^{-4} | 4,376 | 0.03 ± 0.004 | 3.0 × 10^{-3} | 6,285 |
| High-Density Lipoprotein | -0.05 ± 0.004    | 1.7 × 10^{-8}        | 10,660                 | -0.05 ± 0.006          | 4.0 × 10^{-3} | 4,375 | -0.06 ± 0.005 | 2.0 × 10^{-6} | 6,285 |
| Low-Density Lipoprotein  | 0.04 ± 0.004     | 6.3 × 10^{-7}        | 10,661                 | 0.04 ± 0.006           | 4.0 × 10^{-3} | 4,376 | 0.05 ± 0.002 | 2.9 × 10^{-5} | 6,285 |
| HbA1c                    | 0.04 ± 0.006     | 6.0 × 10^{-6}        | 11,751                 | 0.03 ± 0.011           | 4.892 | 0.04 ± 0.008 | 1.1 × 10^{-4} | 6,859 |
| Fasting glucose          | 0.008 ± 0.013    | 0.401                | 10,317                 | -0.008 ± 0.022         | 0.578 | 4.316 | 0.02 ± 0.015 | 0.045 | 6,001 |
| OR (95% CI)              | $p$-value        | Case/Non Case        | OR (95% CI)            | $p$-value | Case/Non Case | OR (95% CI) | $p$-value | Case/Non Case |
| Obesity                  | 1.18 (1.09−1.28) | 2.0 × 10^{-5}        | 2,058/10,480           | 1.14 (1.00−1.29)       | 0.040 | 804/4,444 | 1.22 (1.10−1.35) | 1.3 × 10^{-4} | 1,254/6,036 |
| Metabolic Syndrome       | 1.29 (1.19−1.39) | 1.1 × 10^{-10}       | 2,198/10,317           | 1.21 (1.08−1.35)       | 1.0 × 10^{-3} | 1,016/4,233 | 1.34 (1.20−1.50) | 1.0 × 10^{-7} | 1,182/6,084 |
| Type 2 diabetes          | 1.05 (0.91−1.20) | 0.496                | 470/10,880             | 1.03 (0.85−1.25)       | 0.750 | 243/4,623 | 1.07 (0.88−1.31) | 0.470 | 227/6,257 |

Abbreviations: $\beta$: standardized coefficient, BMI: body mass index, HbA1c: hemoglobin A1c.

#1: All analysis models on intermediate traits were adjusted for confounding effect of age, sex, BMI (with exception on BMI) and smoking habit. Analysis models on diseases were adjusted for confounding effect of age, sex, and smoking habit; as well as, BMI also was adjusted on type 2 diabetes. Sex effect only included in overall model.

#2: Standardized coefficient ($\beta$) means a one-standard deviation higher eosinophil count would result in a $\beta \times SD$ change in the outcome variables.

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95% CI 1.19−1.39 $p = 1.1 \times 10^{-10}$); while in women, with the increased risk of obesity (OR 1.22, 95% CI 1.10−1.35 $p = 1.3 \times 10^{-4}$) and MetS (OR 1.34, 95% CI 1.20−1.50 $p = 1.0 \times 10^{-7}$).

No significant association was found between ln-transformed eosinophil count and intermediate traits or diseases in the cardiac class including blood pressure and renal function indices as well as MI and HTN either in overall or when the analyses were stratified by gender (Table 3 and Table C in S1 File).

As Table 4 presents, we found a one-SD higher of ln-transformed eosinophil count was highly significantly associated with a lower FEV$_1$ and FEV$_1$/FVC ratio. Logistic regression analyses showed that a higher ln-transformed eosinophil count was significantly associated with the increased risk of COPD (OR 1.40, 95% CI 1.25−1.56 $p = 2.7 \times 10^{-5}$) and asthma (OR 1.81, 95% CI 1.61−2.03 $p = 1.0 \times 10^{-23}$). By inclusion of total leucocyte count, the observed significant associations between eosinophil count and pulmonary traits and diseases remained still significant at a $p<7.6 \times 10^{-4}$ (Table D in S1 File, Model III). These results remained significant in men as well as in women (Table 4). In addition evaluation of the relationship between eosinophil count and the intermediate outcomes using partial correlation analysis while controlling for covariates effect, shows the similar results to the multivariate regression analyses (Table E in S1 File).

