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Atopic respiratory diseases and IgE sensitization are associated with leukocyte subset concentrations in 14,440 blood donors

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ABSTRACT

Background and aims: Allergic rhinitis (AR), allergic conjunctivitis (AC), and asthma are characterized by activation of the immune system. The aim of this study was to explore the long-term association between AR, AC, asthma, and specific immunoglobulin E (IgE) and blood platelet and leukocyte differential counts.
Material and methods: In the Danish Blood Donor Study, 14,440 participants from Central Denmark Region had platelet and leukocyte differential counts available and completed a questionnaire regarding AR, AC, and asthma. Of these participants, 8485 were tested for IgE to inhalation allergens.
Results: The prevalence of AR, AC, asthma, and IgE sensitization was 19%, 15%, 9%, and 29%, respectively. AR, AC, asthma, wheeze, and IgE sensitization was associated with increased blood eosinophil concentration even in IgE sensitized participants who did not report any allergy or asthma. The strongest associations were observed for participants with current disease. We found no differences in eosinophil concentration between months without symptoms and months with symptoms of AR and asthma.
Conclusion: AR, AC, asthma, wheezing, and IgE sensitization to inhalation allergens are associated with increased eosinophil concentration. This may reflect a persistent inflammation even in periods without symptomatic disease.

1. Introduction

Allergic rhinitis (AR), allergic conjunctivitis (AC), and asthma are characterized by activation of the immune system and by a type 2 immune response in allergic disease. In sensitized individuals, exposure to inhaled antigens induces immediate immunoglobulin E (IgE)-mediated degranulation of mast cells and basophils causing edema and airway occlusion [1]. The local inflammation induces systemic inflammatory signals and further activation of peripheral blood leukocytes [1–2]. Eosinophils, neutrophils, and basophils circulate in the blood, but in response to allergen exposure, they infiltrate the mucosa. Here, they accelerate the inflammatory cascade in the late-phase of the allergic response [3–4]. Platelets also express IgE receptors and play a role in the pathogenesis by escalating recruitment of leukocytes into tissues, including the lungs [5–7]. Chronic allergen exposure leads to inflammatory increased platelet count [6]. Asthma is characterized by chronic airway inflammation, whereas minimal persistent inflammation at conjunctival and nasal levels is detected in asymptomatic, sensitized

Abbreviations: AR, Allergic rhinitis; AC, Allergic conjunctivitis; ARC, Both allergic rhinitis and allergic conjunctivitis; sIgE, Allergen-specific immunoglobulin E; DBDS, The Danish Blood Donor Study; BMI, Body Mass Index; EDTA, Ethylenediaminetetraacetic acid; NLR, Neutrophil-lymphocyte ratio; PLR, Platelet–lymphocyte ratio.

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individuals continuously exposed to the natural allergens [8]. Allergen exposure increases basophil and neutrophil activity and causes an immediate decrease in blood basophil concentration [9–10]. The blood neutrophil concentration increases but returns to baseline in the late phase [1–3]. Allergen exposure also affects circulating lymphocytes, though changes in blood lymphocyte count are not always observed [3]. Peripheral blood eosinophilia is prominent in chronic asthma and allergic diseases [11–12].

The aim of this study was to explore associations between AR, AC, wheezing, asthma, and allergen-specific IgE (sIgE) sensitization to inhalant allergens and blood platelet and leukocyte differential counts in a Danish cohort of blood donors. In this cohort, the prevalence of AR, AC, asthma, and sIgE sensitization is high (19%, 15%, 9%, and 30%, respectively) [13]. To be accepted as blood donors, however, individuals must be in good overall health and asymptomatic on the date of blood donation. This enables us to examine the persistent impact of AR, AC, asthma, and sIgE sensitization on platelet and leukocyte differential counts in periods without symptomatic disease.

Based on current literature we hypothesized that: 1) participants with asthma, wheezing, AR, AC, and sIgE sensitization would have an increased eosinophil concentration, 2) asthmatic participants would have a higher platelet concentration, 3) the highest platelet and leukocyte concentrations would be found in participants with symptoms of asthma, AR, and AC within the last year, and 4) leukocyte concentrations measured in the same months as reported symptoms would be higher than cell concentrations measured in months with no reported symptoms.

2. Material and methods

2.1. Study population

The participants were unremunerated, voluntary, blood donors from Central Denmark Region, age 18–67 years, who consented to participate in the Danish Blood Donor Study (DBDS): a nationwide population-based study and biobank established in 2010 [14–16]. Participants were included from May 2015 to May 2018. Blood donors with AR, AC, and asthma are permitted to donate blood if they are asymptomatic at the time of donation. Individuals with suspected or confirmed allergy to medication, latex, food, or insect bites are not accepted as blood donors in Denmark.

2.2. Questionnaire and phenotype groups

DBDS participants completed an electronic questionnaire when donating blood [17]. The questionnaire included questions on allergy and asthma based on standardized questions from the European Community Respiratory Health Survey (ECRHS) questionnaire [18].

Allergic rhinitis (AR) was defined as an affirmative answer to the question: “Do you have any nasal allergies including hay fever?”, allergic conjunctivitis (AC) as an affirmative answer to the question: “Do you have any eye allergies including hay fever?”, and ARC as both AR and AC. Asthma was defined as an affirmative answer to the question: “Do you have, or have you ever had asthma?”. Wheezing was defined as an affirmative answer to the question: “Have you had wheezing or whistling in your chest at any time?”. Allergic and asthmatic participants reported months with nasal, eye and/or asthma symptoms, their age at symptom onset (in five- to ten-year age intervals), and time since last symptoms.

Current AR and AC were defined as symptoms of AR and/or AC within the last 12 months. Current asthma was defined when complying with at least one of the following: current use of asthma medicine, asthma attack within the last 12 months or awakened by an attack of coughing or shortness of breath within the last 12 months.

Participants with asthma, AR, and/or AC who did not meet the definition of current disease were classified as previous asthma, AR or AC. More details about the questionnaire and definitions are available in Supplementary Table S1.

The study population was divided into phenotype groups based on questionnaire data and previous findings within the DBDS cohort [13]. ’Only AR’ and ’only AC’ were merged to avoid low numbers and insufficient statistical power. This resulted in six distinct phenotype groups: I) asthma without AR and without AC; II) asthma with either AR or AC (but not both); III) asthma with ARC; IV) ARC without asthma; V) either AR or AC without asthma; and controls (no AR, no AC, and no asthma), see Venn diagram Fig. 1. Based on these groups, we were able to explore associations between multimorbidity (the coexistence of asthma, AR, and AC) as well as asthma as single disease and blood cell concentrations.

