

Short communication

Serum interleukin-17 levels are related to clinical severity in allergic rhinitis

Background: T helper (Th)-17 cells are a subset of T helper lymphocytes that exert regulatory activities. Recently, it has been reported that serum interleukin (IL)-17 levels are high in the most severe cases of birch allergy studied outside the pollen season.

Objective: The aim of this study was to investigate a possible relationship between serum IL-17 levels and clinical parameters in patients with allergic rhinitis studied during the pollen season.

Methods: In all, 56 patients with persistent pollen-induced allergic rhinitis were evaluated during the pollen season. Serum IL-17 levels were evaluated by enzyme-linked immunosorbent assay. Symptoms were assessed by visual analogue scale, drug use was monitored and peripheral eosinophils were counted.

Results: Serum IL-17 levels were significantly related to clinical symptoms, drug use and peripheral eosinophil counts ($P = 0.0001$ for all).

Conclusion: This study provides evidence that serum IL-17 level assessment might be considered to classify allergy severity.

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Allergic rhinitis (AR) is characterized by an inflammatory reaction associated with increased production of T helper (Th)-2-type cytokines, such as interleukin (IL)-4 and IL-13. Indeed, peripheral blood mononuclear cells of AR patients predominantly produce IL-4 with respect to interferon (IFN)- γ expression by Th-1 cells. The recent discovery of another T lymphocyte subset, namely Th-17 cells, has been fundamental to our understanding of how Th-1 cells can actually mediate inflammatory events by producing IFN- γ (1). T helper 17 cells are characterized by the production of various cytokines, including IL-17, IL-6, tumor necrosis factor- α and IL-22.

Previous studies have shown that IL-17 serum levels are increased in several disorders: acute hepatic injury (2), rheumatoid arthritis (3) and McArdle disease (4). Furthermore, anti-IL-17 reduced neutrophil infiltration in an experimental murine asthma model (5), whereas IL-17 induced recruitment and increased survival for airway macrophages (6). These data suggest a regulatory role for IL-17 (7), even though the exact role of Th-17 cells in allergic inflammation remains undefined. One study showed that patients with viral rhinitis had elevated

concentrations of IL-17 in nasal secretions, whereas allergic patients had normal levels (8). However, it has been very recently demonstrated that some patients with AR caused by birch allergy outside the pollen season may have detectable serum IL-17 levels, which are related to various inflammatory parameters (9). Therefore, it has been suggested that IL-17 serum levels might be considered a marker of allergy severity even in the absence of symptoms (9).

Confirmation of this concept would facilitate diagnosis, as assessment of a cytokine at the serum level would certainly simplify clinical evaluation. The previous study was performed on AR patients evaluated outside the pollen season (9). The present study was therefore aimed at investigating whether serum IL-17 levels correlate with clinical parameters in AR patients actively exposed to pollen.

Methods

The study included 56 patients (21 males and 35 females, mean age 35.3 years) with persistent moderate-to-severe AR, evaluated during

the pollen season. Allergic rhinitis was diagnosed according to validated criteria (10).

With the exception of AR, all subjects were healthy, none suffered from any active disorder. Patients with rhinosinusitis and asthma were excluded from the study. All subjects were allergic only to *Parietaria judaica* and grasses as confirmed by allergen-specific conjunctival challenge; other sensitizations were excluded.

The skin prick test and blood sampling for assessment of peripheral eosinophils were performed in all patients. The skin prick test was performed according to the guidelines of the European Academy of Allergy and Clinical Immunology (11). Clinical severity was also evaluated, considering symptom score and drug consumption at the end of the pollen season as previously validated (12). The patient questionnaire included a validated visual analogue scale (VAS) of nasal symptoms (13). Patients had to have VAS score for nasal symptoms ≥ 6 .

During the pollen season, use of levocetirizine drops (10 or 20, corresponding to a 2.5 or 5 mg concentration) on demand as rescue medication was permitted. However, medication was withdrawn at least 1 week before drawing blood samples. The medication scoring (MS) was as follows: not at all (0), occasionally/seldom (1), occasionally/often (2), almost daily (3), continuously with the lowest dose (4), continuously with the maximal dose (5). The study was approved by the local Ethics Committee and performed with the written informed consent of all participants.

The human interleukin 17A Immunoassay (Quantikine IL-17 Immunoassay; R&D Systems Inc., Milan, Italy) employs the quantitative sandwich enzyme immunoassay technique and was performed according to the manufacturer's instructions and expressed as pg/ml. The minimum detectable dose was determined by adding two standard deviations (SD) to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration.

Descriptive statistics were first performed and quantitative parameters are reported as the mean, SD and standard error of the mean (SEM). The nonparametric Wilcoxon test and Spearman's test were used to compare samples and to study respective correlations. The package 'S-Plus' (MathSoft Corp., Needham, MA, USA) was used for all analyses.

Results

All patients had persistent AR with moderate-to-severe symptoms and all were allergic to *Parietaria* and grasses only. The mean serum IL-17 value was 3.11 pg/ml (SD 4.36 pg/ml; SME 0.58 pg/ml). In our centre, the mean value in healthy subjects was 0.85 pg/ml (SD 2.03 pg/ml; SEM 0.77 pg/ml).

A significant positive relationship between IL-17 serum values and symptom severity assessed by VAS was observed ($P = 0.0001$, $r = 0.8$) as reported in Fig. 1. Moreover, serum IL-17 levels and drug use, assessed by medication scoring, were significantly related ($P = 0.0001$, $r = 0.67$). Finally, peripheral eosinophil counts were significantly related to serum IL-17 values ($P = 0.0001$, $r = 0.8$) as described in Fig. 1. In addition, there was a significant relationship between VAS and drug use ($P = 0.0001$; $r = 0.69$) as reported in Fig. 2.