Discussion

In the large population based LifeLines Cohort Study, we investigated the association of eosinophil count with complex diseases and their underlying intermediate phenotypes. We found that higher levels of BMI, TG, TC, LDL, and HbA1c and lower levels of HDL, FEV1, and
Table 3. Multivariate regression results of ln-transformed eosinophil count with studied intermediate traits and diseases in cardiac class.

|                        | Overall¹ | Men | Women |
|------------------------|----------|-----|-------|
|                        | Sβ ± SE² | p-value | N | Sβ ± SE² | p-value | N |
| In-transformed SBP     | 0.007 ± 0.002 | 0.345 | 11,625 | 0.007 ± 0.002 | 0.570 | 4,787 |
| In-transformed DBP     | 0.01 ± 0.002 | 0.244 | 11,625 | 0.008 ± 0.003 | 0.381 | 4,787 |
| In-transformed MAP     | 0.009 ± 0.002 | 0.250 | 11,625 | 0.008 ± 0.002 | 0.534 | 4,787 |
| Pulse Pressure         | 0.003 ± 0.168 | 0.749 | 11,786 | 0.006 ± 0.247 | 0.627 | 4,907 |
| eGFR                   | -0.003 ± 0.167 | 0.616 | 11,560 | 0.004 ± 0.242 | 0.685 | 4,905 |
| In-transformed UACR    | -0.011 ± 0.016 | 0.025 | 11,561 | -0.003 ± 0.025 | 0.798 | 4,903 |

Abbreviations: Sβ: standardized coefficient, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, eGFR: estimated glomerular filtration rate, UACR: Urine Albumin-to-Creatinine Ratio.

¹: All analysis models on intermediate traits and diseases were adjusted for confounding effect of age, age², sex, BMI and smoking habit. Additionally, SBP, DBP, height, overweight and obesity were adjusted in eGFR and UACR models. Sex effect only included in overall model.

²: Standardized coefficient (Sβ) means a one-standard deviation higher eosinophil count would result in a β × SD change in the outcome variables.

FEV1/FVC were significantly associated with higher eosinophil count. We found no association between eosinophil count and FG, blood pressure and renal function indices. Further we observed that higher eosinophil count was significantly associated with a higher prevalence of obesity, MetS, COPD, and asthma, but not with T2D, HTN and MI. In stratified by gender analyses, similar results to overall population were found in women. While, in men higher eosinophil count was significantly associated with higher levels of BMI, TG, TC, and lower levels of HDL; in addition to higher prevalence of MetS, COPD, and asthma. Taking together, in comparison to previous studies presented in Table A in S1 File, using basic models and full

Table 4. Multivariate regression result of ln-transformed eosinophil count with studied intermediate traits and diseases in pulmonary class.

|                        | Overall¹ | Men | Women |
|------------------------|----------|-----|-------|
|                        | Sβ ± SE² | p-value | N | Sβ ± SE² | p-value | N |
| FEV₁                  | -0.05 ± 0.007 | 3.4 × 10⁻²³ | 11,849 | -0.06 ± 0.013 | 7.5 × 10⁻⁹ | 4,903 |
| FEV₁/FVC              | -0.09 ± 0.001 | 6.6 × 10⁻²⁸ | 11,851 | -0.09 ± 0.002 | 2.1 × 10⁻¹² | 4,905 |
| OR (95% CI)           | p-value | Case/Non Case | OR (95% CI) | p-value | Case/Non Case | OR (95% CI) | p-value | Case/Non Case |
| COPD                  | 1.40 (1.25–1.56) | 2.7 × 10⁻⁹ | 1,066/10,342 | 1.40 (1.19–1.64) | 3.6 × 10⁻⁵ | 521/4,200 |
| Asthma                | 1.81 (1.61–2.03) | 1.0 × 10⁻²³ | 869/10,922 | 1.70 (1.40–2.04) | 2.3 × 10⁻⁸ | 334/4,575 |

Abbreviations: Sβ: standardized coefficient, FEV₁: Forced expiratory volume in one second, FVC: Forced vital capacity.

¹: All analysis models on intermediate traits and diseases were adjusted for confounding effect of age, age², sex, smoking habit, and height. Sex effect only included in overall model.