Body Mass Index (BMI) (kg/m^2) was calculated from self-reported weight and height. Smoking habit categories were ‘current smoker’, ‘former smoker’, and ‘never smoker’.

2.3. Register data

Data on age, sex, and current residential municipality were retrieved from Danish registers. All Danish citizens have a unique 10-digit personal identification number (civil registration number) [19], which is used to identify individuals in various data registries. Current residential municipality was classified as either small (less than 100,000 citizens) or large (at least 100,000 citizens).

2.4. Laboratory data

Ethylenediaminetetraacetic acid (EDTA)-stabilized blood samples were taken immediately before each blood donation, as a part of the daily routine in the blood centers in Central Denmark Region. Blood samples from inclusion date were transported to the Department of Clinical Immunology, Aarhus University Hospital, for centrifugation and further analyses. One sample was measured immediately for e.g. platelet and leukocyte differential count with a Sysmex® XT-1800i (Sysmex Corp., Kobe, Japan) according to the manufacturer’s instructions.

A gel-separated EDTA blood sample, drawn at the date of completion of the questionnaire from May 2015 until March 2017, was stored at −20 °C before testing. The samples were tested for IgE antibodies specific for the nine most common respiratory sensitizers by a commercially available enzyme-linked immunosorbent assay (ImmunoCAP Phadiatop; Phadia) at Thermo Fisher Scientific, Allerød, Denmark, with a threshold of 0.35 kU/L. The assay included the following allergens: common silver birch (t3), Timothy grass (g6), mugwort (w6), cat dander

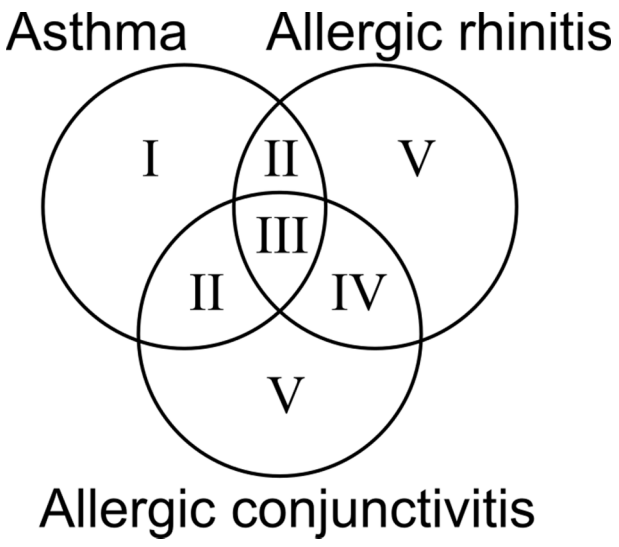

Fig. 1. Venn diagram of the phenotype groups.

(e1), dog dander (e5), horse dander (e3), house dust mites (*Dermatophagoides pteronyssinus* (d1) and *Dermatophagoides farinae* (d2), and molds (*Penicillium chrysogenum* (m1), *Cladosporium herbarum* (m2), *Aspergillus fumigatus* (m3), and *Alternaria alternata* (m6).

2.5. Statistics

Statistical analysis was performed in Stata/MP 16.1 and R 3.6.0. Results were reported as numbers, percentages with 95% confidence intervals (CIs), and medians with interquartile range (IQR). Groups were compared by the Kruskal–Wallis test, and the chi-squared test for categorical data.

We used a linear regression model to explore whether sIgE sensitization, AR, AC, wheezing, or asthma were associated with difference in cell concentrations relative to participants not having any of the conditions. All cell concentrations were log transformed to better approximate the model assumption of normally distributed residuals. The analyses were adjusted *a priori* for age (ten-year strata), smoking behavior (current, former, or never smoker) [20–21], BMI (continuous) [22–23], and current municipality of residence (small/large) [24] based on current literature. Results from the linear regression analyses were presented as percentage difference in cell concentrations with 95% CIs.

We used linear mixed model analysis to compare cell concentrations measured during months with symptoms with cell concentrations measured during months with no reported symptoms. We used all cell concentrations measured before and at the date of the questionnaire response. All samples taken before first reported symptoms or after last reported symptoms were considered to be from a symptomless month, regardless of which month they were from. A mixed model was used to counteract the effects of repeated measures. We did not adjust the mixed models since the variables we adjusted for were close to being constant for individual donors bringing about severe overfitting when combining with the random effect. A p-value of 0.05 or below was considered statistical significant. Due to multiple testing p-values were Bonferroni corrected. We tested for interactions. If sex modified the effects of the exposures on the outcome, analyses were stratified by sex; otherwise, overall pooled estimates of the effects adjusted by sex were calculated. Results were based on complete case analyses.

2.6. Ethics

The DBDS was approved by the Research Ethics Committee in the Central Demark Region (M-20090237). The biobank and research database were approved by the Danish Data Protection Agency (P-2019-99). Oral and written informed consent was obtained from all participants before enrolment into the study.

3. Results

A total of 14,440 blood donors from the Central Denmark Region had cell count measured at the date of completion of the questionnaire, see flowchart in Fig. 2. Of these participants, 3576 (25%) reported AR, AC and/or asthma and had at least one platelet and leukocyte differential count measurement in a month with symptoms as well as a measurement in a month with no symptoms. sIgE sensitization to inhalant allergens was measured in 8485 (59%) of the participants.

For demographics of the study population, stratified by the six allergy and asthma phenotype groups, see Table 1. The study population comprised 51% men and a median age of 39 years. The prevalence of AR, AC, asthma, and sIgE sensitization was 19%, 15%, 9%, and 29%, respectively.

3.1. Association between AR, AC, wheezing, asthma, and sIgE sensitization and blood cell concentrations

Except for asthma alone (phenotype group I), the adjusted linear regression analysis showed that the eosinophil concentration was higher in all allergy and asthma phenotype groups relative to controls, after Bonferroni correction, see Table 2. Participants with both allergy and asthma (phenotype groups II and III) had the highest eosinophil concentration (significantly higher than allergy alone (phenotype groups IV and V)).

We observed a higher basophil concentration in participants with both asthma and ARC (phenotype group III) relative to controls.

