Local and systemic immune markers of persistent airway inflammation induced by house dust mite allergens

NUOLATINIO KVĖPAVIMO TAKŲ UŽDEGIMO, SUKELTO NAMŲ DULKIŲ ERKIŲ ALERGENO, VIETINIAI IR SISTEMINIAI IMUNINIAI ŽYMENYS

LAURA TAMAŠAUSKIENĖ
LSMU MA MF Imunologijos ir alergologijos klinika

Summary. Laura Tamašauskienė successfully defended a doctoral dissertation "Local and Systemic Immune Markers of Persistent Airway Inflammation Induced by House Dust Mite Allergens" at the open session of the Medical Research Council of the Lithuanian University of Health Sciences on August 30, 2022. The Dissertation has been prepared at the Department of Immunology and Allergology of Faculty of Medicine of Lithuanian University of Health Sciences during the period of the 2018–2022 year. The article presents the main results of the dissertation.
Scientific Supervisor:
Prof. Dr. Brigita Šitkauskienė (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).
The Defense Council:
prof. Dr. Elona Juozaitytė, chairperson (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);
prof. Dr. Juozas Kupčinskas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);
prof. Dr. Andrius Macas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);
assoc. Prof. Dr. Vincas Urbonas (Vilnius University, Medical and Health Sciences, Medicine – M 001);
dr. Nicholas Brodszki (Lund University, Medical and Health Sciences, Medicine – M 001).

Santrauka. 2022 m. rugpjūčio 30 d. Lietuvos sveikatos mokslų universiteto viešajame Lietuvos sveikatos mokslų universiteto Medicinos mokslo krypties tarybos posėdyje Laura Tamašauskienė sėkmingai apgynė daktaro disertaciją „ NUOLATINIO KVĖPAVIMO TAKŲ UŽDEGIMO, SUKELTO NAMŲ DULKIŲ ERKIŲ ALERGENO, VIETINIAI IR SISTEMINIAI IMUNINIAI ŽYMENYS ". Disertacija rengta 2018–2022 metais Lietuvos sveikatos mokslų universiteto Medicinos fakulteto Imunologijos ir alergologijos klinikoje. Šiame straipsnyje pateikiami pagrindiniai disertacijos rezultatai.
Mokslinė vadovė:
prof. dr. Brigita Šitkauskienė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).
Disertacijos gynimo taryba:
prof. dr. Elona Juozaitytė, pirmininkė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
prof. dr. Juozas Kupčinskas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
prof. dr. Andrius Macas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
doc. dr. Vincas Urbonas (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
dr. Nicholas Brodszki (Lundo Universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

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INTRODUCTION

Allergic airway diseases – allergic rhinitis (AR) and allergic asthma (AA) – cause a great burden worldwide (1–5). The prevalence of these diseases increases despite modern methods of treatment and better access to them (1, 5, 6). AR and AA, which usually affect children and young adults, are highly associated with poorer quality of life, disturbed social life, daily activity and increased leave days at school or work (7, 8). There are a lot of factors that are suspected to be important for the risk of development and severity of allergic diseases such as genetic predisposition, allergen exposure, dietary changes, air pollution, microbiota changes, etc. (9). There is some evidence that low vitamin D level is associated with increased risk and more severe forms of allergic diseases (10, 11). It is suggested that vitamin

D may participate in immune regulation by increased production of anti-inflammatory cytokines and may reduce the level of pro-inflammatory cytokines (Fig. 1) (12–15). Active vitamin D form activates the vitamin D receptor (VDR), regulating the expression of genes involved in calcium metabolism, proliferation, differentiation, apoptosis and immunity. The VDR is a member of the nuclear receptor superfamily located in macrophages, dendritic cells (DCs), activated T cells and other types of cells in about 30 different tissue (16). VDR genetic variants have also been studied as a potential factor for autoimmune diseases and allergic diseases since they may influence VDR activity (17, 18).

Staphylococcus aureus is a frequent gram-positive opportunistic pathogen that colonizes the anterior nares of humans (19). There is evidence that nasal *Staphylococcus aureus* carriage is more frequent in patients with persistent AR caused by HDM or asthma than in healthy individuals (20–22). It is thought that *Staphylococcus aureus*-derived proteases may induce allergic airway inflammation (23). However, some experimental studies revealed that nasal commensal *Staphylococcus aureus* from subjects with AR mediated anti-allergic effects (24).

It is known that in allergic airway diseases, the main role belongs to T lymphocyte helper (Th) 2 producing interleukin (IL) 4, IL-5 and IL-13 (type 2 immunity) (25–27). Type 1 immunity consists of Th1 and its produced cytokines such as interferon-γ (IFN-γ) and type 3 immunity consists of Th17 cells producing IL-17 and IL-22 (25). It is thought that type 1 and type 3 immunity is important in the development of autoimmune disorders or chronic non-allergic airway inflammation (25, 27). However, there is increasing evidence that not only Th2, but other subtypes of T lymphocytes, such as recently discovered Th17 and Th22, may be involved in the pathogenesis of allergic airway diseases (Fig. 2).

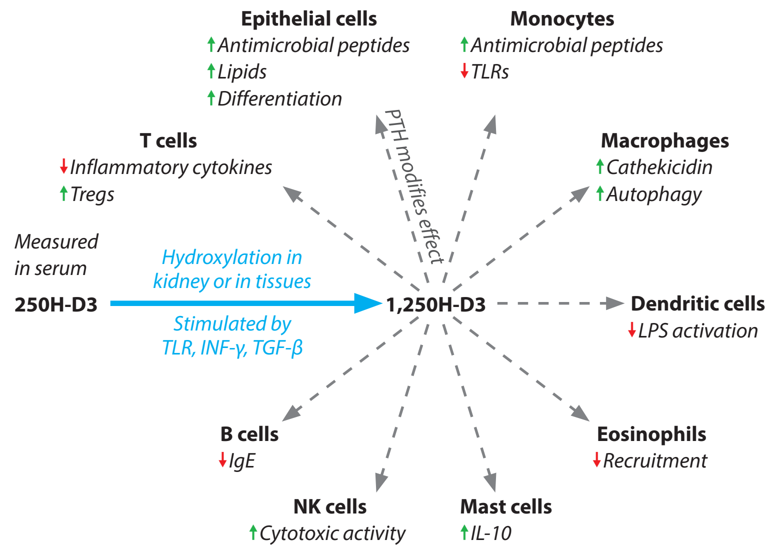

Figure 1. The main role of vitamin D in allergic airway diseases

Adapted according to Agrawal et al. Curr Allergy Asthma Rep. 2010.