²: Standardized coefficient (Sβ) means a one-standard deviation higher eosinophil count would result in a β × SD change in the outcome variables.

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models we could find consistently significant association of eosinophil count with traits and diseases in metabolic and pulmonary classes.

In the present study, eosinophil count was significantly higher in participants with higher BMI, triglyceride, cholesterol, LDL, and lower HDL level. Besides, higher eosinophil count was significantly associated with higher BMI in women. Lately, several studies have reported inconsistent associations between eosinophil count and metabolic traits, as was summarized in Table A in S1 File. Our findings are in line with one study which reported that eosinophil count was significantly higher in people with hyper-triglyceridemia and hyper-cholesterolemia [44] and a previously reported significant reverse association between eosinophil count and HDL level [45]. Another study found no association between eosinophil count and high-LDL level [46]. Consistent with a previous reported association between eosinophil count and lipid indices in our data and those from others [45, 46], participants with higher eosinophil count showed a significantly higher risk for obesity and MetS. These findings were in concordance with those of previous studies as summarized in Table A in S1 File [13, 18, 47–49] on MetS [13, 18, 45, 49] in Asian (Korean) [13, 45] and in Caucasian (Spanish) populations [49]. Taken together, our findings corroborate those of previous reports and confirm that a higher eosinophil count is significantly associated with a higher level of lipid indices such as high TC, high LDL, high TG, and low HDL level and with higher risk for obesity and MetS. Higher levels of eosinophil counts seems to be a significant risk factor for metabolic disorders associated with lipid disturbances.

We found no significant association between eosinophil count and FG; likewise, eosinophil count showed no significant relationship to T2D. To date, few studies have described the relationship between eosinophil count and glucose level. The case-control study by Xu et al. and the cross-sectional study by Zhu et al. found that a lower eosinophil count was associated with hyperglycemia [17, 50]. As summarized in Table A in S1 File, others also found no significant relationship between eosinophil count and the risk for T2D [18, 47, 48]. However, one cross-sectional study found that the mean eosinophil count increased with progression of diabetes [51], which is likely to be a reflection of disease severity. Taken together, our finding of a lack of association between eosinophil count and glycaemia adhere with earlier reports of no significant association between eosinophil count and T2D. Therefore, it is unlikely that eosinophil count is a strong determinant of glycaemia or T2D and to play a major role in glycemic control.

We found no association between eosinophil count and SBP, DBP, PP, MAP, HTN or MI. Likewise, a few previous epidemiological studies (Table A in S1 File) were inconsistent on association between eosinophil count and HTN. While one study found no significant association between eosinophil count and HTN [18]; another study has reported otherwise [13]. Unlike our finding, a few reports showed that higher eosinophil count was associated with coronary heart disease [6, 52, 53]. In our study the association of eosinophil count and MI did not reach significance after multiple testing correction. Observing this conflicting result with our findings, we investigated the power of our study. Indeed we estimated that this study had a suboptimal power of 54.1% to detect the association of eosinophil count with MI due to a low number of subjects with MI (N = 190, Table 1) in our study. When we additionally explored the association of indices of renal function, which are closely related to cardiovascular parameters, we observed no relationship between eosinophil count and eGFR and UACR as well. The lack of association of renal parameters and hypertension, as opposed to the association with metabolic traits, remains interesting in terms of pathway dissection—as clinically the metabolic syndrome, hypertension and renal damage are very closely associated and this might be at the phenotypic level. Putting together, though eosinophil count is unlikely to be significantly associated with the indices of blood pressure, the hypothesis that higher eosinophil count is a
risk factor for MI remains yet to be addressed. Future well-powered population based longitudi-
nal studies are required to test this hypothesis.