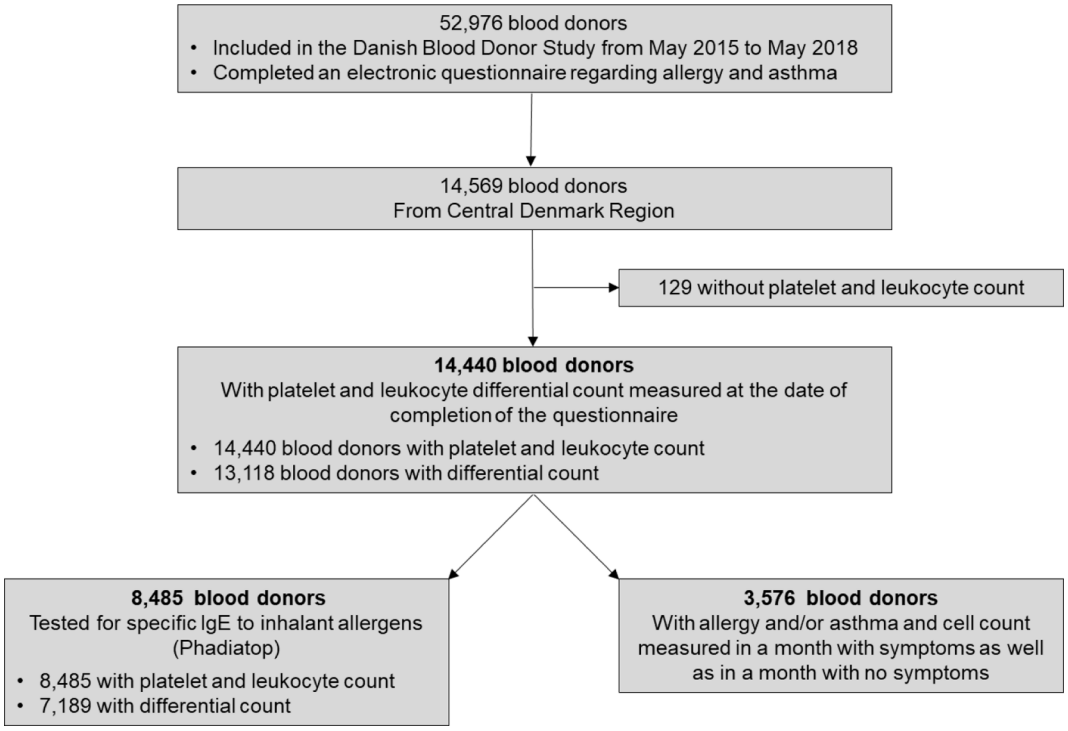

Fig. 2. Flowchart.

Table 1
Demographic of the study population stratified by allergy and asthma phenotype groups.

			Controls	Asthma (I) (no AR, no AC)	Asthma, and AR or AC (II)	Asthma and ARC (III)	ARC (IV) (no asthma)	AR or AC (IV) (no asthma)	Comparison
N (%)			10,724 (74.3)	618 (4.28)	168 (1.16)	433 (3.00)	1522 (10.5)	802 (5.55)	
		N	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	
Females		7013	49.3 (48.4–50.3)	48.7 (44.8–52.6)	50.0 (42.5–57.5)	46.4 (41.8–51.1)	42.6 (40.1–45.1)	48.8 (45.3–52.2)	**
Current smokers		1698	12.2 (11.6–12.8)	13.7 (11.2–16.7)	11.4 (7.41–17.1)	12.1 (9.32–15.5)	8.89 (7.55–10.4)	11.7 (9.62–14.1)	*
Former smokers		3056	21.5 (20.7–22.3)	20.9 (17.8–24.3)	22.2 (16.5–29.0)	17.4 (14.1–21.3)	20.3 (18.3–22.4)	24.2 (21.4–27.4)	NS
Large municipality		6684	45.8 (44.9–46.8)	51.2 (47.2–55.2)	47.6 (39.9–55.5)	51.5 (46.7–56.3)	48.6 (46.1–51.2)	44.5 (41.0–48.0)	NS
Wheeze		1812	5.57 (5.14–6.02)	64.0 (60.1–67.8)	79.0 (72.1–84.9)	80.1 (76.0–83.8)	15.4 (13.7–17.4)	11.1 (9.02–13.5)	**
Age strata (years)									
18–24		2302	15.5 (14.8–16.2)	28.0 (24.6–31.7)	15.5 (10.8–21.7)	22.2 (18.5–26.3)	15.1 (13.4–17.0)	13.6 (11.4–16.1)	**
25–34		3678	25.3 (24.4–26.1)	35.4 (31.8–39.3)	35.7 (28.9–43.2)	28.6 (24.6–33.1)	24.3 (22.2–26.5)	22.4 (19.7–25.5)	
35–44		3124	21.1 (20.3–21.9)	16.7 (13.9–19.8)	26.2 (20.1–33.3)	25.4 (21.5–29.7)	26.8 (24.6–29.1)	22.4 (19.7–25.5)	
45–54		3113	21.9 (21.1–22.7)	12.3 (9.94–15.1)	11.3 (7.36–17.0)	16.9 (13.6–20.7)	22.5 (20.4–24.6)	25.3 (22.4–28.4)	
55–67		2226	16.3 (15.6–17.0)	7.61 (5.77–9.97)	11.3 (7.36–17.0)	6.93 (4.90–9.72)	11.3 (9.81–13.0)	16.2 (13.8–18.9)	
	Sex	N	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	
Age		14,440	40 (28–51)	30 (25–42)	35 (27–44)	34 (26–44)	39 (28–49)	42 (30–51)	**
BMI (kg/m^2)		14,415	25 (23–28)	25 (23–28)	25 (23–28)	25 (23–28)	25 (23–27)	25 (23–28)	NS
Eosinophils (×10^6/L)	Female	6329	130 (80.0–190)	140 (80.0–230)	180 (120–310)	160 (120–280)	150 (100–220)	140 (90.0–220)	**
	Male	6770	140 (90.0–210)	160 (90.0–230)	170 (120–290)	190 (130–280)	150 (100–230)	160 (100–250)	**
Basophils (×10^6/L)	Female	6313	30.0 (20.0–40.0)	30.0 (20.0–40.0)	30.0 (20.0–40.0)	30.0 (20.0–40.0)	30.0 (20.0–40.0)	30.0 (20.0–40.0)	NS
	Male	6747	30.0 (20.0–40.0)	30.0 (20.0–40.0)	30.0 (20.0–40.0)	30.0 (20.0–40.0)	30.0 (20.0–40.0)	30.0 (20.0–40.0)	NS
Neutrophils (×10^9/L)	Female	6340	3.70 (2.93–4.59)	3.97 (3.09–4.94)	3.79 (3.29–4.87)	3.84 (2.93–4.79)	3.60 (2.78–4.54)	3.67 (2.94–4.47)	*
	Male	6777	3.18 (2.60–3.90)	3.19 (2.53–3.94)	2.98 (2.42–3.93)	3.06 (2.36–3.78)	3.05 (2.48–3.76)	3.15 (2.58–3.89)	NS
Lymphocytes (×10^9/L)	Female	6341	2.09 (1.73–2.49)	2.18 (1.87–2.57)	2.05 (1.64–2.45)	2.12 (1.78–2.62)	2.04 (1.70–2.44)	2.01 (1.70–2.43)	NS
	Male	6777	1.88 (1.56–2.24)	1.85 (1.54–2.17)	1.78 (1.57–2.13)	1.91 (1.54–2.24)	1.81 (1.48–2.18)	1.85 (1.55–2.19)	NS
Platelets (×10^9/L)	Female	7013	261 (225–298)	264 (224–309)	259 (228–298)	259 (222–295)	259 (220–299)	261 (227–297)	NS
	Male	7427	233 (202–268)	239 (209–273)	241 (218–273)	241 (207–279)	231 (201–267)	236 (208–271)	*
IgE sensitization									
N (%)			6307 (74.3)	358 (4.22)	101 (1.19)	236 (2.78)	879 (10.4)	494 (5.82)	
		N	% (CI)	% (CI)	% (CI)	% (CI)	% (CI)	% (CI)	
Sensitized		2470	15.5 (14.7–16.5)	32.4 (27.8–37.4)	73.3 (63.9–80.9)	91.1 (86.8–94.1)	86.6 (84.2–88.7)	60.5 (56.2–64.7)	**