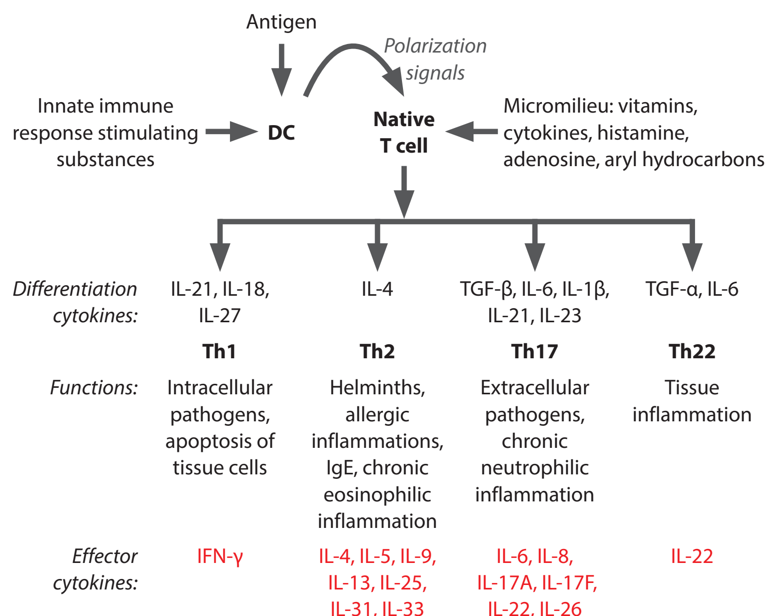

Figure 2. Overview of Th1, Th2, Th17 and Th22

Adapted according to Akdis et al. Allergy Clin Immunol. 2012;129(6):1431–8.

AR and AA are heterogenous diseases and have a variety of symptoms, of which severity and response to treatment differs. Moreover, AR and AA are very often diagnosed together, which is why the hypothesis of united airway disease was proposed (28, 29). It is known that during allergic airway diseases both local and systemic inflammation develops. There is evidence that local and systemic inflammatory markers may not always reflect each other which is why not always analysis of serum is enough (30, 31). In addition, most of the research with novel biomarkers is conducted in severe forms of allergic diseases nowadays, and studies with mild and moderate forms are lacking. Milder forms of diseases not always are identified, diagnosed and treated properly and this can lead to the development of severe forms associated with poor quality of life and delayed treatment interventions.

Despite growing knowledge of the pathogenesis of AR and AA and the discovery of novel biomarkers and even modern individualized therapies, the diagnosis and treatment of these diseases are challenging. For better management of allergic airway diseases, early diagnosis

and evaluation of the type and level of inflammation are particularly important (5, 32). This can ensure a personalized approach, treatment options and prognosis for the current patient especially when common medications are not effective enough. That is why this study analyses systemic and local cytokine profiles in persistent allergic airway inflammation in patients with mild or moderate AR and/or AA. The aim was to investigate local and systemic immune markers of persistent airway inflammation induced by house dust mite allergen.

METHODS

Study population

Patients (18–60 yrs.) with persistent AR diagnosed according to the guidelines of Allergic Rhinitis and its Impact on Asthma (ARIA) (5) and having symptoms for at least 2 years, patients with AR and AA diagnosed according to the guidelines of Global Initiative for Asthma (GINA) (1) and having symptoms for at least 2 years and healthy individuals without AR and AA or other diseases that can negatively impact the results, were involved into the study.

Information about the duration of disease symptoms and data about weight, height, and BMI were obtained. Only subjects who stopped using vitamin D supplements at least three months before the study were involved. The study was performed in the Department of Immunology and Allergology of the Hospital of Lithuanian University of Health Sciences during the period of 2018–2022. The study was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-28). Subjects gave their written informed consent.

A summary of the study protocol is presented in Fig. 3.

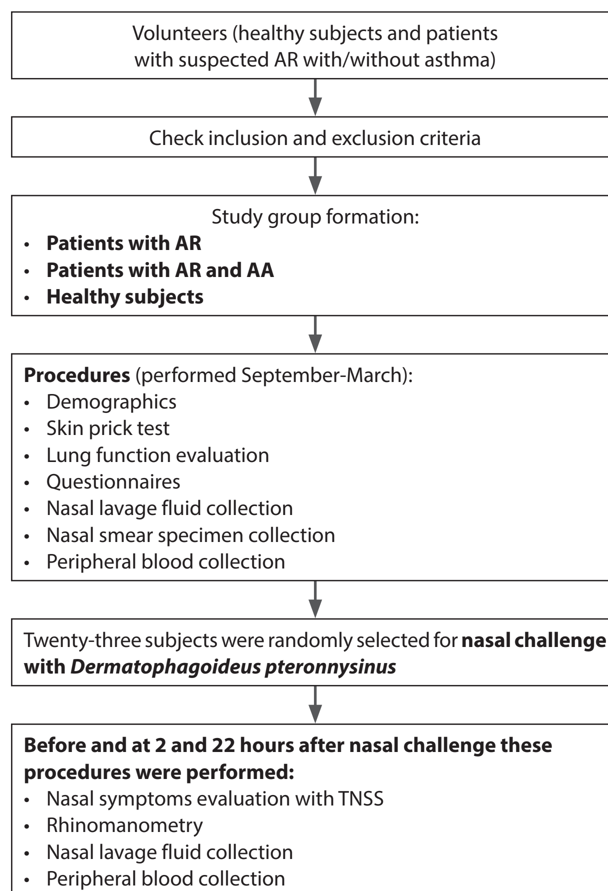

Figure 3. Summary of study protocol

Assessment of symptoms severity and quality of life

All subjects were asked to complete Total Nasal Symptom Score (TNSS) and Pittsburgh Sleep Quality Index (PSQI) (33, 34). Patients with AR completed the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) (35). Patients with AA additionally were asked to complete the Asthma Control Test (ACT) (36) and the Asthma Quality of Life Questionnaire (AQLQ) (37). The permission to use validated questionnaires (Lithuanian versions) was received.

