

Original article

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Soluble CD14 and total IgE in the serum of atopic and non-atopic adolescents in relation to environmental factors: a pilot study

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The CD14 receptor is expressed on the surfaces of monocytes, macrophages, and, to a lesser extent, of activated granulocytes and B lymphocytes (mCD14). It also exists in soluble form in the serum (sCD14), in which it binds microbial compounds and indoor allergens. The objectives of this study were to see whether serum sCD14 concentrations could be used as a marker of atopic disorders and to estimate the effects of environmental factors (tobacco smoke exposure, childhood residence in urban or rural areas, and having a pet) on sCD14 and IgE values. Mass fraction of sCD14 and total IgE were determined in the sera of atopic (N=53) and non-atopic (N=35) participants using the standard enzyme-linked immunosorbent assay. Exposure to pets and environmental tobacco smoke was estimated based on the International Study of Asthma and Allergies in Childhood questionnaire (ISAAC). Median sCD14 concentration was significantly lower in atopic than in non-atopic girls (3.49 vs. 3.83 $\mu\text{g mL}^{-1}$; $p < 0.010$). The number of smokers at home positively correlated with the sCD14 level in atopics, and urban atopics had significantly lower sCD14 levels than rural atopics (3.47 vs. 3.92 $\mu\text{g mL}^{-1}$, $p = 0.028$). Median total IgE concentration was significantly lower in atopic pet owners than in atopics with no pets (161 vs. 252 kIU L^{-1} ; $p = 0.021$). In conclusion, while sex and environmental factors might be involved in sCD14 expression, particularly in atopics, we found no correlation between sCD14 and total IgE concentrations. The usefulness of sCD14 as a marker of atopic disorders should be investigated further, particularly in relation to the severity of allergic disorders.

KEY WORDS: *allergy; atopy; environmental tobacco smoke; immunoglobulin E; sCD14; pets*

The incidence of atopic disorders such as asthma, allergic rhinoconjunctivitis, and atopic eczema has increased over the past two decades in many countries including Croatia (1). Atopy is a complex genetic disease that results from the interplay between gene variants and environmental influences (2). The level of specific IgE in serum is a good marker of sensitisation but not of clinically symptomatic allergy, and new markers of atopic diseases have been investigated (3). One such potential marker is the cluster of differentiation 14 (CD14), a multifunctional receptor on the macrophages, monocytes, and dendritic cells that binds lipopolysaccharides (LPS) and other bacterial wall compounds, fungal wall components (β -glucan, mannans), and indoor allergens (dust mite and cockroach allergens) (4-7). In serum it takes soluble form (sCD14), and its concentrations have been associated with serum IgE and asthma (8). The CD14-LPS complex plays a functional role in the maturation of the Th1-type response, suppressing the Th2-type response, which may result in weakened IgE response and atopic phenotype (8). As sCD14 levels seem to vary with age, sex, and genetic variants (9), its clinical relevance as a possible marker of atopic diseases

is still not clear. Recent studies in children and adults reported apparently contradictory results; some have associated lower serum sCD14 with atopy (10) or sensitisation to house dust mites (6), and some reported increased sCD14 levels in various allergic diseases such as allergic alveolitis and atopic dermatitis (11-14). These contradicting effects of sCD14 can be explained by the fact that higher sCD14 levels in asthmatic patients are secondary to allergic inflammation. Thus, Kusunoki et al. (15) have proposed that CD14 can have a dual effect on atopy; it inhibits IgE production before allergic inflammation and stimulates IgE after its onset.

As environmental factors such as pets and tobacco smoke exposure are associated with the development of atopy in children (2, 16), and the relationship between sCD14 levels and environmental factors has been poorly investigated in atopic patients, understanding this relationship may be important for identifying the mechanisms that regulate IgE production.

Therefore, the aim of our study was to: (i) determine serum sCD14 concentrations in atopic and non-atopics adolescents, (ii) investigate the association between sCD14 concentrations and environmental characteristics (childhood rural or urban residence, tobacco smoke exposure, pet keeping), and (iii) see if values sCD14 concentrations

correlate with total IgE in both atopic and non-atopic adolescents.