Results are presented as numbers (N), percentages with corresponding 95% confidence intervals (CI), and medians with interquartile ranges (IQR). Cell concentrations corresponding to the date of the questionnaire response. ARC: both AR and AC; Sensitized: allergen-specific immunoglobulin E (Phadiatop) ≥ 0.35 kU/L.
Groups were compared by Kruskall-Wallis test, and chi-squared test for categorical data. P-values were Bonferroni corrected, *$p < 0.05$; **$p < 0.001$; NS: non-significant. In total 176 (1.2%) could not be categorized due to missing data in self-reported allergic rhinitis (AR), allergic conjunctivitis (AC), or asthma.

Furthermore, neutrophil and lymphocyte concentrations were lower in participants with ARC without asthma (phenotype group IV) relative to controls. The lymphocyte concentration was also lower in participants with asthma and either AR or AC (group II). However, after Bonferroni correction none of these differences were statistical significant, see Table 2. As expected, the analyses showed independent effects of age, sex, BMI, current residential municipality, and current smoking.

Current asthma and allergy, regardless of single diseases and multimorbidity (phenotype groups I–V), were associated with higher eosinophil concentrations. For male participants current asthma (phenotype groups I–III) was also associated with higher platelet concentration, see Supplementary Table S2.

Compared with non-sensitization, sIgE sensitization was associated with higher eosinophil concentration and lower neutrophil concentration, see Table 3. For the sIgE sensitized individuals, wheezing was also associated with increased eosinophil concentration, see Supplementary Table S3a and Table S3b.

To increase the size of each phenotype group, the allergic participants were merged (phenotype groups II + III and IV + V), and the study population was then divided into four distinct phenotype groups before stratifying each of the groups in sIgE sensitized and non-sensitized, see Supplementary Table S4. In controls and in participants with both

Table 2
Association between allergy and asthma phenotypes and cell count measurements.

	Phenotype groups	N	Percentage difference (95% CI) Crude	Adjusted
	Eosinophils			
	Controls	9700	REF	REF
I	Asthma (no AR, no AC)	571	6.70 (0.96–12.8)	8.72 (2.90–14.9)
II	Asthma, and either AR or AC	157	**39.8 (26.1–55.0)**	**40.7 (27.1–55.9)**
III	Asthma and ARC	414	**37.3 (28.8–46.5)**	**38.9 (30.3–48.1)**
IV	ARC (no asthma)	1393	**15.6 (11.4–19.9)**	**16.4 (12.3–20.8)**
V	Either AR or AC (no asthma)	723	**16.3 (10.7–22.2)**	**16.5 (10.9–22.4)**
	Basophils			
	Controls	9974	REF	REF
I	Asthma (no AR, no AC)	572	4.13 (-0.65–9.14)	5.71 (0.83–10.8)
II	Asthma, and either AR or AC	157	9.11 (-0.06–19.1)	10.2 (0.91–20.3)
III	Asthma and ARC	411	7.86 (2.09–14.0)	8.94 (3.12–15.1)
IV	ARC (no asthma)	1386	−0.64 (-3.70–2.53)	0.25 (-2.85–3.45)
V	Either AR or AC (no asthma)	719	4.58 (0.26–9.09)	4.37 (0.05–8.87)
	Neutrophils			
	Controls	9711	REF	REF
I	Asthma (no AR, no AC)	575	3.16 (0.28–6.11)	2.29 (-0.46–5.11)
II	Asthma, and either AR or AC	157	4.48 (-0.92–10.2)	3.69 (-1.43–9.08)
III	Asthma and ARC	414	−0.86 (-4.08–2.47)	−1.17 (-4.25–2.00)
IV	ARC (no asthma)	1396	**−4.04 (-5.83; −2.21)**	−2.70 (-4.45; −0.93)
V	Either AR or AC (no asthma)	723	−1.26 (-3.73–1.28)	−1.29 (-3.66–1.14)
	Lymphocytes			
	Controls	9712	REF	REF
I	Asthma (no AR, no AC)	575	1.38 (-0.99–3.81)	−1.33 (-3.55–0.94)
II	Asthma, and either AR or AC	157	−3.38 (-7.57–1.00)	−4.83 (-8.78; −0.72)
III	Asthma and ARC	414	1.15 (-1.61–3.99)	0.41 (-2.21–3.10)
IV	ARC (no asthma)	1396	**−3.24 (-4.75; −1.70)**	−2.18 (-3.65; −0.69)
V	Either AR or AC (no asthma)	723	−1.47 (-3.54–0.65)	−0.96 (-2.95–1.08)
	Platelets			
	Controls	10,722	REF	REF
I	Asthma (no AR, no AC)	617	1.82 (0.00–3.69)	0.79 (-0.95–2.56)
II	Asthma, and either AR or AC	168	2.44 (-0.99–5.98)	2.15 (-1.11–5.53)
III	Asthma and ARC	433	1.33 (-0.82–3.53)	1.23 (-0.82–3.33)
IV	ARC (no asthma)	1522	−1.54 (-2.71; −0.35)	−0.60 (-1.74–0.55)
V	Either AR or AC (no asthma)	802	1.15 (-0.45–2.79)	1.16 (-0.39–2.72)

Linear regression analyses with log transformed cell concentrations. Results are presented as percentage difference with corresponding 95% confidence intervals (CI). Crude: no adjustment. Adjusted: adjusted for sex, current residential municipality (small/large), BMI (continuous), smoking habit (former, current, or never smoker), and age (ten-year strata). Statistically significant results ($p < 0.05$), after Bonferroni correction, are marked in bold. AR: allergic rhinitis. AR: allergic conjunctivitis. ARC: both AR and AC.

asthma and allergy (phenotype groups II and III) the eosinophil concentration was higher in sIgE sensitized relative to non-sensitized, after Bonferroni correction.

