



Preliminary report

Sublingual immunotherapy may affect serum neopterin: Preliminary findings

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ABSTRACT

Background: Neopterin is a protein produced by monocytes/macrophages. It has been considered a biomarker of immune activation in several disorders. However, there are few studies in allergic rhinitis (AR).

This study evaluated the serum neopterin levels in patients with pollen-induced AR or treated with sublingual immunotherapy (SLIT) and in healthy subjects.

Methods: 146 subjects (70 males, median age 30.5 years): 56 with moderate–severe persistent AR, 40 with AR and successfully treated with 2 year SLIT, and 50 healthy controls were consecutively evaluated outside the pollen season. A skin prick test and blood sampling for assessing serum neopterin levels were performed in all subjects.

Results: SLIT-treated patients had lower neopterin levels than untreated patients and healthy subjects (respectively $p = 0.012$ and $p = 0.0001$).

Conclusions: Neopterin is a biomarker for immune activation, SLIT may affect serum neopterin probably as a consequence of Treg response to SLIT.

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1. Introduction

Neopterin is a substance of low molecular mass that becomes biosynthesized from guanosine-triphosphate and is produced preferentially by human monocytes–macrophages. Interferon- γ was identified as the most potent cytokine that induces significant production of neopterin [1]. The extent of neopterin formation should reflect the combined effect of positively- and negatively-regulating influences on the population of monocytes–macrophages activated by interferon- γ . Increased neopterin concentrations in body-fluids, such as serum or urine, are connected with diseases linked with cellular immune reaction, e.g. infections, autoimmune diseases, inflammatory diseases, organ transplantation rejection and certain malignant diseases [1]. In all these different disorders the cellular immune system is involved in the pathogenesis and/or affected by the underlying disease process, and neopterin concentrations are very closely linked with the progression of these diseases. Therefore, neopterin may be considered a diagnostic marker able to measure the degree of activation of the immune system.

Allergic rhinitis (AR) is typically an immune-mediated disease characterized by a dysregulation of T cell response. Therefore, it may be interesting to investigate the possible role of neopterin in AR. Studies that evaluated neopterin in AR are still rare. When neopterin

concentrations were analyzed in the neonatal cord blood it was observed that neopterin was unable to predict atopic disease, including AR, in early childhood [2]. Another study evaluated whether there was a relationship between the outcome of pollen specific immunotherapy and neopterin changes [3]. Also this study did not provide positive findings probably because the patients were evaluated outside the pollen season. On the other hand, it is well known that sublingual immunotherapy is able to induce a tolerance toward the causal allergen [4].

Therefore, this preliminary study was performed to measure serum neopterin levels in AR patients outside the allergen exposure or successfully cured with specific sublingual immunotherapy (SLIT) and in healthy subjects.

2. Materials and methods

Ninety-six allergic patients (47 males, median age: 33.2 years) suffering from persistent allergic rhinitis were included in this study; they were subdivided in two groups: 40 patients successfully treated with SLIT (group 1; 21 males, median age 32.8 years) and 56 patients untreated with SLIT (group 2; 26 males, median age 33.9 years). All allergic patients were sensitized to pollens (i.e. grasses, *Parietaria*, birch, and olive tree) alone and suffered from moderate–severe persistent allergic rhinitis since at least 4 years.

Group 1 included patients successfully treated with SLIT. SLIT treatment lasted 2 years: a pre-co-seasonal schedule was followed. Patients were treated with *Parietaria* pollen extract manufactured by

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Anallergo, Florence, Italy. All of them were responders to SLIT. The response to SLIT was documented by both improvement of symptoms and reduction of drug use.

Group 2 included patients previously treated with drug therapy alone.

The diagnosis of persistent allergic rhinitis was made according to the ARIA document [4]: brief presence of both typical symptoms (including nasal itching, sneezing, rhinorrhea, and nasal obstruction) and positive skin prick test for allergens whose exposure was causing symptom occurrence.

In addition, 50 healthy non-allergic subjects were recruited as control group (group 3; 23 males, median age 27.5 years): they had to be nonsensitized (such as negative to the skin prick test for the most common aeroallergens) and never suffered from allergic disorders (including food allergy, drug allergy, atopic dermatitis, and respiratory allergy).

All subjects were evaluated during the winter, such as when pollen exposure is absent. So patients were without allergic inflammation and symptoms.

The Ethical Committee approved the study and an informed consent was obtained by each subjects.

Neopterin concentrations in patients and healthy subjects were determined using Neopterin-MW EIA kit (DRG Instruments GmbH) that employs an enzyme immunoassay technique to quantify neopterin in a serum sample and was performed according to the manufacturer's instructions and expressed as nmol/L. The minimum detectable dose of neopterin is approximately 0.8 nmol/L serum [1,5].