MATERIALS AND METHODS

Participants

The study included 88 adolescents (53 atopic and 35 non-atopic) with median age of 19 years (range 18-19 years). The participants were selected from a database containing data on 440 students (a highly homogenous population), who had been recruited from the University of Zagreb (Croatia) to participate in a larger study investigating the influence of various genetic and environmental factors on allergic respiratory diseases (17). We excluded adolescents with an acute infectious disease and those currently under antibiotic treatment. A modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (18) was completed by a physician for each participant to determine the presence of respiratory and skin atopic symptoms in the previous year and to take family history of allergies. It included questions on the symptoms of asthma (wheezing), rhinitis (runny nose, itchy nose, blocked nose, sneezing), and dermatitis (skin redness, itching, vesicles, and rash) as well as questions on exposure to various environmental and lifestyle risk factors (rural-urban life in the childhood, smoking status, environmental tobacco smoke exposure, and having a pet). Respiratory atopic symptoms were defined as the presence of wheezing and/or at least two of the above mentioned rhinitis symptoms and positive skin prick test (SPT) on at least one standard inhalatory allergen. Atopic skin symptoms were defined as the presence of at least two of the above mentioned skin symptoms and positive SPT. The participants were categorised according to the smoking status as never smokers, ex-smokers (if they ceased to smoke at least one year before the study), and current smokers. Since the number of ex-smokers was very low (three participants), only two groups were defined for statistical analysis: non-smokers (comprising never smokers and ex-smokers) and smokers (current smokers). Environmental tobacco smoke exposure (passive smoke exposure) was assessed according to the number of smokers at home.

The study was designed in accordance with the Helsinki Declaration and approved by the Ethics Committee of the Institute for Medical Research and Occupational Health, Zagreb. Each participant was informed about the study protocol and signed a consent form. The participants were matched according to (in order of priority): 1) sex, 2) childhood urban/rural residence, 3) number of smokers at home, and 4) smoking. The smoking status was considered as the least relevant matching factor due to a relatively short period of smoking (median 3 years) in the participating adolescents. Even though we aimed at the 1:1 ratio between

non-atopic and atopic participants, we had to settle for the 1:1.5 ratio due to a limited number of participants from rural areas.

Methods

All participants underwent standard skin prick testing (SPT) with a preparation of common inhalatory allergens: grass pollen mixture, birch, hazel, weed (*Ambrosia elatior*; *Artemisia vulgaris*) pollens, house dust mites (HDM) (*Dermatophagoides pteronyssinus*, *D. farinae*), storage mite *Lepidoglyphus destructor*, moulds (*Cladosporium herbarum*, *Alternaria alternata*), and cat and dog dander (all from Allergopharma, Reinbeck, Germany). SPT included testing with positive control solution of histamine (10 mg mL^{-1}) and negative buffer control solution (Allergopharma, Reinbeck, Germany). Skin reaction (weal) was evaluated after 15 min. Wheals with mean diameter $(D+d)/2$ (where "D" is the largest diameter and "d" is perpendicular diameter at midpoint of D) larger than negative control by more than 3 mm were considered positive. Atopy was defined as positive SPT to at least one of the tested allergens.

Blood samples were taken in sterile Vacutainer® tubes between 8 and 10 a.m. after an overnight fast and allowed to clot for one hour at room temperature. They were centrifuged at 1800 g for 10 min and stored in pyrogen-free polyethylene tubes at $-20 \text{ }^{\circ}\text{C}$ until further analysis.