Our results showed that a higher eosinophil count was significantly associated with a lower
FEV$_1$ and FEV$_1$/FVC ratio in multivariate models controlling for potential confounders. We
have also previously reported that asthmatic patients with lower FEV$_1$ had higher eosinophil
count in blood [23, 54] and in sputum [55, 56], which endorsed previous studies on association
of eosinophil count with low lung function in asthmatic patients [57–60]. Also, we have previ-
ously shown that the pattern and strength of the association between eosinophil and lung func-
tion differs according to gender, smoking habit, and presence of skin test reactivity [23, 54].
Additionally, we found that a higher eosinophil count was linked to higher risk for asthma and
COPD. Our findings were consistent with previous studies which showed, eosinophil level
were higher in asthmatic [60–62] and COPD [62–64] patients. Nevertheless, treatment strategy
directed at normalization of eosinophil remains inconclusive regarding the critical role of
eosinophil in asthma and COPD pathogenesis [65–67]. Taken together, our findings align
with those aforementioned studies (Table A in S1 File) suggesting that eosinophil count is
likely to be a risk factor for low lung function, airway obstruction, COPD and asthma. Blood
eosinophils are derived from bone marrow myeloid progenitors, and they mature in the
peripheral circulation. The main factor regulating eosinophils is interleukin-5, but also other
cytokines are important in eosinophil survival, such as IL3 and GMCSF [68]. Eosinophils traf-
fic to the site of inflammation and can have important local effects, e.g. asthma in the airway.

The relation between the chronic disease and blood eosinophils can be twofold, either eosin-
ophils being part of the causal pathway, or diseases that are associated with local production of
cytokines which then stimulate increase of eosinophil count in the peripheral circulation (i.e.
chemokines produced by adipose tissue)[69]. In the present study, we focused on eosinophil
count and found strong associations with metabolic and pulmonary outcomes which fall within
aforementioned previous observations (Table A in S1 File). When studying the role of inflam-
matory multifunctional cells in complex diseases, the complex interaction out of functional
dependencies among these cells is a limiting factor for definite conclusions. Nevertheless, the
findings remained significant for associations between eosinophil count and TC, LDL, pulmo-
nary outcomes, even when we corrected for leukocytes count. This suggests despite a labyrin-
thine functional dependency between various type of leukocytes, eosinophil count maintained
its independent effects on these outcomes. For other metabolic traits and diseases the signifi-
cant findings presented in the Table 2, turned to be statistically non-significant after including
leukocyte count into the regression model. We therefore propose proper follow-up functional
or methodological oriented studies such as mediation and moderation analyses using longitu-
dinal observational studies, and a causal modeling such as Mendelian randomization approach
to distinguish between causal, pleiotropic or co-stimulatory nature of observed significant asso-
ciations between eosinophil count and the studied complex outcomes.

**Limitations**

This study has a number of limitations; firstly, it remains to be tested whether the association
of eosinophil count with traits and diseases was independent of other inflammatory biomark-
ers; a next is whether the identified significant associations between eosinophil count and traits
and disease outcomes are causal. Appropriate approaches, such as the use of genetic determi-
nants of eosinophil count, are required to test the causal nature of these associations. Thirdly,
our study is cross sectional; longitudinal measures of eosinophil count with the setting of pro-
spective cohort studies are required to further validate and specify the role of eosinophils in
the development of complex diseases.
Conclusion

Higher eosinophil count is significantly associated with metabolic traits including BMI, TG, TC, HDL, LDL, HbA1c, and with lung function indices such as FEV$_1$ and FEV$_1$/FVC level, and is likely to be a common factor for obesity, MetS, COPD, and asthma and perhaps for MI but not for HTN. These association hold in both men and women, with an exception of obesity. Hence, higher eosinophil count may serve as an informative biomarker to identify individuals at higher risk for associated complex diseases and may have an additive predictive value when combined with known predictors. Future large scale genetic and cellular studies preferably embedded within longitudinal cohorts are needed to establish the causal nature of eosinophil count in complex traits and diseases. When confirmed, these findings may ultimately have a clinical implication such as prediction or intervention, which remains to be determined.

Supporting Information

S1 File. Supplementary Text and Figures.

(DOCX)