Descriptive statistics were first performed, and quantitative parameters were reported as the medians with first and third quartiles (IQ) due to the skewed distribution. Analysis of the serum neopterin levels in different groups of patients was performed by means of the non-parametric analysis of variance (Kruskal–Wallis's test); the Wilcoxon's test was used for *post-hoc* comparisons. All tests were two sided and a *p*-value less than 0.05 was considered statistically significant. The “S-Plus” software package (MathSoft Corp.) was used for all analyses.

3. Results

The comparison between AR patients and healthy subjects showed no significant difference in median serum concentrations of neopterin (AR patients: median 5.0 nmol/L; IQ 4.5–6.95 nmol/L. Healthy subjects: median 1.78 nmol/L; IQ 1.7–2.8 nmol/L; $p < 0.0001$). The comparisons between SLIT-treated patients (median 4.7 nmol/L; IQ 3.9–5.4 nmol/L) and AR patients and non-allergic subjects showed a significant difference in median serum concentrations of neopterin ($p = 0.012$ and $p = 0.0001$, respectively), as reported in Fig. 1.

4. Discussion

AR is sustained by an inflammatory reaction. Various populations of effector and regulatory T cells have been shown to play a crucial role in allergic inflammation, mainly concerning Th2 cells [6]. Th2-derived cytokines, such as IL-4 and IL-13, are the primary pathogenic factors in inducing, maintaining, and amplifying inflammatory allergic inflammation. IL-4 and IL-13 orchestrate allergic inflammation promoting IgE synthesis, up-regulating adhesion molecules selective for eosinophil recruitment, and causing increased mucus production and airway hyperreactivity. On the other hand, there is accumulating evidence that Th1-related cytokines, such as IFN- γ and IL-12, may suppress and counteract this Th2 response and vice-versa, as there is functional dichotomy between Th1 and Th2 cells [6]. Therefore, allergic reaction is typically characterized by an activation of immune system.

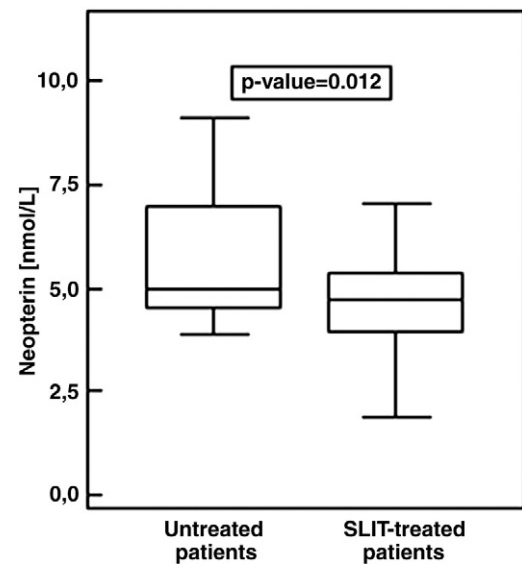


Fig. 1. Neopterin concentrations in untreated (AR) patients and SLIT-treated AR patients. Concentrations are represented as medians (horizontal lines), quartiles (25th and 75th percentiles, boxes), and ranges (vertical lines) (*p*-value of group comparison is given in the figure).

Allergen-specific immunotherapy, including SLIT, is highly effective in the treatment of AR, as it is the only treatment which leads to a life-long tolerance against previously disease-causing allergens, resulting from the restoration of normal immunity [7]. Successfully SLIT-treated patients may show a cellular and humoral immune response superimposable to healthy subjects [8]. In particular, sublingual immunotherapy is able to induce a tolerance toward the causal allergen. This effect mainly depends on allergen-specific Treg cell induction [8]. Treg cells exert their activity producing two main cytokines: IL-10 and TGF- β . Therefore, it has been reported that SLIT is able to significantly increase IL-10 production [7,8]. On the other hand, it has been reported that IL-10 significantly reduces neopterin production [9].

As neopterin has been proposed as a biomarker of activation of the immune system, this study was designed for assessing serum neopterin in AR patients considering two models: uncured patients and successfully SLIT-treated patients evaluated outside the pollen season, such as without allergic inflammation and consequently symptoms.

This study shows that SLIT may significantly affect serum neopterin levels. This phenomenon might depend on the Treg response to SLIT able to induce IL-10 production that may in turn inhibit neopterin production.

However, it has been very recently reported that allergic patients with allergic rhinitis may have impaired serum levels of neopterin and also of tryptophan and kynurenine [10]. An analysis of indoleamine 2,3-dioxygenase activity might also be of relevance to neopterin levels. Therefore, further study should be addressed to this issue.

In conclusion, this preliminary study demonstrates that serum neopterin could be a biomarker of achieved immune tolerance toward the causal allergen in allergic patients successfully treated with SLIT. Further studies should be performed for confirming these findings.

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