The concentration of sCD14 was determined with a commercial Enzo Life Science kit (Farmingdale, NY, USA; Cat No. ALX-850-302) using the solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Briefly, serum samples were diluted with phosphate buffer saline with Tween 20 (PBS-T) at a ratio 1:200. Coated microtitre plates were incubated with diluted serum samples, standards ($3.12\text{-}50.0 \text{ ng mL}^{-1}$), and reference serum and shaken for one hour at room temperature. After three washes, detecting antibody was added and the plates incubated in the shaker for another hour at room temperature. After washing, the substrate (TMB, 3,3',5,5'-tetramethylbenzidine) was added to all wells and incubated at room temperature for 13 ± 1 min without shaking, and the reaction was quenched by adding the stopping solution. Recombinant human CD14 was used as reference standard. The optical absorbance of each well was read at 450 nm using a plate reader (IASON, Graz, Austria). The sCD14 concentrations were calculated using linear regression analysis and were expressed in $\mu\text{g mL}^{-1}$. All serum samples were measured in triplicate. The limit of detection (LOD) was $0.701 \mu\text{g mL}^{-1}$ and intra- and inter-assay imprecisions (expressed as coefficients of variation) for sCD14 were 2.8 % and 6.4 %, respectively. The analytical inaccuracy shown as bias was 2.7 %. Lipemic and haemolysed serums were not included in sCD14 measurements (as per manufacturer's instructions). Total IgE concentration was determined using ELISA with a

commercial kit (Adaltis, Guidonia Montecelio Italy). The optical absorbance was read at 450 nm using a plate reader (IASON, Graz, Austria). Total IgE concentrations were calculated using linear regression analysis, and the results are expressed in kIU L⁻¹.

Statistical analysis

For statistical analysis we used Stata/SE 11.2 for Windows (StataCorp LP, College Station, TX, USA). Differences between groups were analysed with Pearson's chi-square test (or Fisher's exact test if any expected cell frequency in contingency table was ≤5) for categorical variables and with Mann-Whitney U test for non-normally distributed continuous variables (age, smoking index, serum sCD14, and total IgE). Respiratory and skin symptoms were analysed in atopic subjects only, because only a few non-atopics had them and their symptoms most probably had different mechanisms. Correlation between two variables was analysed using Spearman's correlation. In atopics, we used sCD14 and logarithmically transformed IgE levels (to obtain normal distribution) as dependent variables for multiple linear regression analysis, and sex, childhood urban/rural residence, smoking status (smoker vs. non-smoker), and number of smokers at home as predictors of atopy in children. Atopic symptoms were also used as predictors in order to analyse the relationship between sCD14 and IgE levels and clinical expression of atopy in

the participants. The effect of having a pet on sCD14 and IgE levels was analysed using separate models in urban residents only, since in rural residents contact with domestic animals is already presumed. In these models sex, atopic symptoms, and smoking were used as predictors. All models were tested for normality and homoscedasticity of residuals and multicollinearity. Although sCD14 data were moderately skewed and could not be normalised by standard mathematical transformations, the analysis of residuals showed that the assumptions of linear regression were met. We considered *p*<0.05 as statistically significant. Considering the asymmetrical shape of distribution, serum IgE and serum sCD14 concentrations are presented as medians and 25th-75th interquartile ranges.

RESULTS

Characteristics of atopic and non-atopic participants are presented in Table 1. There were no significant differences in age, sex ratio, and environmental and lifestyle characteristics (rural vs. urban, smoking status, having a pet) between the two groups. As expected, atopic participants reported four and two times more respiratory and skin symptoms than non-atopics, respectively. Mother's history of atopy was also nine times more prevalent in

Table 1 Demography and clinical characteristics of participants

	Non-atopics (N=35)	Atopics (N=53)	<i>p</i> -value
Personal characteristics			
Male, N	17	26	0.964
Age in years (range)	19	19	0.346
Mother's history of atopy, N	1	15*	0.002*
Father's history of atopy, N	5	7	1.00
Symptoms, N			
Wheezing or rhinitis symptoms**	4	25	<0.001**
Skin symptoms	4	13	0.128
Smoking status, N			
Non-smokers	29	36	0.264
Ex-smokers	1	2	
Smokers	5	15	
Smoking index for current smokers	30	39	1.00
Number of smokers at home, N			
0	14	27	
1	12	13	0.533
>1	9	13	
Urban-rural residence***, N			
Urban from birth to now	21	31	
Rural from birth to now	7	12	
Moved from rural to urban	1	5	0.342
Moved from urban to rural	3	1	
Pet, N			
At present	8	16	0.541

*Statistically significant difference (Pearson's chi-square test; *p*<0.05); **statistically significant difference (Mann-Whitney U test; *p*<0.05); wheezing, rhinitis, or skin symptoms refer to the previous year; ***for two non-atopic and four atopic participants urban/rural type of residence in early childhood was unknown

atopics than in non-atopics. Father's history of atopy did not differ significantly between the two groups.