3.2. Comparison of platelet and leukocyte counts in months with symptoms and in months with no symptoms

We found no difference in the neutrophil concentration during months with and without symptoms, see Table 4. The eosinophil concentration was slightly higher during months with symptoms of AC than during month with no AC symptoms. No differences in eosinophil concentration were found for symptoms of asthma and AR. Basophil and platelet concentrations were lower during months with symptoms of asthma, AR, and AC than during months without symptoms. However, the differences were small.

Table 3
Association between IgE sensitization and platelet and leukocyte concentrations.

	N	Median (IQR)	Percentage difference (95% CI) Crude	Adjusted
Eosinophils ($\times 10^6$/L)				
Non-sensitized	5051	130 (90.0–200)	REF	REF
Sensitized	2131	160 (110–240)	**22.1 (18.2–26.1)**	**23.0 (19.1–27.0)**
Basophils ($\times 10^6$/L)				
Non-sensitized	5031	30.0 (20.0–40.0)	REF	REF
Sensitized	2117	30.0 (20.0–40.0)	0.49 (-2.29–3.35)	2.46 (-0.40–5.40)
Neutrophils ($\times 10^9$/L)				
Non-sensitized	5057	3.45 (2.75–4.31)	REF	REF
Sensitized	2131	3.28 (2.64–4.17)	**−3.89 (-5.52; −2.24)**	**−2.44 (-4.04; −0.83)**
Lymphocytes ($\times 10^9$/L)				
Non-sensitized	5058	1.97 (1.64–2.36)	REF	REF
Sensitized	2131	1.94 (1.63–2.31)	−1.15 (-2.53–0.25)	−0.51 (-1.86–0.85)
Platelets ($\times 10^9$/L)				
Non-sensitized	6015	246 (212–283)	REF	REF
Sensitized	2470	241 (209–281)	−0.88 (-1.90–0.16)	0.23 (-0.77–1.24)

Results are presented as numbers (N), medians with interquartile ranges (IQR), and the linear regression analyses, with log transformed cell concentrations, as percentage difference with corresponding 95% confidence intervals (CI). Crude: no adjustment. Adjusted: adjusted for sex, current residential municipality (small/large), BMI (continuous), smoking habit (former, current, or never smoker), and age (ten-year strata). Statistically significant results ($p < 0.05$), after Bonferroni correction, are marked in bold. Sensitized: allergen-specific immunoglobulin E (Phadiatop) $\geq$ 0.35 kU/l. Non-sensitized: allergen-specific immunoglobulin E (Phadiatop) $<$ 0.35 kU/l.

3.3. Missing data

Missing data in at least one of the following variables: asthma, AR, AC, smoking, and BMI, were present in 2.4% (N = 349) of the study population. No one had missing data in all five variables. A total of 176 (1.2%) participants could not be categorized into the phenotype groups due to missing data in AR, AC, or asthma. All participants had complete data on age, sex, and current residential municipality. Participants with missing leukocyte differential counts were evenly distributed across the allergy and asthma phenotypes. Missing leukocyte differential counts were due to technical problems in the daily routine of the blood center, and not related to specific periods of time.

4. Discussion

In this large study of healthy individuals, blood eosinophil

Table 4
Platelet and leukocyte concentrations in and out of symptom season among participants with asthma, allergic rhinitis, and allergic conjunctivitis.

Cell type	Symptom season	Blood donors, N #	Cell counts, N ‡	Median (IQR)	Coef. (95% CI)	p-values
Asthma						
Eosinophils	Out of season	1226	7349	160 (100–250)	156 (150–161)	NS
	In season	199	578	180 (100–270)	156 (148–164)	
Basophils	Out of season	1225	7352	30.0 (20.0–50.0)	30.1 (29.3–30.9)	**
	In season	199	577	30.0 (20.0–40.0)	26.6 (25.4–27.8)	
Platelets	Out of season	1268	10,697	251 (218–289)	251 (248–254)	**
	In season	416	1955	253 (214–290)	246 (243–249)	
Neutrophils	Out of season	1231	7474	3.48 (2.78–4.40)	3.54 (3.49–3.60)	NS
	In season	219	634	3.49 (2.74–4.40)	3.54 (3.44–3.64)	
Allergic rhinitis						
Eosinophils	Out of season	2767	12,946	150 (100–240)	155 (151–158)	NS
	In season	1787	4818	160 (100–250)	158 (154–162)	
Basophils	Out of season	2765	12,932	30.0 (20.0–50.0)	30.3 (29.8–30.9)	**
	In season	1786	4801	30.0 (20.0–40.0)	26.1 (25.6–26.7)	
Platelets	Out of season	2794	13,912	247 (214–284)	247 (246–249)	**
	In season	2727	15,601	243 (211–278)	242 (240–244)	
Neutrophils	Out of season	2769	12,992	3.35 (2.66–4.18)	3.38 (3.34–3.42)	NS
	In season	1860	5159	3.37 (2.70–4.21)	3.41 (3.37–3.45)	
Allergic conjunctivitis						
Eosinophils	Out of season	2174	10,328	150 (100–230)	152 (149–156)	*
	In season	1374	3659	160 (100–250)	157 (153–162)	
Basophils	Out of season	2173	10,304	30.0 (20.0–40.0)	29.9 (29.3–30.5)	**
	In season	1373	3644	30.0 (20.0–40.0)	25.8 (25.2–26.4)	
Platelets	Out of season	2198	10,997	247 (214–283)	247 (245–249)	**
	In season	2155	2181	242 (209–278)	242 (240–244)	
Neutrophils	Out of season	2176	10,358	3.35 (2.67–4.19)	3.38 (3.34–3.42)	NS
	In season	1429	3916	3.35 (2.67–4.18)	3.40 (3.35–3.45)	

Results are presented as numbers (N) and medians with interquartile ranges (IQR). The linear mixed model analysis with log transformed cell concentrations. Results are presented as exponential function coefficients (Coef.) and corresponding 95% confidence intervals (CI). P-values were Bonferroni corrected, *$p < 0.05$; **$p < 0.001$; NS: non-significant.
The number of blood donors with at least one cell count measured in and out of symptom season, respectively.
‡ The number of cell count measurements in and out of symptom season, respectively.

concentration was higher in all allergy and asthma phenotype groups relative to controls, with the highest concentrations in the allergy and asthma multimorbidity groups (phenotype groups II and III). sIgE sensitization was also associated with higher eosinophil concentrations, even in participants who did not report any AR, AC, or asthma. Furthermore, sIgE sensitization was associated with a decreased neutrophil concentration. Overall, no differences between the phenotype groups were seen for basophil, lymphocyte, neutrophil, and platelet concentrations. However, relative to controls, a tendency towards decreased lymphocyte and neutrophil concentrations was seen in participants with allergic multimorbidity, while the opposite was observed for basophil concentration in participants with allergy and asthma multimorbidity. In male participants, current asthma was associated with a small increased platelet concentration.