Evaluation of allergic sensitization

Allergic sensitization was determined by a skin prick test or allergen-specific IgE test. Skin prick test was performed according to the standard protocol with standard inhalant allergens (Diater, Spain). Measurement of allergen-specific IgE was performed using standard immunoblot analysis according to the manufacturer's instructions (Euroimmun, Germany). Total IgE in serum was measured using enzyme immunoassays (AIA-FAC IgEII Tosoh Bioscience, Japan).

Lung function measurement

Lung function for all subjects was evaluated using the pneumotachometric spirometer (Smart PFT, Gmbh, Germany). All subjects were asked to avoid the use of short-acting β2-agonists for at least 8 h before the testing. Forced Expiratory Volume in 1 s (FEV_1), Forced Vital Capacity (FVC) and FEV_1/FVC ratio were measured. The best value of the three measurements was selected.

The nasal challenge with *Dermatophagoideus pteronnysinus*

The nasal challenge was performed according to EAACI recommendations (38). *Dermatophagoideus pteronnysinus* allergen (Inmunotek, S.L., Spain) for provocation was prepared according to the manufacturer's recommendations. During the allergen application, the patient had to hold his breath to avoid inhaling the allergen into the lower airways. The applicator of the delivery device was inserted into the nasal vestibule and pointed upward and laterally toward the medial canthus of the eye to deposit allergen on the inferior and the middle turbinate mucosa when spraying the solution into the nose. After 2 and 22 hours, after the nasal challenge, peripheral blood and nasal lavage fluid were collected.

Peripheral blood collection and processing

Peripheral vein puncture was performed for all subjects. Blood for analysis was collected during the period of September – March. Blood samples were drawn into KEDTA tubes for investigation for a complete blood count. Blood eosinophilia was defined as a blood count >0.3×10^9/l (39). Blood samples were also drawn into serum tubes. Serum tubes were stored at room temperature for 30–60 minutes and centrifuged at 3500 rpm for 10 minutes. Serum was separated and frozen at –80°C for further analysis.

Nasal smear and nasal lavage fluid specimen collection and processing

Nasal smears of patients were obtained by gently swabbing the nasal inferior turbinate with a cotton-tipped swab. The sample was then placed on the surface of a glass microscope slide and stained with Giemsa stain for eosinophil detection. All specimens were examined by a qualified pathologist. Eosinophil inflammation was diagnosed when the eosinophil count was ≥10% (39).

A microbiological swab sample was taken from one nostril for confirmation of *Staphylococcus aureus* carriage.

Nasal lavage fluid was collected for all subjects using 5 ml isotone saline per nostril with a reclined neck (about 30° from the horizontal) and closed soft palate. After 30 seconds the subject flexed the neck draining lavage fluid into a sterile vessel. Nasal lavage fluid was frozen at –80°C for further analysis (cytokine measurement).

Laboratory evaluation of cytokines and vitamin D

Measurements of IL-10, IL-13, IL-17, IL-22, IL-33, and IFN-γ in serum and nasal lavage were performed by ELISA using commercial kits (Elabscience Biotechnology Inc., USA) with Euroimmun Analyzer I (Germany). Intra-assay precision coefficient of variability for IL-10 was 5.42%, for IL-13 – 5.58%, for IL-17 – 5.04%, for IL-22 – 5.51%, for IL-33 – 4.99% and for IFN-γ – 4.89%.

25(OH)D level was measured using a one-step delayed competitive enzyme immunoassay using commercial kits (ST AIA-PACK 25-OH Vitamin D) with an automated enzyme immunoassay analyzer (TOSOH AIA-2000). Vitamin D deficiency was defined as a 25(OH)D below 50 nmol/l, and vitamin D insufficiency as a 25(OH)D of 50–72.5 nmol/l (40).

Investigation of single nucleotide polymorphisms (SNPs) of the VDR gene

SNPs of the VDR gene were investigated for patients with AR and AA and healthy individuals. DNA from peripheral blood samples was extracted using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Ten SNPs (rs7975232, rs1544410, rs731236, rs3847987, rs2228570, rs4588, rs7041, rs4725, rs11168293, and rs3733359) on the 12q13.11 chromosomal region were analyzed in this study using TaqMan SNP Genotyping Assay probes.

Statistical methods

Statistical analysis was performed using the statistical program SPSS 27. The sample size was estimated according to data from the pilot study using sample size calculation formulas.

Non-parametric statistical methods were applied for non-parametric data analysis (Mann–Whitney U and Kruskal–Wallis H tests) and parametric statistical methods were applied for parametric data analysis (one-way ANOVA) for comparison of variables between studied groups. Chi-Square (χ^2) test was used to examine the differences between categorical variables.

A comparison of variables before and after nasal challenge with HDM allergen was performed using the Friedman test.

Methods of correlation (Spearman's coefficient) were used to find associations between variables.

Logistic and multinomial regression analysis was applied to find risk factors for the development of allergic airway diseases.

A P value of <0.05 was considered statistically significant.

RESULTS

Study population, demographic data and symptoms score and quality of life

Eighty-one subjects were involved in the study. Demographic characteristics are presented in table 1. The duration of nasal symptoms was slightly longer in patients with AR and AA compared with patients with AR only. FVC in percentage was significantly lower in all patients with allergic airway diseases than in healthy individuals (Table 1). The tendency of lower FEV_1 percentage in patients with allergic airway diseases than in healthy individuals was also observed (p=0.08).

TNSS score was significantly higher in patients with allergic airway diseases than in healthy individuals (Table 2). PSQI score was significantly higher in all patients with allergic airway diseases than in healthy individuals.