We found a significantly lower sCD14 concentration in atopics than in non-atopics [3.55 (3.19-3.92) vs. 3.82 (3.53-4.01) $\mu\text{g mL}^{-1}$, $p < 0.049$] (Table 2). Median sCD14 concentration in female atopics was significantly lower than in female non-atopics ($p < 0.010$), but male adolescents did not differ significantly in sCD14 levels ($p = 0.709$) (Figure 1a). Median IgE concentration was 12.5 times higher in atopics than non-atopics (Table 2). Differences were significant in both sexes but more pronounced in female adolescents (Figure 1b). We found no correlation between IgE and sCD14 levels in either atopics ($\rho = -0.046$, $p = 0.746$) nor non-atopics ($\rho = 0.149$, $p = 0.393$). Figure 2 shows sCD14 concentrations in atopics and non-atopics according to the residence in childhood (urban-rural). Urban atopics had significantly lower sCD14 levels [3.47 (3.18-3.83) $\mu\text{g mL}^{-1}$] than rural atopics [3.92 (3.72-4.03) $\mu\text{g mL}^{-1}$, $p = 0.028$], which was confirmed by multiple linear regression controlled for sex, atopic symptoms, and smoking as predictors (coefficient_{rural vs urban} = -0.32, 95 % CI -0.58 to -0.05, $p = 0.019$, adjusted $R^2 = 0.24$, $P_{\text{model}} = 0.005$). In contrast, there were no significant differences in sCD14 levels between urban and rural non-atopics.

Smokers and non-smokers did not significantly differ in sCD14 levels within the atopic and non-atopic groups (data not presented). However, environmental tobacco smoke exposure (number of smokers at home) positively correlated with sCD14 concentrations in the atopic group, in which near-significant Spearman's correlation was found ($\rho = 0.270$, $p = 0.051$). This correlation became significant when we used the multiple regression model with sex, atopic symptoms, urban vs. rural residence, and smoking as predictors (coeff. = 0.20, 95 % CI 0.06-0.34, $p = 0.007$). Such a trend was not observed in non-atopic participants.

Owning a pet did not significantly affect sCD14 levels among atopics, even though we did observe a slight correlation ($p = 0.211$).

In contrast, atopic pet owners had significantly lower IgE levels than atopics with no pets [161 (36.9-209) vs. 252 (135-356) kIU L^{-1} , $p = 0.021$] (Figure 3). The result was confirmed by multiple linear regression, with sex, atopic symptoms, and smoking as predictors. IgE levels (logarithmically transformed) inversely correlated with pet keeping at home (coeff. = -1.28, 95 % CI -2.22 to -0.33, $p = 0.021$, adjusted $R^2 = 0.44$, $P_{\text{model}} = 0.001$). Non-atopics

showed no significant relationship between IgE levels and keeping a pet.

Total IgE levels did not significantly correlate with tobacco smoke exposure and urban-rural residence in either group (p ranging from 0.104 to 0.906).

We found no correlation between sCD14 levels and rhinitis or asthma-like symptoms in atopics, but 19 % lower sCD14 levels were found in male atopics with skin symptoms than in asymptomatic male atopics [3.13 (3.12-3.19) vs. 3.88 (3.51-4.07), $p = 0.017$].

DISCUSSION

Lower serum sCD14 levels in atopic adolescents found in our study corroborate reports from Sweden and Taiwan, which showed that atopic children had significantly lower serum sCD14 than non-atopic children (6, 11). In contrast, other studies reported higher serum sCD14 levels in allergic alveolitis (12), atopic dermatitis (13), and asthma (14, 15, 20). Moreover, several authors found no differences in sCD14 concentrations between atopics and non-atopics (21, 22). These inconsistencies in findings can be explained in several ways. Beside methodological differences between the studies, one reason for the inconsistencies may be the role of disease status on serum sCD14, because acute inflammation and acute atopic asthma have been associated with a higher production of CD14 (20, 23-25). In contrast, mild atopic manifestations may be associated with milder changes in sCD14 levels, as was observed in the our and several other studies (6, 11, 19). In our study, atopics presented no severe clinical features of atopic disease. Those with atopic skin symptoms had a mild increase sCD14 levels, and those with rhinitis or asthma-like symptoms showed no increase. This is why we believe that the observed inconsistencies between earlier reports could be explained by the association between the acuteness of atopic diseases and CD14 expression (14, 23). Other reasons for the inconsistencies may be the influence of microbial and allergen load interacting with CD14 as well as the CD14 gene polymorphisms (19, 26).