Overall, we found no convincing differences between blood eosinophil and neutrophil concentrations measured during symptomatic months compared with asymptomatic months. Basophil and platelet concentrations were slightly lower during symptomatic months compared with asymptomatic months, however, the differences were relatively small and may not be clinical relevant.

Because blood donors are generally healthier than the background adult population [25], associations between AR, AC, asthma, and sIgE sensitization and blood cell concentrations might be underestimated in this study. Individuals with severe asthma are not included in our study population. Allergic multimorbidity is strongly associated with severe asthma [26–29] and they have overlapping cellular pathways [30]. The prevalence of asthma with AR is lower in the DBDS cohort compared with another study using the ERCHS cohort (47% *vs* 64.5%) [13,31].

The current study, however, indicates that associations persist even in currently asymptomatic participants. Furthermore, as expected, we found higher eosinophil concentrations in participants with allergy, asthma, and sIgE sensitization relative to controls, which is consistent with other studies that link the activities of eosinophils to airway inflammation and remodeling [11]. Similarly to other studies, wheezing was associated with increased eosinophil concentration [32]. In a subanalysis of asthma and wheezing, we found that wheezing alone as well as having both asthma and wheezing were associated with increased eosinophil concentration in sensitized participants. The association between asthma alone and increased eosinophil concentration was weaker, possibly due to the absence of participants with severe asthma in our study population.

The persistent peripheral blood eosinophilia may be caused by chronic inflammation even in asymptomatic participants with allergy and asthma. This might also be the reason for the increased basophil concentration in participants with both ARC and asthma, as well as the small increase in platelet concentration in male participants with current asthma, which are in accordance with other studies [33,34].

Even though allergen exposure can impact circulating lymphocyte subtypes, changes in blood lymphocyte concentration are not always observed [3].

sIgE sensitization was associated with a small decrease in neutrophil concentration. The decrease could be due to the chronic type 2 immune milieu, in which interleukin (IL)-4 receptor signaling leads to inhibited neutrophil effector function and decreased blood neutrophils [35]. Conversely, IgE also stimulates IL-8 production by neutrophils [36] causing them to migrate toward the site of inflammation.

We speculate that cell concentrations could be affected by anti-allergic or anti-asthmatic medication, but blood donors with AR, AC, and asthma were only allowed to donate blood if they were asymptomatic on inhalation treatment. Also, a previous study on local treatment with glucocorticoids observed only limited effects on peripheral blood immune cells [37].

Neutrophil-lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR) are suggested as parameters of systemic inflammation and disease severity in asthma and AR [38–40]. We used a linear regression model to explore whether NLR and PLR were associated with AR, AC, asthma, or

sIgE sensitization in this population. However, since this was not our main analysis results are presented in Supplementary Material.

The strengths of this study are the large, otherwise healthy, well-characterized study population, the high participation rate [15], the low number of unanswered questions (approximately 2%), and a prevalence of AR, AC, and asthma in DBDS that is only slightly lower than in the general adult Danish population [13,41].

Limitations comprise the absence of individuals with severe asthma, the use of self-reported outcomes, and the cross-sectional design that only allows exploration of associations and not causal effects. We were not able to explore associations between single allergic diseases and blood cell concentrations, inasmuch as participants with AR and AC were merged for most analyses to avoid low numbers and insufficient power in these groups.

In this study, we examined associations with absolute cell counts; however, the DBDS cohort facilitates further explorations of the impact of allergy, asthma, and sIgE sensitization on peripheral blood leukocytes, such as cytokine secretion and functional analyses of neutrophils, basophils, and platelets.

5. Conclusion

AR, AC, asthma, wheezing, and sIgE sensitization to inhalation allergens are associated with increased eosinophil concentration. This may reflect a persistent inflammation even in periods without symptomatic disease.

Author contribution

SM, CE, and TS designed the study. SM drafted the manuscript. JKB and SM performed the statistical analyses with contribution from KR, TS, and CE. All authors interpreted and discussed the results. CE, BKM, KMD, MSP, KAK, OBP, SRO, LJH, LWT, and SM made substantial contribution to acquisition of data. All authors contributed to revising the manuscript critically and approved the final draft for publication.

CRediT authorship contribution statement

Susan Mikkelsen: Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Visualization, Writing - original draft, Writing - review & editing. **Jens Kjærgaard Boldsen:** Formal analysis, Investigation, Methodology, Resources, Visualization, Writing - review & editing. **Bjarne Kuno Møller:** Conceptualization, Project administration, Resources, Supervision, Writing - review & editing. **Khoa Manh Dinh:** Investigation, Project administration, Resources, Writing - review & editing. **Klaus Rostgaard:** Formal analysis, Methodology, Resources, Writing - review & editing. **Mikkel Steen Petersen:** Software, Resources, Writing - review & editing. **Kathrine Agergård Kaspersen:** Resources, Writing - review & editing. **Ole Birger Pedersen:** Funding acquisition, Project administration, Resources, Writing - review & editing. **Lise Wegner Thørner:** Funding acquisition, Project administration, Resources, Writing - review & editing. **Linda Jenny Handgaard:** Project administration, Resources, Writing - review & editing. **Sisse Rye Ostrowski:** Project administration, Resources, Writing - review & editing. **Torben Sigsgaard:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing - review & editing. **Christian Erikstrup:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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References

[1] S. Lavinskiene, J. Jeroch, K. Malakauskas, K. Malakaskas, I. Bajoriuniene, J. Jackute, R. Sakalauskas, Peripheral blood neutrophil activity during Dermatophagoides pteronyssinus-induced late-phase airway inflammation in patients with allergic rhinitis and asthma, Inflammation. 35 (2012) 1600–1609, https://doi.org/10.1007/s10753-012-9475-0.