Peripheral blood cells, total IgE and Staphylococcus aureus, and eosinophil count in nasal smear

Eosinophil count in peripheral blood and serum IgE were significantly higher in patients with allergic airway diseases in comparison with healthy individuals

(table 3). Neutrophil count in percentage was significantly lower in patients with allergic airway diseases than in a control group. Eosinophils count in nasal smear was significantly higher in all patients with allergic airway diseases and in AR only patients than in healthy individuals and a tendency was observed that eosinophil counts in nasal smear was higher in patients with AR and AA than in healthy individuals. A tendency that positive *Staphylococcus aureus* in nasal smear was more frequent in patients with allergic airway diseases than in healthy individuals was observed (Table 3).

Cytokines level in serum and nasal lavage fluid

A tendency was observed that serum IL-22 level was higher in patients with allergic airway diseases than in healthy individuals (p=0.09). Serum IL-17 and IL-33 level tended to be higher in patients with AR and AA than in patients with AR only and healthy individuals (p=0.06 and p=0.08, respectively).

IL-10 concentration in serum was significantly higher in patients with AR only than in patients with AR and AA. Serum IL-13 level was higher in patients with AR than in patients with healthy individuals and patients with AR and AA. IFN-γ level in nasal lavage fluid was higher in patients with AR and AA than in patients with AR only and in healthy individuals.

All cytokine level in serum and nasal lavage fluid is illustrated in Fig. 4 and Fig. 5.

Relation between immune markers, symptom severity, and quality of life

Serum IL-17 positively correlated with total IgE in patients with allergic airway diseases and in patients with AR and AA (rs=0.74, p<0.01). IL-17 in nasal lavage fluid had a negative link with eosinophils in a nasal smear in patients with AR and AA (rs=-0.51, p<0.05). IL-22 in nasal lavage fluid had a negative correlation with

Table 1. Demographic characteristics and lung function of patients with allergic airway diseases and healthy individuals

	Patients with allergic airway diseases (N=63)	Patients with AR (N=42)	Patients with AR and AA (N=21)	Control group (n=18)
Male/ female, N	25/38	17/25	8/13	3/15
Age, years, mean±SEM	31.22±1.22	30.12±1.50	33.43±2.10	34.06±2.85
BMI, kg/m^2	25.24±0.78	24.92±1.03	25.95±1.06	24.53±0.81
Weight, kg	75.67±2.27	75.91±2.95	75.20±3.40	72.13±2.53
Duration of rhinitis symptoms, years, mean±SEM	11.96±1.30	10.71±1.60	14.46±2.27	N/A
Duration of asthma symptoms, years, mean±SEM,	9.13±2.48	N/A	11.33±2.80	N/A
FEV$_1$, l, mean±SEM	3.67±0.12	3.76±0.14	3.50±0.22	3.57±0.16
FVC, l, mean±SEM	4.19±0.14	4.34±0.17	3.84±0.22	4.12±0.18
FEV$_1$/FVC, %, mean±SEM	84.13±0.75	84.40±0.92	83.56±1.33	82.85±1.32
FEV$_1$, % of predicted, mean±SEM	99.12±1.04	98.58±1.12	100.25±2.27	106.00±3.30
FVC, % of predicted, mean±SEM	**96.90±1.08***	96.85±1.29	97.00±2.04	105.21±3.44

*p<0.05 compared with control group.

Table 2. Patients with allergic airway diseases and healthy individuals' severity of symptoms[1]

	Patients with allergic airway diseases (N=63)	Patients with AR (N=42)	Patients with AR and AA (N=21)	Control group (n=18)
TNSS	**4.25±0.30****	**4.55±0.36****	**3.67±0.50****	0.67±0.30
ACT	19.19±0.93	N/A	19.19±0.93	N/A
AQLQ	5.51±0.28	N/A	5.51±0.28	N/A
RQLQ	1.66±0.16	1.79±0.19	1.38±0.30	N/A
PSQI	**7.47±0.53***	7.35±0.57	7.75±1.17	5.69±0.52

[1]Data are presented as mean±SEM. **p<0.01 compared with control group. *p<0.05 compared with control group.

Table 3. Peripheral blood cells, serum total IgE level, and eosinophil count in a nasal smear in patients with allergic airway diseases and healthy individuals[1]

	Patients with Allergic Airway Diseases (N=63)	Patients with AR (N=42)	Patients with AR and AA (N=21)	Control group (n=18)
Eosinophils, $\times 10^9$/l	**0.25±0.02****	**0.22±0.02***	**0.31±0.05****	0.13±0.03
Eosinophils, %	**3.98±0.34****	**3.77±0.38****	**4.40±0.70****	2.13±0.42
Neutrophils, $\times 10^9$/l	3.45±0.15	3.31±0.18	3.72±0.26	3.88±0.24
Neutrophils, %	**55.65±0.93***	**55.43±1.29***	**56.11±1.10***	60.74±1.81
Total IgE, kU/l	**307.03±47.18****	**313.34±64.48****	**294.40±60.32****	40.47±11.91
Eosinophils in nasal smear, %	**12.57±2.89***	**11.43±3.21***	14.79±5.90	3.94±1.83
Positive *Staphylococcus aureus* in nasal smear, n (%)	16 (26.20)	8 (19.50)	8 (40.00)	3 (16.70)

[1]Data are presented as mean±SEM. *p<0.05 compared with control group. **p<0.01 compared with control group. #p<0.01 compared with AR.

blood eosinophil count in patients with AR and AA (rs=-0.51, p<0.05). IL-33 in serum positively correlated with total IgE in patients with allergic airway diseases. IL-33 in nasal lavage fluid positively correlated with blood eosinophils in patients with allergic airway diseases and in patients with AR and AA (rs=0.66, p<0.05). No significant correlation was found between cytokines and other inflammatory markers in patients with AR only.

IL-10 in nasal lavage fluid had a positive link with the duration of rhinitis symptoms in all patients with allergic airway diseases and in patients with AR only (rs=0.49, p<0.01). Serum IL-10, IL-22, and IFN-γ were positively related to lung function parameters in these patients. IL-17 and IL-33 in nasal lavage fluid were positively associated with the duration of asthma symptoms (rs=0.60, p<0.05 and rs=0.64, p<0.05, respectively). IFN-γ in nasal lavage fluid negatively correlated with TNSS and RQLQ in patients with allergic airway diseases and AR only (rs=-0.30, p<0.05). IL-13 in nasal lavage fluid had a positive correlation with PSQI component 2 (rs=0.33, p<0.05) and serum IL-13 had a positive link with PSQI component 6 (rs=0.33, p<0.05) in patients with allergic airway diseases. IL-13 in nasal lavage fluid positively correlated with TNSS domain assessing itching/ sneezing (rs=0.24, p<0.05) in patients with AR only. Serum IL-22 negatively correlated with PSQI score (rs=-0.52, p<0.05), whereas IL-22 in nasal lavage fluid positively correlated with PSQI score (rs=0.64, p<0.05) in patients with AR and AA.