Sex and age can also affect CD14 expression (16). In our study, atopic girls showed significantly lower serum sCD14 levels than atopic boys. This is in accordance with the findings of LeVan et al. (19) that male subjects had higher sCD14 levels than female subjects. These results may indicate that atopy affects innate immunity differently

Table 2 Serum concentrations of sCD14 and total IgE in the participants

	Non-atopics (N=35)	Atopics (N= 53)	p-value
sCD14 ($\mu\text{g mL}^{-1}$)	3.82 (3.53-4.01)	3.55 (3.19-3.92)	0.049*
Total IgE (kIU L^{-1})	15.8 (3.4-79.6)	196.8 (84.4-318.4)	<0.001*

Data are shown as median and interquartile range; *statistically significant difference (Mann-Whitney U test; $p < 0.05$)

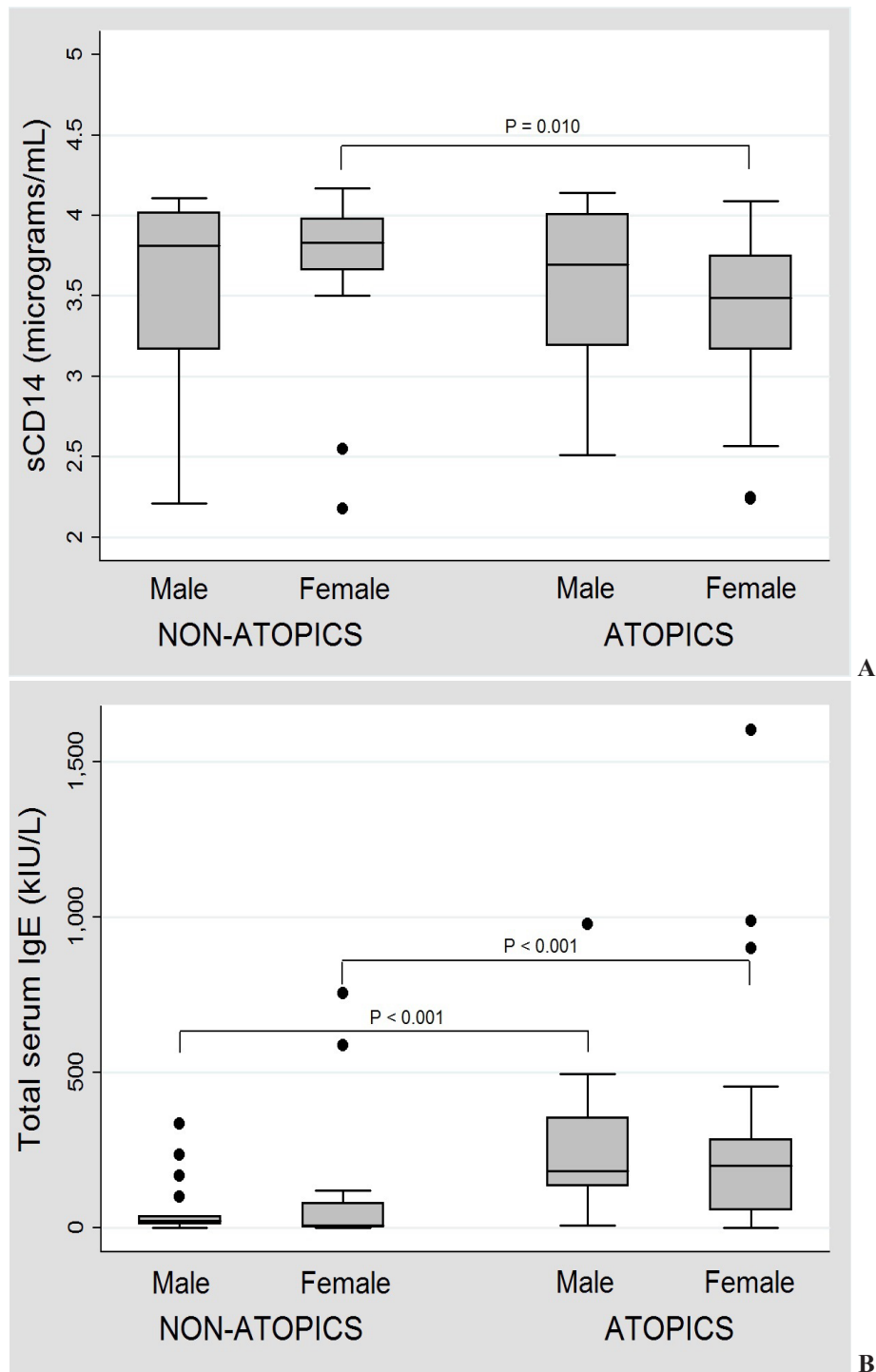


Figure 1 Serum sCD14 (A) and total IgE (B) concentrations in non-atopic and atopic adolescents in relation to sex. The box shows the median and interquartile range (25-75 %), and the whisker corresponds to 1.5 times the interquartile range

in women and men, suppressing CD14 activity more in women than in men. Furthermore, atopy-related increase in total IgE was much more pronounced in girls than in boys, which may be related to genetically based sex differences in IgE regulatory mechanisms (27). Our results showed that atopic adolescents who grew up in rural communities had higher sCD14 levels than the atopics raised in urban settings (Figure 2), which confirms earlier reports such as the one by Lauener et al. (28). They suggested that CD14 and Toll-like receptor 2 (TLR 2) might serve as biological markers of exposure to a higher bacterial load, which can explain higher sCD14 in adolescents who grew up in a rural area. Another study (29) reported that higher expression of CD14 and TLR genes was related to protective factors (farming environments, endotoxin, extracellular polysaccharides) against the development of asthma and wheezing in children.