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References

1. Sampson AP. The role of eosinophils and neutrophils in inflammation. Clin Exp Allergy. 2000; 30: 22–27. PMID: 10849470
2. Behm CA, Ovington KS. The role of eosinophils in parasitic helminth infections: insights from genetically modified mice. Parasitol Today. 2000; 16: 202–209. PMID: 10782080
3. Niccoli G, Ferrante G, Cosentino N, Conte M, Belloni F, Marino M, et al. Eosinophil cationic protein: A new biomarker of coronary atherosclerosis. Atherosclerosis. 2010; 211: 606–611. doi: 10.1016/j.atherosclerosis.2010.02.036 PMID: 20307883
4. Lippi G, Montagnana M, Salvagno GL, Franchini M, Targher G, Guidi GC. Eosinophilia and first-line coagulation testing. J Thromb Thrombolysis. 2009; 28: 90–93. doi: 10.1007/s11239-008-0247-5 PMID: 19618227
5. Lacy P, Moqbel R. Immune effector functions of eosinophils in allergic airway inflammation. Curr Opin Allergy Clin Immunol. 2001; 1: 79–84. PMID: 11964674
6. Umemoto S, Suzuki N, Fuji K, Fujii A, Fujii T, Iwami T, et al. Eosinophil counts and plasma fibrinogen in patients with vasospastic angina pectoris. Am J Cardiol. 2000; 85: 715–719. PMID: 12000045
7. Gleich GJ. The eosinophil and bronchial asthma: current understanding. J Allergy Clin Immunol. 1990; 85: 422–436. PMID: 2406322
8. Menzies-Gow A, Robinson DS. Eosinophilic cytokines (interleukin-5), and antieosinophilic therapy in asthma. Curr Opin Pulm Med. 2002; 8: 33–38. PMID: 11753121
9. Belda J, Giner J, Casan P, Sanchis J. Mild exacerbations and eosinophilic inflammation in patients with stable, well-controlled asthma after 1 year of follow-up. Chest. 2001; 119: 1011–1017. PMID: 11296162
10. Petsky HL, Kynaston JA, Turner C, Li AM, Cates CJ, Lasserson TJ, et al. Tailored interventions based on sputum eosinophils versus clinical symptoms for asthma in children and adults. Cochrane Database Syst Rev. 2007; 2: CD005603.
11. Johansson MW. Activation states of blood eosinophils in asthma. Clin Exp Allergy. 2014; 44: 482–498. doi: 10.1111/cea.12292 PMID: 24552191
12. Weng M, Baron DM, Bloch KD, Luster AD, Lee JJ, Medoff BD. Eosinophils are necessary for pulmonary arterial remodeling in a mouse model of eosinophilic inflammation-induced pulmonary hypertension. Am J Phsiol Lung Cell Mol Physiol. 2011; 301: L927–936.
13. Kim DJ, Noh JH, Lee BW, Choi YH, Chung JH, Min YK, et al. The associations of total and differential white blood cell counts with obesity, hypertension, dyslipidemia and glucose intolerance in a Korean population. J Korean Med Sci. 2008; 23: 193–198. doi: 10.3346/jkms.2008.23.2.193 PMID: 18436999
14. Lampinen M, Backman M, Wingqvist O et al. Different regulation of eosinophil activity in Crohn's disease compared with ulcerative colitis. J Leukoc Biol. 2008; 84: 1392–1399. doi: 10.1189/jlb.0807513 PMID: 18801925
15. Rothenberg ME, Mishra A, Brandt EB, Hogan SP. Gastrointestinal eosinophils. Immunol Rev. 2001; 179: 139–155. PMID: 11292017
16. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science. 2011; 332: 243–247. doi: 10.1126/science.1201475 PMID: 21436399
17. Zhu L, Tu T, Xu M, Xu Y, Li M, Wang T, et al. Eosinophil inversely associates with type 2 diabetes and insulin resistance in Chinese adults. PLoS One. 2013; 8: e67613. doi: 10.1371/journal.pone.0067613 PMID: 23894289
18. Meng W, Zhang C, Zhang Q, Song X, Lin H, Zhang D, et al. Association between leukocyte and metabolic syndrome in urban Han Chinese: a longitudinal cohort study. PLoS One. 2012; 7: e49875. doi: 10.1371/journal.pone.0049875 PMID: 23209610
19. Postma DS. Gender differences in asthma development and progression. Gend Med. 2007; 4: S133–146. PMID: 18156099
20. Mensinga TT, Schouten JP, Rijcken B, Weiss ST, van der Lende R. Host factors and environmental determinants associated with skin test reactivity and eosinophilia in a community-based population study. Ann Epidemiol. 1994; 4: 382–392. PMID: 7981846
21. Banos G, Guainer V, Perez-Torres I. Sex steroid hormones, cardiovascular diseases and the metabolic syndrome. Cardiovasc Hematol Agents Med Chem. 2011; 9: 137–146. PMID: 21745183
22. Siroux V, Curt F, Orlyssczyn MP, Maccario J, Kauffmann F. Role of gender and hormone-related events on IgE, atopy, and eosinophil in the Epidemiological Study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy. J Allergy Clin Immunol. 2004; 114: 491–498. doi: 10.1016/j.jaci.2004.05.027 PMID: 15356546
23. Wang X, Mensinga TT, Schouten JP, Rijcken B, Weiss ST. Determinants of maximally attained level of pulmonary function. Am J Respir Crit Care Med. 2004; 169: 941–949. doi: 10.1164/rcrm.220101 PMID: 15072985