Eosinophil in nasal smear and eosinophil in blood had a positive correlation (rs=0.35, p<0.01) in patients with allergic airway diseases. In these patients' serum, IL-22 negatively correlated with IL-22 in nasal lavage fluid (especially in patients with AA and AR: rs=-0.78, p<0.01), whereas serum IFN-γ positively correlated with IFN-γ in nasal lavage fluid (especially in patients with AA and AR: rs=0.67, p<0.01). Positive correlations between these cytokines in serum and nasal lavage fluid were found in patients with AR and AA only: IL-10 (rs=0.54, p<0.05) and IL-13 (rs=0.66, p<0.01).

Serum IL-17 is highly positively correlated with serum IL-33 as well as IL-17 and IL-33 in nasal lavage fluid. Other significant positive correlations were found between serum IL-22 and serum IL-10, IL-17, IL-33, and IFN-γ.

All correlations in patients with allergic airway diseases are presented in Table 4.

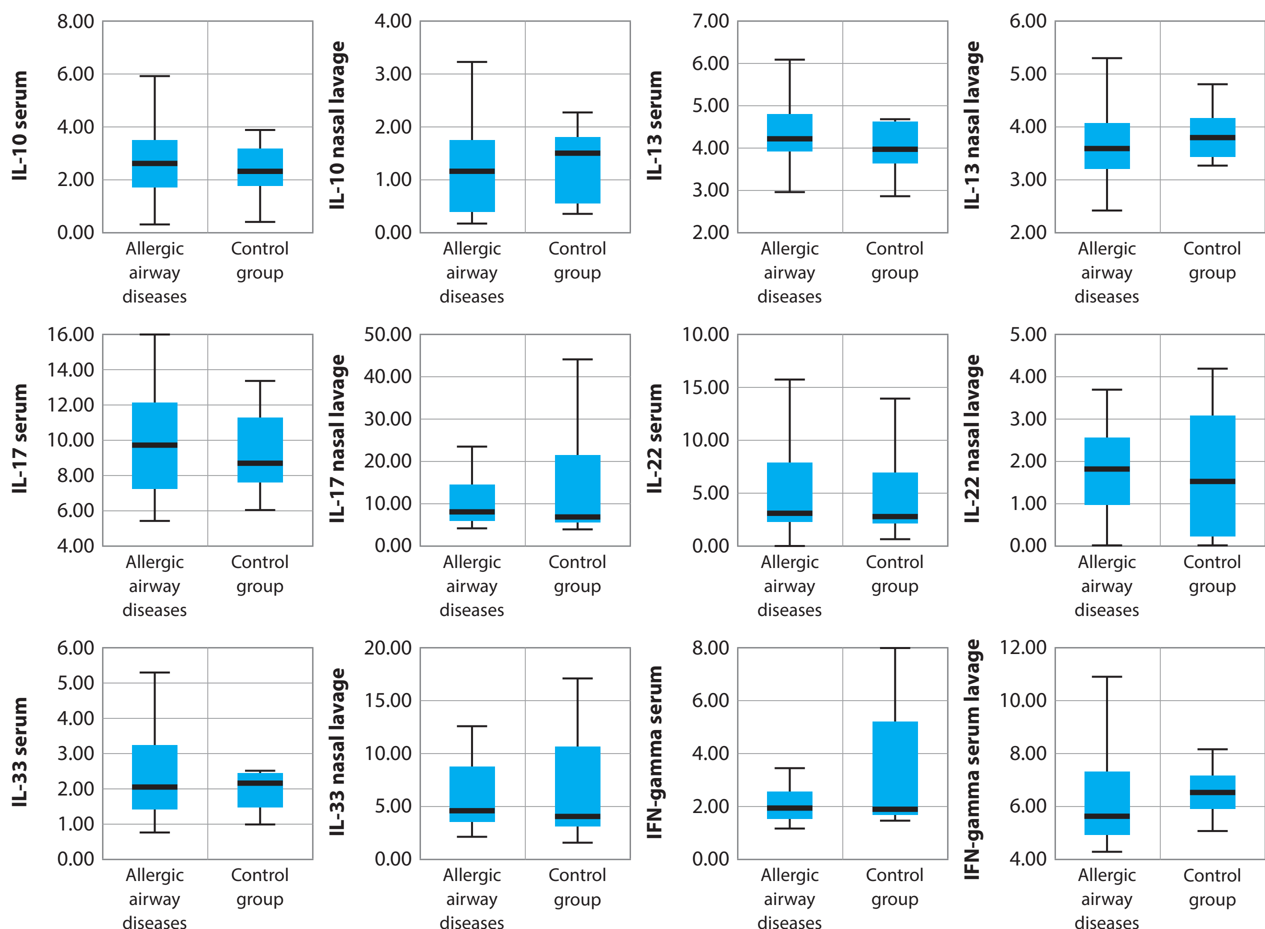

Figure 4. Cytokines level in serum and nasal lavage fluid in patients with allergic airway diseases and healthy individuals