Air pollutants such as those contained in environmental tobacco smoke present a considerable health problem to the general population, including non-smokers (16, 30-32). Some studies suggest that active and passive smoking present the risk of IgE sensitisation and atopic conditions, atopic rhinitis and dermatitis in particular (32, 33). Our study however found no correlation between passive smoking and elevated IgE in either atopic or non-atopic adolescents, but in atopics passive smoking showed a borderline significant effect on sCD14 levels. This effect might be explained by chronic exposure to bacterial endotoxins in cigarette smoke and by gene-smoking interaction (34, 35).

Unlike some researchers (36, 37), we found no association between sCD14 levels and active smoking, but

this may be owed to the young age of the participants (median 19 years) and short smoking period of 3 years.

Many studies have investigated the effect of pet keeping on the development of atopic diseases in children (2, 38). Interestingly, our results showed significantly lower IgE levels in the serum of atopic pet owners than in atopics without pets (Figure 3). This may have something to do with the so called “hygiene theory”, which says that pets are carriers of endotoxins, and exposure to endotoxin is responsible for the switch from IgE to IgG₄ production, modified Th2 immune response, and beneficial effect of pet exposure against the risk of atopy (39). Several studies have found that having a pet may protect against the development of atopic diseases in childhood (2, 40, 41). However, the effect of pet keeping seems to depend on the type of the pet, exposure duration, and the genetic setup (41, 42). In contrast to IgE, we found no correlation between sCD14 levels and pet exposure.

Furthermore, we found no correlation between sCD14 and total IgE serum concentrations either in atopic or non-atopic adolescents. This lack of association was also reported by other authors (10, 15). The mechanism through which CD14 might act in atopy is not known. Activation of CD14 appears to upregulate IL-4 and IL-12-dependent IgE production. Several studies have reported that certain CD14 polymorphisms may lead to a more severe allergic reaction, that is, a stronger IgE response (6, 9, 19, 21, 25). In contrast, polymorphisms of Toll-like receptors 2 and 4, which are LPS receptors too, were not associated with allergy and asthma when compared with controls (43).