24. Song JW, Chung KC. Observational Studies: Cohort and Case-Control Studies. Plast Reconstr Surg. 2010; 126: 2234–2242. doi: 10.1097/PRS.0b013e3181f44abc PMID: 20697313
25. Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navig S, Slaets JP, et al. Universal risk factors for multifactorial diseases: Lifelines: a three-generation population-based study. Eur J Epidemiol. 2008; 23: 67–74. doi: 10.1007/s10654-007-9204-4 PMID: 18075776
26. Scholtens S, Smidt N, Swertz MA, Bakker SJ, Dotinga A, Vonk JM, et al. Cohort Profile: Lifelines, a three-generation cohort study and biobank. Int J Epidemiol. 2015; 44: 1172–1180. doi: 10.1093/ije/dyu229 PMID: 25502107
27. Janssen I, Katzmarzyk PT, Ross R. Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines. Arch Intern Med. 2002; 162: 2074–2079. PMID: 12374515
28. Li N, van der Sijde MR, LifeLines Cohort Study Group, Bakker SJ, Dullaart RP, van der Harst P, et al. Pleiotropic effects of lipid genes on plasma glucose, HbA1c, and HOMA-IR levels. Diabetes. 2014; 63: 3149–3158. doi: 10.2337/db13-1800 PMID: 24722249
29. Janssen H, Stolk RP, Nolte IM, Kema IP, Wolffenbuttel BH, Snieder H. Determinants of HbA1c in nondiabetic Dutch adults: genetic loci and clinical and lifestyle parameters, and their interactions in the Lifelines Cohort Study. J Intern Med. 2013; 273: 283–293. doi: 10.1111/joim.12010 PMID: 23121487
30. Anonymous Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults—The Evidence Report. National Institutes of Health. Obes Res. 1998; 6: 51S–209S. PMID: 9813653
31. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive Summary. Crit Pathw Cardiol. 2005; 4: 198–203. PMID: 18340209
32. Basevi V, Di Mario S, Morciano C, Nonino F, Magrini N. Comment on: American Diabetes Association. Standards of medical care in diabetes—2011. Diabetes Care 2011; 34(Suppl. 1):S11–S61. Diabetes Care. 2011; 34: e53; author reply e54.
33. American Diabetes Association. Executive summary: Standards of medical care in diabetes—2012. Diabetes Care. 2012; 35: S4–S10. doi: 10.2337/dc12-s004 PMID: 22187471
34. International Expert Committee. International expert committee report on the role of the A1c assay in the diagnosis of diabetes. Diabetes Care. 2009; 32: 1327–1334. doi: 10.2337/dc09-0933 PMID: 19502545
35. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab. 2008; 93: 2447–2453. doi: 10.1210/jc.2007-2174 PMID: 18460560
36. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011; 478: 103–109. doi: 10.1038/nature10405 PMID: 21909115
37. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al, CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009; 150: 604–612. PMID: 19414839
38. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002; 39: S1–266. PMID: 11904577
39. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2007; 25: 1105–1187. doi: 10.1097/HJH.0b013e3281c975a PMID: 17563527
40. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014; 43: 343–373. doi: 10.1183/09031936.00202013 PMID: 24337046
41. The National Heart, Lung, and Blood Institute. Guidelines for the Diagnosis and Management of Asthma. NIH Publication. 2007; 8: 1–60.
42. de Jong K, Boezen HM, Kromhout H, Vermeulen R, Postma DS, Vonk JM, et al. Pesticides and other occupational exposures are associated with airway obstruction: the LifeLines cohort study. Occup Environ Med. 2014; 71: 88–96. doi: 10.1136/oemed-2013-101639 PMID: 24142985
43. Langholz B: Case–Control Study, Nested. John Wiley & Sons. 2005; p.646–655.
44. Huang ZS, Chien KL, Yang CY, Tsai KS, Wang CH. Peripheral differential leukocyte counts in humans vary with hyperlipidemia, smoking, and body mass index. Lipids. 2001; 36: 237–245. PMID: 11337978
45. Shim WS, Kim HJ, Kang ES, Ahn CW, Lim SK, Lee HC, et al. The association of total and differential white blood cell count with metabolic syndrome in type 2 diabetic patients. Diabetes Res Clin Pract. 2006; 73: 284–291. doi: 10.1016/j.diabres.2006.02.001 PMID: 16563549
46. Oda E. Longitudinal associations between lymphocyte count and LDL cholesterol in a health screening population. Journal of Clinical & Translational Endocrinology. 2014; 1: 49–53.
47. Babio N, Ibarrola-Jurado N, Bullo M, Martinez-Gonzalez M, Warna-J, Salaverr I, et al. White blood cell counts as risk markers of developing metabolic syndrome and its components in the PREDIMED study. PLoS One. 2013; 8: e58354. doi: 10.1371/journal.pone.0058354 PMID: 23526980
59. Talini D, Novelli F, Bacci E, Bartoli M, Cianchetti S, Costa F, et al. Sputum eosinophilia is a determinant of FEV1 decline in occupational asthma: results of an observational study. BMJ Open. 2015; 5: e005748. doi: 10.1136/bmjopen-2014-005748 PMID: 25564139

60. Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. Am J Respir Crit Care Med. 2000; 161: 64–72. doi: 10.1164/ajrccm.161.1.9809100 PMID: 10619799

61. Jansen DF, Rijcken B, Schouten JP, Kraan J, Weiss ST, Timens W, et al. The relationship of skin test positivity, high serum total IgE levels, and peripheral blood eosinophilia to symptomatic and asymptomatic airway hyperresponsiveness. Am J Respir Crit Care Med. 1999; 159: 924–931. doi: 10.1164/ajrccm.159.3.9804024 PMID: 10051274

62. Gorska K, Krenke R, Korczynski P, Kosciuch J, Domagala-Kulawik J, Chazan R. Eosinophilic airway inflammation in chronic obstructive pulmonary disease and asthma. J Physiol Pharmacol. 2008; 59: 261–270. PMID: 19218650

63. Bafadhel M, McKenna S, Terry S, Mistry V, Reid C, Haldar P, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. Am J Respir Crit Care Med. 2011; 184: 662–671. doi: 10.1164/rccm.201104-0597OC PMID: 21680942

64. Hospers JJ, Schouten JP, Weiss ST, Rijcken B, Postma DS. Asthma attacks with eosinophilia predict mortality from chronic obstructive pulmonary disease in a general population sample. Am J Respir Crit Care Med. 1999; 160: 1869–1874. doi: 10.1164/ajrccm.160.6.9811041 PMID: 10588599

65. Ullmann N, Bossley CJ, Fleming L, Silvestri M, Bush A, Saglani S. Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. Allergy. 2013; 68: 402–406. doi: 10.1111/all.12101 PMID: 23347007

66. Medoff BD, Okamoto Y, Leyton P, Weng M, Sandall BP, Raher MJ, et al. Adiponectin deficiency increases allergic airway inflammation and pulmonary vascular remodeling. Am J Respir Cell Mol Biol. 2009; 41: 397–406. doi: 10.1165/rcmb.2008-0415OC PMID: 19168697

67. Lee SH, Park JS, Park CS. The search for genetic variants and epigenetics related to asthma. Allergy Asthma Immunol Res. 2011; 3: 236–244. doi: 10.4168/aair.2011.3.4.236 PMID: 21966603

68. Sriaroon P, Ballow M. Biological Modulators in Eosinophilic Diseases. Clin Rev Allergy Immunol. 2016; 50: 252–272. doi: 10.1007/s12016-014-8444-9 PMID: 25129490

69. Exley MA, Hand L, O'Shea D, Lynch L. Interplay between the immune system and adipose tissue in obesity. J Endocrinol. 2014; 223: R41–48. doi: 10.1530/JOE-13-0516 PMID: 25228503