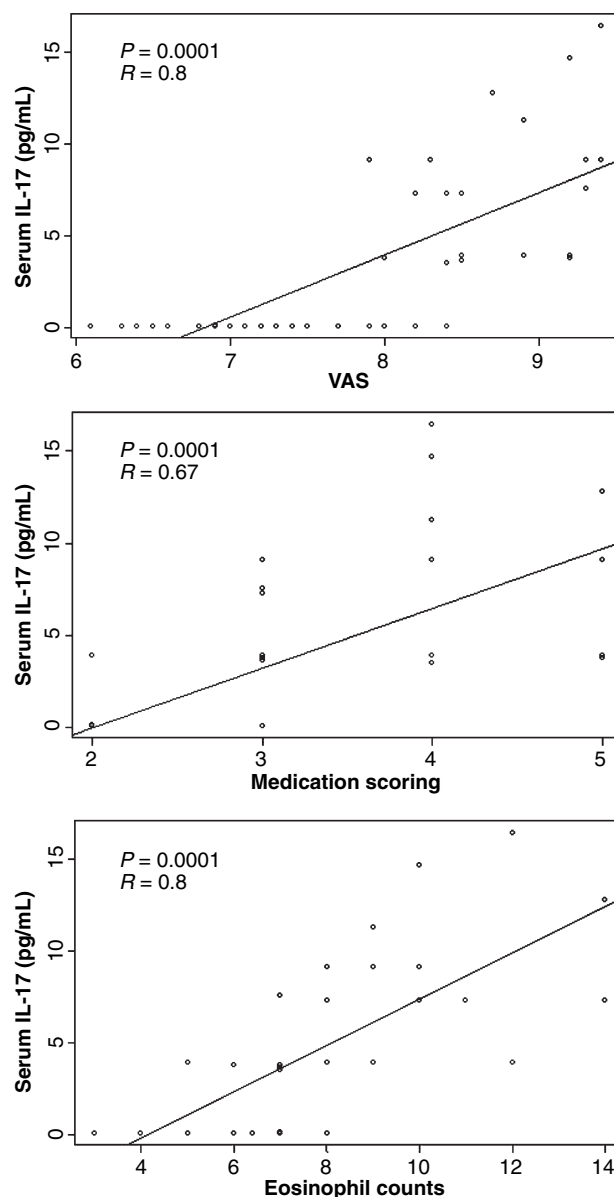


Figure 1. Correlations between serum interleukin-17 and visual analogue scale (VAS), medication scoring and eosinophil counts [expressed as Spearman's correlation coefficient (r) and P -value].

Discussion

The present study evidences that serum IL-17 levels are significantly related to some clinical and inflammatory parameters in patients with persistent moderate-to-severe AR evaluated during the pollen season. These findings are clinically relevant and demonstrate that serum IL-17 levels correlate well with clinical severity as documented by symptom score and drug consumption. In addition, serum IL-17 levels correlated with allergic inflammation as documented by the significant relationship observed with peripheral eosinophils. Therefore, serum IL-17 levels

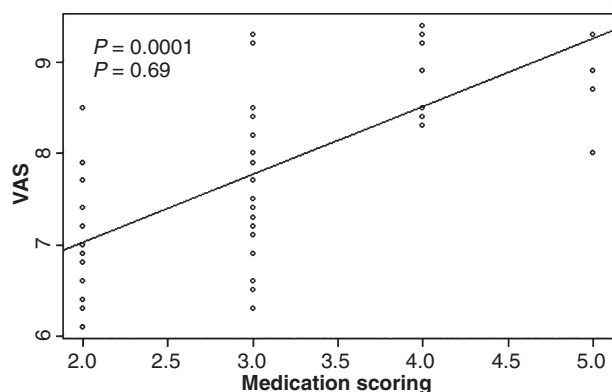


Figure 2. Correlation between VAS and medication scoring [expressed as Spearman's correlation coefficient (r) and P -value].

evaluated in patients with pollen allergy might be considered a marker of allergy severity. This concept confirms a previous study evidencing that serum IL-17 levels were detectable in the most severe cases of birch allergy evaluated outside the pollen season. In fact, that study provided evidence that the highest IL-17 serum levels were detectable in those patients with the highest total and specific IgE levels and peripheral eosinophils and also the lowest allergen threshold dose for conjunctival challenge (6). Therefore, to evaluate whether serum IL-17 correlated with clinical parameters, symptomatic patients in this study were recruited during the pollen season.

The results of these studies are partially in conflict with previous investigations (8, 14), probably because the

patient characteristics evaluated were different as well as the site of observation. Klemens et al. showed that only patients with viral rhinitis had increased IL-17 in nasal secretions (8) and Lei et al. reported that IL-17 serum levels in asthmatic patients are similar to normal controls (14). Also, in this study, contrasting results might be explained by the different clinical characteristics of patients, mainly concerning the type of sensitization. Moreover, neither of the studies mentioned above considered the clinical severity of patients. Indeed, the present study demonstrates that serum IL-17 correlates well with allergy severity. This phenomenon is not a complete surprise, however, as it might be dependent on a chronic allergic inflammation to pollens in sensitized patients. In this scenario, Th-17 cells may be implicated in chronic events of allergic reaction that endure over time (15). In fact, allergic inflammation is typically continuous and persistent until allergen exposure occurs.

In conclusion, this study provides evidence that serum IL-17 levels are significantly related to allergy severity and confirms our previous study conducted outside the pollen season. Thus, increased IL-17 serum levels might be considered a marker of allergy severity in AR patients.

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