This highlights the complexity of the interaction between innate and adaptive immune responses and

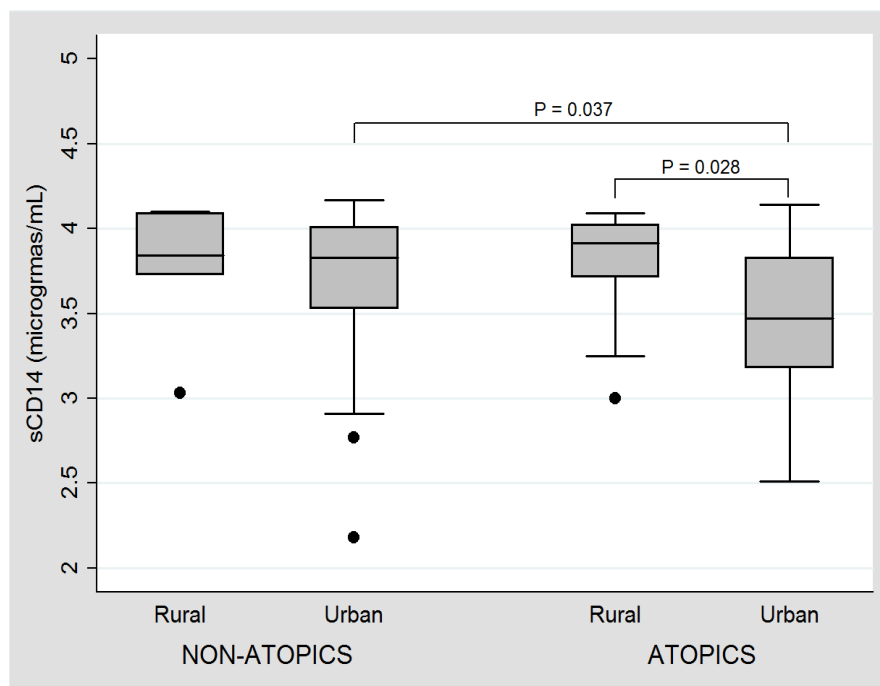


Figure 2 Serum sCD14 concentrations in non-atopic and atopic adolescents in relation to childhood residence (rural-urban). The box shows the median and interquartile range (25-75 %), and the whisker corresponds to 1.5 times the interquartile range

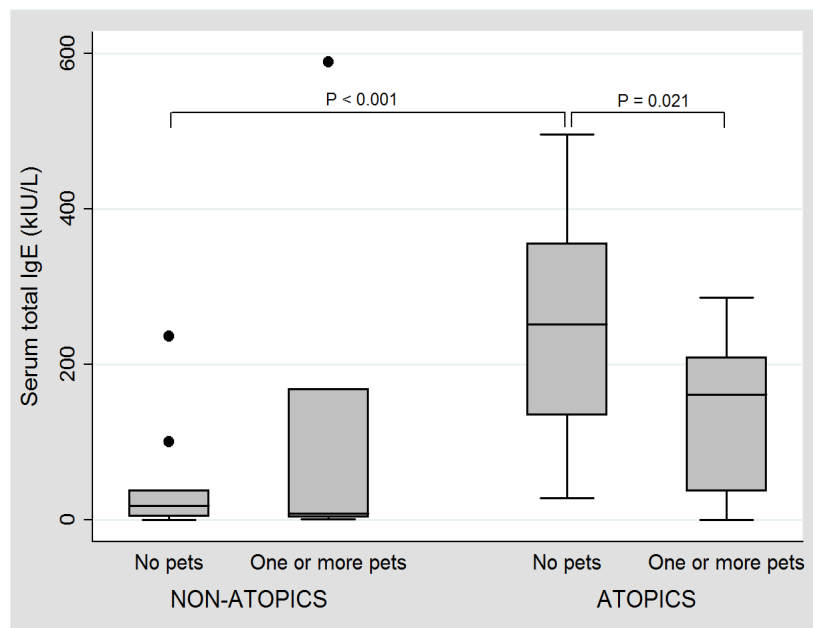


Figure 3 Total serum IgE in urban non-atopic and atopic adolescents in relation to keeping a pet (a dog, cat, or a bird) at home at the time of the study. The box shows the median and interquartile range (25-75 %), and the whisker corresponds to 1.5 times the interquartile range

suggests that environmental and lifestyle factors should be considered if we seek to understand the role of sCD14 in allergic development (30). Understanding this interaction may improve insights into atopy and asthma pathogenesis and result in new preventive strategies (34).