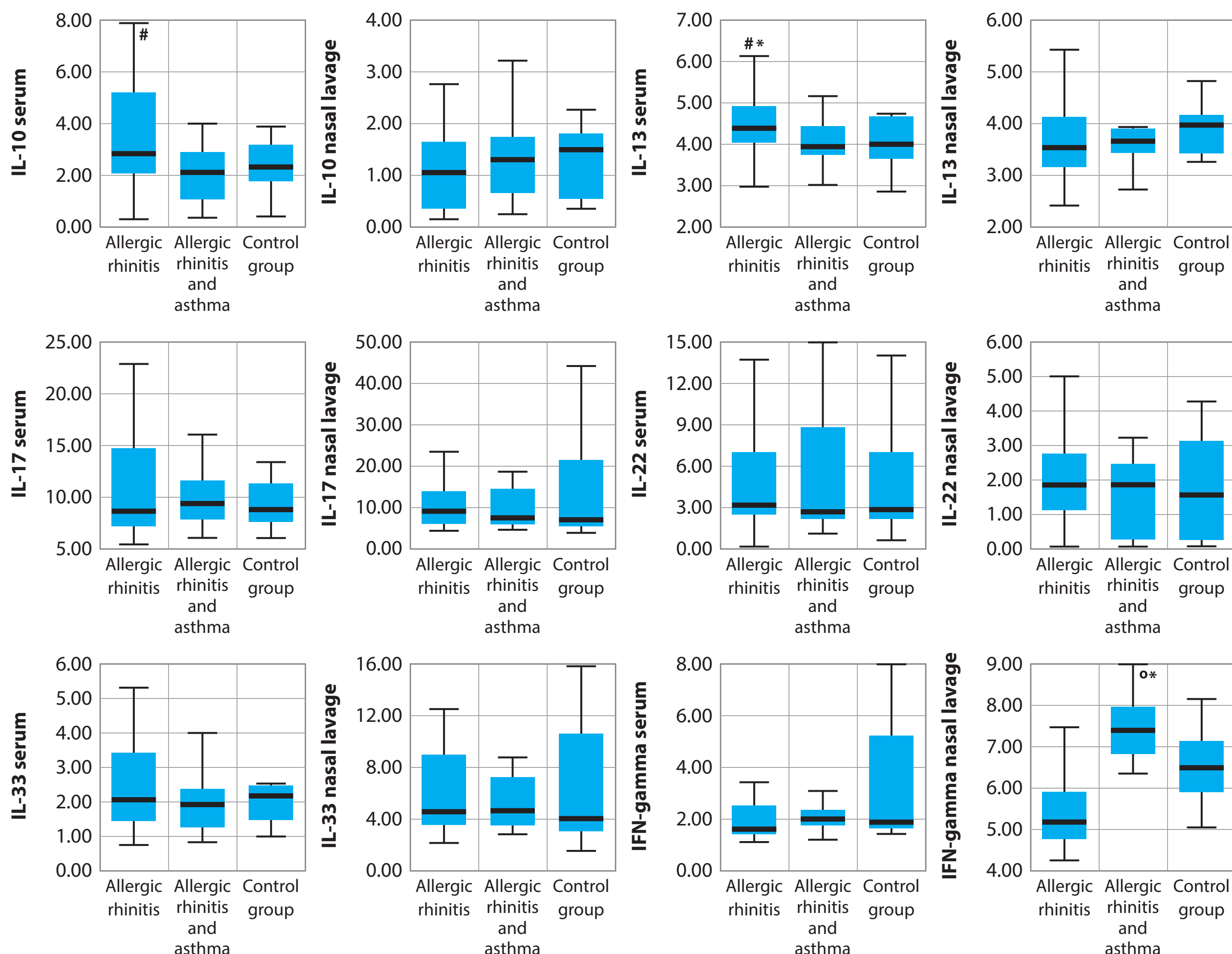

Figure 5. Cytokines level in serum and nasal lavage in patients with AR, AA, and AR and healthy individuals

*p<0.05 compared with control group. #p<0.05 compared with allergic rhinitis and asthma. °p<0.01 compared with allergic rhinitis.

Table 4. Correlation between local and systemic immune markers in patients with allergic airway diseases

	Serum IL-22	IL-22 in nasal lavage	Serum IL-13	IL-13 in nasal lavage	Serum IL-10	IL-10 in nasal lavage	Serum IL-17	IL-17 in nasal lavage	Serum IL-33	IL-33 in nasal lavage	Serum IFN-γ	IFN-γ in nasal lavage
Serum IL-22	–	**–0.35****	**0.35****	0.06	**0.49****	**0.28***	**0.50****	–0.05	**0.53****	0.07	**0.55****	0.19
IL-22 in nasal lavage	**–0.35****	–	–0.08	0.10	–0.08	–0.06	–0.01	0.16	–0.06	0.17	–0.24	–0.12
Serum IL-13	**0.35****	–0.08	–	0.17	**0.60****	0.12	0.21	0.13	**0.30***	0.19	0.16	0.03
IL-13 in nasal lavage	0.06	0.10	0.17	–	0.23	**0.27***	–0.01	**0.50****	0.15	**0.49****	0.18	**0.31***
Serum IL-10	**0.49****	–0.08	**0.60****	0.23	–	0.20	**0.30***	0.30	**0.42****	**0.34****	**0.34****	0.13
IL-10 in nasal lavage	**0.28***	–0.06	0.12	**0.27***	0.20	–	–0.04	0.05	–0.14	0.22	0.23	**0.37****
Serum IL-17	**0.50***	–0.01	0.21	–0.01	0.28*	–0.04	–	–0.01	**0.72****	–0.02	**0.50****	0.18
IL-17 in nasal lavage	–0.05	0.16	0.13	**0.45****	0.25	0.05	–0.01	–	0.12	**0.90****	0.06	0.16
Serum IL-33	**0.53****	–0.06	**0.30***	0.15	**0.42****	–0.14	**0.72****	0.12	–	0.09	**0.43****	0.15
IL-33 in nasal lavage	0.07	0.17	0.19	**0.49****	**0.34****	0.22	–0.02	**0.90****	0.09	–	0.12	**0.27***
Serum IFN-γ	**0.55****	–0.24	0.16	0.18	**0.34****	0.23	**0.50****	0.06	**0.43****	0.12	–	**0.37****
IFN-γ in nasal lavage	0.19	–0.12	0.03	**0.31***	0.13	**0.37****	0.18	0.16	0.15	0.27*	**0.37****	–

*p<0.05; **p<0.01.