[2] A. Togias, Systemic effects of local allergic disease, J. Allergy Clin. Immunol. 113 (2004) S8–14, https://doi.org/10.1016/j.jaci.2003.09.051.

[3] G. Jordakieva, E. Jensen-Jarolim, The impact of allergen exposure and specific immunotherapy on circulating blood cells in allergic rhinitis, World Allergy Organ. J. 11 (2018), https://doi.org/10.1186/s40413-018-0197-0.

[4] K. Hosoki, I. Boldogh, S. Sur, Neutrophil recruitment by allergens contribute to allergic sensitization and allergic inflammation, Curr. Opin. Allergy Clin. Immunol. 16 (2016) 45–50, https://doi.org/10.1097/ACI.0000000000000231.

[5] M. Idzko, S. Pitchford, C. Page, Role of platelets in allergic airway inflammation, J. Allergy Clin. Immunol. 135 (2015) 1416–1423, https://doi.org/10.1016/j.jaci.2015.04.028.

[6] M. Dogru, A. Aktas, S. Ozturkmen, Mean platelet volume increased in children with asthma, Pediatr. Allergy Immunol, Off. Publ. Eur. Soc. Pediatr. Allergy Immunol. 26 (2015) 823–826, https://doi.org/10.1111/pai.12381.

[7] M. Joseph, A.S. Gounni, J.P. Kusnierz, H. Vorng, M. Sarfati, J.P. Kinet, A.B. Tonnel, A. Capron, M. Capron, Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors, Eur. J. Immunol. 27 (1997) 2212–2218, https://doi.org/10.1002/eji.1830270914.

[8] G. Ciprandi, S. Buscaglia, G. Pesce, C. Pronzato, V. Ricca, S. Parmiani, M. Bagnasco, G.W. Canonica, Minimal persistent inflammation is present at mucosal level in patients with asymptomatic rhinitis and mite allergy, J. Allergy Clin. Immunol. 96 (1995) 971–979, https://doi.org/10.1016/s0091-6749(95)70235-0.

[9] H.J. Hoffmann, T. Skjold, M. Raithel, K. Adolf, O. Hilberg, R. Dahl, Response of respiratory flour allergics in an ingested flour challenge may involve plasmacytoid dendritic cells, CD25+ and CD152+ T cells, Int. Arch. Allergy Immunol. 140 (2006) 252–260, https://doi.org/10.1159/000093251.

[10] S. Mikkelsen, B.M. Bibby, M.K.B. Dolberg, R. Dahl, H.J. Hoffmann, Basophil sensitivity through CD63 or CD203c is a functional measure for specific immunotherapy, Clin. Mol. Allergy CMA. 8 (2010) 2, https://doi.org/10.1186/1476-7961-8-2.

[11] P. Venge, The eosinophil and airway remodelling in asthma, Clin. Respir. J. 4 (Suppl 1) (2010) 15–19, https://doi.org/10.1111/j.1752-699X.2010.00192.x.

[12] H.F. Rosenberg, S. Phipps, P.S. Foster, Eosinophil trafficking in allergy and asthma, J. Allergy Clin. Immunol. 119 (2007) 1303–1310; quiz 1311–1312. https://doi.org/10.1016/j.jaci.2007.03.048.