To the best of our knowledge, this is the first study that has investigated associations between sCD14 levels and lifestyle risk factors in atopic adolescents in Croatia.

Our study has several limitations, including the small number of participants and the lack of quantitative environmental exposure data (such as urinary nicotine and cotinine for tobacco smoke exposure). However, the questionnaire data on smoking habits and environmental smoke have been validated previously, and we have assumed that reported tobacco smoke exposure adequately reflects lifestyle characteristics such as cigarette smoking. An advantage is that we studied a very homogenous group of adolescents, which excluded age-dependent variations in sCD14 levels. Furthermore, ELISA showed very good analytical properties (low intra and inter-assay imprecision and high accuracy).

CONCLUSION

We have shown that serum sCD14 in adolescents depends on sex and environmental factors (urban-rural residence and tobacco smoke exposure), but found no correlation between sCD14 and total IgE concentrations. The clinical usefulness of sCD14 as a marker of atopy should be investigated further in occupational settings, particularly those with endotoxin-rich aerosols and those associated with a high prevalence of allergic diseases and occupational asthma. Further longitudinal studies on large

samples are needed to better understand the possible gene-environment sCD14 interactions in the development of atopic diseases.

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Topivi receptor CD14 i ukupni IgE u serumu mladih atopičara odrasle dobi u ovisnosti o čimbenicima iz okoliša: pilot istraživanje

Receptor CD14 izražen je na površini monocita, makrofaga, te u manjoj mjeri na aktiviranim granulocitima i B limfocitima (mCD14). Također, CD14 postoji u topivom obliku u serumu (sCD14), koji ima važnu ulogu u vezanju nekih komponenata mikroorganizama i alergena unutarnjeg okoliša. Cilj je ovog istraživanja bio utvrditi mogu li se vrijednosti koncentracije sCD14 u serumu koristiti kao biljeg atopičnih poremećaja, te ustanoviti utjecaj čimbenika okoliša (pušenje, stanovanje u gradskom-ruralnom području tijekom djetinjstva te kućni ljubimci) na vrijednosti sCD14 i IgE. Koncentracija sCD14 i ukupni IgE određeni su u serumu atopičara (N=53) i neatopičara (N=35) standardnim enzim-immunokemijskim metodama. Izloženost okolišnim čimbenicima (kućni ljubimci, pušenje) procijenjeni su prema Upitniku iz Međunarodne studije o astmi i alergiji (ISAAC). Medijan koncentracija sCD14 bio je značajno niži u atopičnih djevojaka nego u neatopičnih (3.49 vs. 3.83 $\mu\text{g mL}^{-1}$; $p < 0.010$). Koncentracija sCD14 u ispitnika atopičara bila je pozitivno povezana s brojem pušača u kući te značajno niža u atopičara s prebivalištem u gradu u odnosu na atopičare s prebivalištem na selu (3.47 vs. 3.92 $\mu\text{g mL}^{-1}$, $p = 0.028$). Pronašli smo značajno nižu koncentraciju ukupnog IgE u atopičara koji imaju kućne ljubimce negoli u atopičara bez kućnog ljubimca (161 vs. 252 kIU L^{-1} ; $p = 0.021$). Zaključno, premda spol i okolišni čimbenici utječu na izražajnost sCD14, osobito u atopičara, nismo pronašli korelaciju između koncentracija sCD14 i ukupnog IgE. Značenje određivanja sCD14 kao biljega atopičnih poremećaja trebala bi se dalje ispitivati, naročito u vezi sa stupnjem ozbiljnosti alergijskih poremećaja.

KLJUČNE RIJEČI: *alergija; atopija; sCD 14; duhanski dim iz okoliša; imunoglobulin E; kućni ljubimci*