Table 5. Risk for development of allergic airway diseases

	B	Sig.	Exp (B)	95% Confidence Interval for Exp(B) Lower bound	Upper bound
Risk for development of AR for healthy individuals					
Serum IFN-γ	–0.71	0.02	0.49	0.274	0.874
Age	–0.08	0.04	0.92	0.851	0.996
Risk for development of AA for patients with AR					
Serum IL-22	0.14	0.02	1.15	1.024	1.299
Serum IL-10	–0.27	0.01	0.07	0.009	0.504
IL-10 in nasal lavage	2.30	0.03	9.98	1.198	83.138
IL-33 in nasal lavage	0.57	0.05	1.77	1.011	3.091
Vitamin D	–0.09	0.03	0.91	0.842	0.990
Risk for development of AA for all subjects					
Vitamin D	–0.07	0.01	0.93	0.881	0.984
Serum IL-22	0.10	0.03	1.10	1.013	1.202
Serum IL-10	–1.64	0.01	0.19	0.06	0.62
IL-10 in nasal lavage	1.32	0.05	3.75	0.999	14.060
IL-33 in nasal lavage	0.44	0.01	1.56	1.108	2.189

#Only significant predictors are provided.

Risk factors for allergic airway diseases

Logistic regression analysis showed that an increase in serum IFN-γ and age decreased the risk of AR for healthy individuals (Table 5). The risk for the development of AA for patients with AR and for all subjects increased when levels of IL-22 in serum and IL-10 and IL-33 in nasal lavage fluid increased. This risk decreased when serum IL-10 and vitamin D levels were higher (Table 5).

Comparison of cytokine level in patients with allergic airway diseases according to Staphylococcus aureus colonization, vitamin D status, and eosinophil count

Levels of IL-13, IL-17, IL-33, and IFN-γ in nasal lavage fluid were higher in patients with allergic airway diseases with *Staphylococcus aureus* carriage than in patients without *Staphylococcus aureus* carriage (Table 6).

Table 6. Cytokines level in patients with allergic airway diseases according to the presence of Staphylococcus aureus in nasal smear[1]

	Positive *Staphylococcus aureus* in nasal smear (N=17)	Negative *Staphylococcus aureus* in nasal smear (N=46)
Serum IL-10, pg/ml	3.03 (2.96)	2.56 (1.45)
IL-10 in nasal lavage, pg/ml	1.22 (5.22)	1.07 (1.39)
Serum IL-13, pg/ml	4.20 (0.98)	4.40 (0.87)
IL-13 in nasal lavage, pg/ml	**3.84 (1.47)***	3.46 (0.87)
Serum IL-17, pg/ml	8.75 (4.76)	8.38 (5.22)
IL-17 in nasal lavage, pg/ml	**12.77 (13.11)****	6.88 (5.82)
Serum IL-22, pg/ml, pg/ml	2.92 (12.03)	3.82 (6.81)
IL-22 in nasal lavage, pg/ml	1.80 (1.98)	1.73 (2.07)
Serum IL-33, pg/ml, pg/ml	1.70 (2.55)	2.07 (1.88)
IL-33 in nasal lavage, pg/ml	**6.80 (8.51)***	4.17 (3.01)
Serum IFN-γ, pg/ml	2.24 (3.29)	1.69 (1.06)
IFN-γ in nasal lavage, pg/ml	**7.62 (4.57)****	5.39 (1.94)

[1]Data are presented as median (interquartile range). *p<0.05; **p<0.01.

Patients with blood eosinophil count $>0.3\times10^9$/l (n=13) had significantly higher IL-13 levels in nasal lavage fluid than patients with blood eosinophil count $<0.3\times10^9$/l (n=50) (median 3.99 (IR 1.16) vs. median 3.50 (IR 0.88), p <0.05).

A significantly higher level of IL-10 in nasal lavage fluid was observed in patients with vitamin D level ≥ 75 nmol/l (N=8) than in patients with vitamin D level < 75 nmol/l (N=55) (median 1.92 (IR 1.21) vs. median 0.87 (IR 1.32), p<0.05).

Cytokines level before and after nasal challenge with *Dermatophagoideus pteronnysinus* and the relation between cytokines and clinical parameters

Level of IL-13, IL-17, IL-22, and IL-33 was measured in serum and nasal lavage fluid before and at 2 and at 22 hours after nasal challenge with allergen. A tendency was observed that levels of IL-13, IL-17, IL-22, and IL-33 in serum and IL-17 and IL-33 levels in nasal lavage fluid before nasal challenge were higher in patients with allergic airway diseases than in healthy individuals (p<0.15) (Fig. 6).

Serum and nasal lavage fluid IL-22 levels significantly increased after nasal challenge in patients with allergic airway diseases (Fig. 6). Serum IL-22 increased more in patients with AR and AA at 2 and 22 hours after nasal challenge in comparison with baseline data (38.73 (35.87) vs. 19.37 (117.33) vs. 2.54 (9.87), p<0.05, respectively), but IL-22 level in nasal lavage fluid increased significantly only in patients with AR. Serum IL-33 tended to increase after nasal challenge in patients with allergic airway diseases whereas IL-33 in nasal lavage fluid decreased. IL-17 levels in serum and nasal lavage fluid significantly decreased after nasal challenge in this group of subjects. IL-13 level in serum tended to increase after 2 and 22 hours compared with baseline only in patients with AR and AA (32.56 (33.39) vs. 11.90 (112.02) vs. 3.88 (1.96), respectively). IL-13 level in nasal lavage fluid decreased at 22 hours after nasal challenge compared with baseline data in patients with allergic airway diseases.