[13] S. Mikkelsen, K.M. Dinh, J.K. Boldsen, et al., Combinations of self-reported rhinitis, conjunctivitis, and asthma predicts IgE sensitization in more than 25,000 Danes, Clin. Transl. Allergy (2021) 1–23, https://doi.org/10.1002/clt2.12013.
[14] The Danish Blood Donor Study, Accessed April, 2021, 2021. www.dbds.dk.
[15] O.B. Pedersen, C. Erikstrup, S.R. Kotzé, E. Sørensen, M.S. Petersen, K. Grau, H. Ullum, The Danish Blood Donor Study: a large, prospective cohort and biobank for medical research, Vox Sang. 102 (2012) 271, https://doi.org/10.1111/j.1423-0410.2011.01553.x.
[16] K.S. Burgdorf, O.B.V. Pedersen, E. Sørensen, L.W. Thørner, H. Hjalgrim, C. Erikstrup, H. Ullum, Extending the gift of donation: blood donor public health studies, ISBT Sci. Ser. 10 (2015) 225–230, https://doi.org/10.1111/voxs.12119.
[17] K.S. Burgdorf, N. Felsted, S. Mikkelsen, M.H. Nielsen, L.W. Thørner, O.B. Pedersen, E. Sørensen, K.R. Nielsen, M.T. Bruun, T. Werge, C. Erikstrup, T. Hansen, H. Ullum, Digital questionnaire platform in the Danish Blood Donor Study, Comput. Methods Programs Biomed. 135 (2016) 101–104, https://doi.org/10.1016/j.cmpb.2016.07.023.
[18] European Community Respiratory Health Survey, Accessed November 2019, 2019. http://www.ecrhs.org/.
[19] C.B. Pedersen, H. Gøtzsche, J.O. Møller, P.B. Mortensen, The Danish Civil Registration System. A cohort of eight million persons, Dan. Med. Bull. 53 (2006) 441–449.
[20] K.M. Pedersen, Y. Çolak, C. Ellervik, H.C. Hasselbalch, S.E. Bojesen, B. G. Nordestgaard, Smoking and Increased White and Red Blood Cells, Arterioscler. Thromb. Vasc. Biol. 39 (2019) 965–977, https://doi.org/10.1161/ATVBAHA.118.312338.
[21] T. Higuchi, F. Omata, K. Tsuchihashi, K. Higashioka, R. Koyamada, S. Okada, Current cigarette smoking is a reversible cause of elevated white blood cell count: Cross-sectional and longitudinal studies, Prev. Med. Rep. 4 (2016) 417–422, https://doi.org/10.1016/j.pmedr.2016.08.009.
[22] Y. Herishanu, O. Rogowski, A. Polliack, R. Marilus, Leukocytosis in obese individuals: possible link in patients with unexplained persistent neutrophilia, Eur. J. Haematol. 76 (2006) 516–520, https://doi.org/10.1111/j.1600-0609.2006.00658.x.
[23] Y. Furuncuoğlu, S. Tulgar, A.N. Dogan, S. Cakar, Y.K. Tulgar, B. Cakiroglu, How obesity affects the neutrophil/lymphocyte and platelet/lymphocyte ratio, systemic immune-inflammatory index and platelet indices: a retrospective study, Eur. Rev. Med. Pharmacol. Sci. 20 (2016) 1300–1306.
[24] Z. Zhang, T.-C. Chan, C. Guo, L. Chang, C. Lin, Y.C. Chuang, W.K. Jiang, K.F. Ho, T. Tam, K.S. Woo, A.K.H. Lau, X.Q. Lao, Long-term exposure to ambient particulate matter (PM2.5) is associated with platelet counts in adults, Environ. Pollut. 240 (2018) 432–439, https://doi.org/10.1016/j.envpol.2018.04.123.
[25] F. Atsma, I. Veldhuizen, A. Verbeek, W. de Kort, F. de Vegt, Healthy donor effect: its magnitude in health research among blood donors, Transfusion (Paris). 51 (2011) 1820–1828, https://doi.org/10.1111/j.1537-2995.2010.03055.x.
[26] S. Toppila-Salmi, S. Chanoine, J. Karjalainen, J. Pekkanen, J. Bousquet, V. Siroux, Risk of adult-onset asthma increases with the number of allergic multimorbidities and decreases with age, Allergy. 74 (2019) 2406–2416, https://doi.org/10.1111/all.13971.
[27] S. Chanoine, M. Sanchez, I. Pin, S. Temam, N. Le Moual, A. Fournier, C. Pison, J. Bousquet, P. Bedouch, M.-C. Boutron-Ruault, R. Varraso, V. Siroux, Multimorbidity medications and poor asthma prognosis, Eur. Respir. J. 51 (2018), https://doi.org/10.1183/13993003.02114-2017.
[28] C. Porsbjerg, A. Menzies-Gow, Co-morbidities in severe asthma: Clinical impact and management, Respirol. Carlton Vic. 22 (2017) 651–661, https://doi.org/10.1111/resp.13026.
[29] R.D.C.C.M. Brito, G.A.P. da Silva, M.E.F.A. Motta, M.C.A. Brito, The association of rhinoconjunctivitis and asthma symptoms in adolescents, Rev. Port. Pneumol. 15 (2009) 613–628.
[30] D. Aguilar, M. Pinart, G.H. Koppelman, Y. Saeys, M.C. Nawijn, D.S. Postma, M. Akdis, C. Auffray, S. Ballereau, M. Benet, J. García-Aymerich, J.R. González, S. Guerra, T. Keil, M. Kogevinas, B. Lambrecht, N. Lemonnier, E. Melen, J. Sunyer, R. Valenta, S. Valverde, M. Wickman, J. Bousquet, B. Oliva, J.M. Antó, Computational analysis of multimorbidity between asthma, eczema and rhinitis, PloS One. 12 (2017), e0179125, https://doi.org/10.1371/journal.pone.0179125.
[31] A. Boudier, S. Chanoine, S. Accordini, J.M. Anto, X. Basagaña, J. Bousquet, P. Demoly, J. Garcia-Aymerich, F. Gormand, J. Heinrich, C. Janson, N. Künzli, R. Matran, C. Pison, C. Raherison, J. Sunyer, R. Varraso, D. Jarvis, B. Leynaert, I. Pin, V. Siroux, Data-driven adult asthma phenotypes based on clinical characteristics are associated with asthma outcomes twenty years later, Allergy. 74 (2019) 953–963, https://doi.org/10.1111/all.13697.
[32] A. Malinovschi, J.A. Fonseca, T. Jacinto, K. Alving, C. Janson, Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subjects, J. Allergy Clin. Immunol. 132 (821–827) (2013) e1–5, https://doi.org/10.1016/j.jaci.2013.06.007.
[33] L. Chamitava, L. Cazzoletti, M. Ferrari, V. Garcia-Larsen, A. Jalil, P. Degan, A. G. Fois, E. Zinellu, S.S. Fois, A.M. Fratta Pasini, M. Nicolis, M. Olivieri, A. Corsico, R. Bono, P. Pirina, M.E. Zanolin, Biomarkers of Oxidative Stress and Inflammation in Chronic Airway Diseases, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21124339.
[34] S.A. Shah, C.P. Page, S.C. Pitchford, Platelet-Eosinophil Interactions As a Potential Therapeutic Target in Allergic Inflammation and Asthma, Front. Med. 4 (2017) 129, https://doi.org/10.3389/fmed.2017.00129.
[35] C. Egholm, L.E.M. Heeb, D. Impellizzieri, O. Boyman, The Regulatory Effects of Interleukin-4 Receptor Signaling on Neutrophils in Type 2 Immune Responses, Front. Immunol. 10 (2019), https://doi.org/10.3389/fimmu.2019.02507.
[36] J. Monteseirín, P. Chacón, A. Vega, R.E. Bekay, M. Alvarez, G. Alba, M. Conde, J. Jiménez, J.A. Asturias, A. Martínez, J. Conde, E. Pintado, F.J. Bedoya, F. Sobrino, Human neutrophils synthesize IL-8 in an IgE-mediated activation, J. Leukoc. Biol. 76 (2004) 692–700, https://doi.org/10.1189/jlb.0903441.
[37] H. Zhang, L.O. Cardell, J. Bjorkander, M. Benson, H. Wang, Comprehensive profiling of peripheral immune cells and subsets in patients with intermittent allergic rhinitis compared to healthy controls and after treatment with glucocorticoids, Inflammation. 36 (2013) 821–829, https://doi.org/10.1007/s10753-013-9608-0.
[38] M. Dogru, R.G. Yesiltepe Mutlu, The evaluation of neutrophil–lymphocyte ratio in children with asthma, Allergol. Immunopathol. (Madr.). 44 (2016) 292–296, https://doi.org/10.1016/j.aller.2015.09.005.
[39] M. Dogru, M.F. Evcimik, A.A. Cirik, Is neutrophil–lymphocyte ratio associated with the severity of allergic rhinitis in children? Eur. Arch. Otorhinolaryngol. 273 (2016) 3175–3178, https://doi.org/10.1007/s00405-015-3819-y.
[40] A.E. Göker, E. Ekincioglu, M.H. Alagöz, R. Hummatov, M.E. Arkan, A. Baskadem Yilmazer, A. Güngör Doğuşlu, Y. Uyar, The association of allergic rhinitis severity with neutrophil–lymphocyte and platelet–lymphocyte ratio in adults, Eur. Arch. Otorhinolaryngol. 276 (2019) 3383–3388, https://doi.org/10.1007/s00405-019-05640-0.
[41] L.R. Skadhauge, J. Baelum, H.C. Siersted, D.L. Sherson, S. Dahl, G.F. Thomsen, O. Omland, E. Taudorf, T.I. Sigsgaard, The occurrence of asthma among young adults. A population-based study in five west Danish counties, Ugeskr. Laeger. 167 (2005) 648–651.