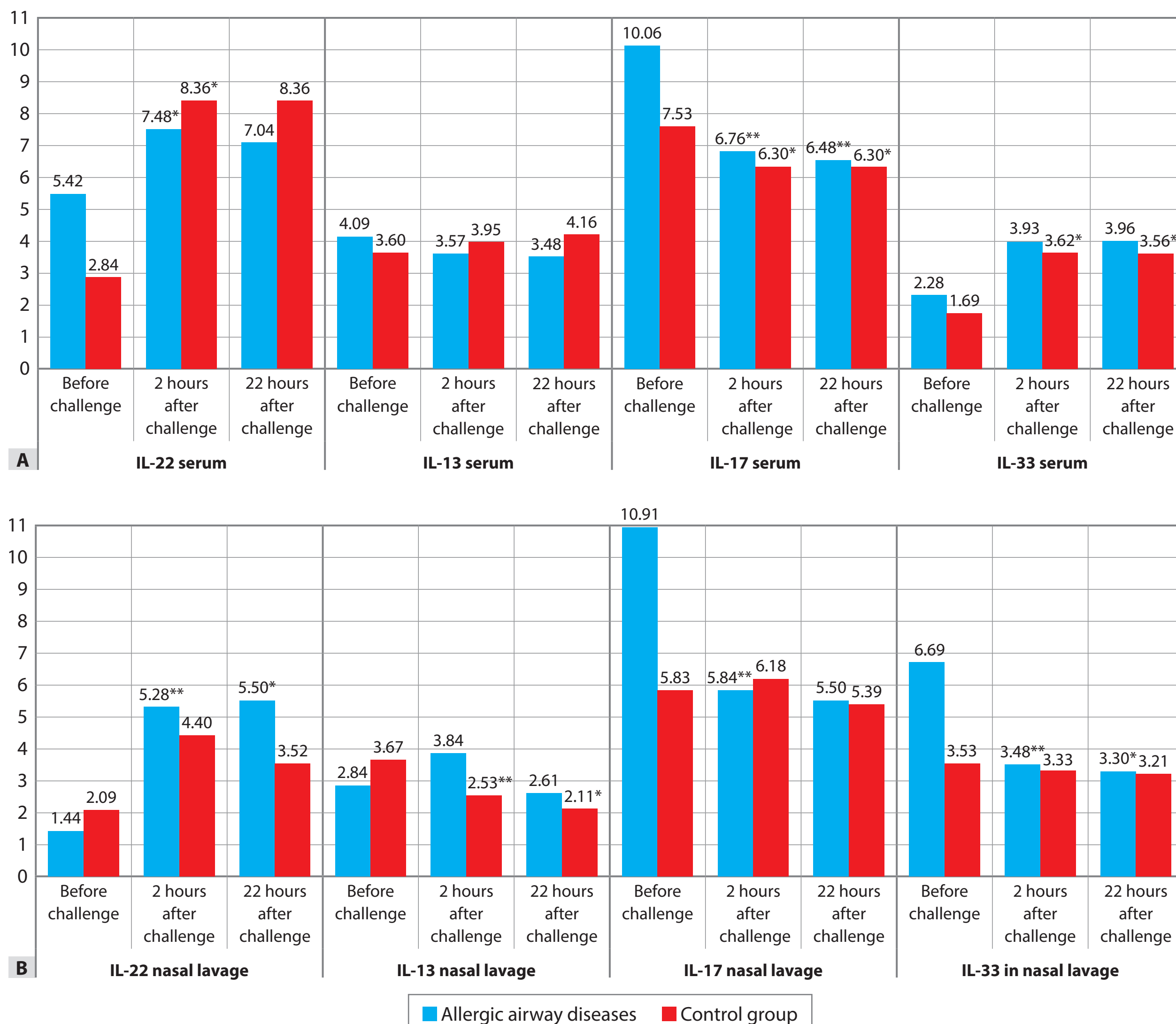

Fig. 6. Cytokines level before and after nasal challenge with Dermatophagoideus pteronnysinus in serum (a) and nasal lavage fluid (b)[1]

[1]Data are presented as median. **p<0.01 compared with data before challenge in certain group. *p<0.05 compared with data before challenge in certain group

SNPs of the VDR gene

The genetic analysis results revealed that the rs731236 A allele was more common in patients with AR and AA than in healthy individuals (table 9). A tendency was observed that the rs4588 TT genotype was more prevalent in patients with AR and AA (p=0.10). No other differences were found in the studied SNPs of a VDR gene.

CONCLUSIONS

1. Patients having allergic rhinitis with or without allergic asthma have more severe nasal symptoms, especially sneezing and nasal secretion and worse sleep quality than healthy individuals.
2. Analysis of immune markers revealed:
   - T2 type inflammatory cytokine IL-13 is higher only in serum from patients with allergic rhinitis and is related to nasal symptoms and sleep quality; serum IL-13 and IL-33 are related to systemic eosinophilia in an allergic airway, whereas anti-inflammatory cytokine IL-10 did not differ from healthy subjects but was related to lung function and lower risk of allergic asthma.
   - Th1 cytokine IFN-γ is higher in nasal secretion from patients with allergic rhinitis and allergic asthma and is related negatively to nasal symptoms showing its anti-inflammatory properties.
   - Levels of IL-17 and IL-22 did not differ significantly between the groups but were negatively related to eosinophilia; IL-22 showed a higher risk for allergic asthma development.
   - Nasal carriage of *Staphylococcus aureus* showed an increase of IL-13, IL-17, IL-33, and IFN-γ in nasal lavage fluid from patients with allergic airway diseases.

3. Levels of nasal eosinophils and cytokines IL-10, IL-13, and IFN-γ are related to the same immune markers in the blood whereas IL-22 showed a negative correlation and IL-17 and IL-33 did not correlate between local and systemic compartments.
4. The nasal challenge with *Dermatophagoides pteronyssinus* allergen revealed an increase of IL-22 in nasal secretion and serum and an increase of IL-33 only in serum, whereas IL-17 levels after provocation decreased in both compartments.
5. Assessment of allergic asthma risk showed that a higher vitamin D level is associated with a lower risk of this disease development. The presence of vitamin D receptor gene rs731236 A allele is more prevalent in patients with allergic rhinitis and allergic asthma than in healthy individuals.

CLINICAL RELEVANCE

The results of the study provided additional knowledge about the pathogenesis of allergic airway diseases. It was found that novel cytokines such as IL-17, IL-22, and IL-33 together with Th1 and Th2 secreted cytokines, vitamin D level and VDR gene polymorphisms were important in the development of these diseases and can be predictors for their development. Differences in immune markers and their interactions between patients with AR only and AR and AA provide a more comprehensive view of the disparity of these diseases. Differences in levels of cytokines between serum and nasal lavage fluid illustrate that systemic inflammation does not always reflect local inflammation and suggest thinking about the investigation of local biomarkers in clinical practice. Moreover, adequate vitamin D level was associated with a lower risk of asthma development suggesting that vitamin D level should be measured, and if it is lower than recommended supplements should be considered.

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