

## Elevated neopterin levels in non-allergic asthma

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### Abstract

Neopterin is synthesized by human monocyte-derived macrophages upon stimulation with interferon- $\gamma$  (IFN- $\gamma$ ). Measurement of neopterin concentration is useful to monitor cell-mediated (Th1-type) immune activation.

In this study, we aimed to analyze the behaviour of neopterin in long lasting asthma considering its role as a marker of the Th1 environment and to establish the distinction between patients belonging either to the allergic or the non-allergic population, particularly in the elderly where asthma is often under diagnosed. Therefore we evaluated allergic parameters such as skin prick tests, IgE and hemogram (eosinophils count), and we compared our findings with neopterin values found in an age-matched control population.

A group of individuals older than 65 was selected. It included 64 asthmatic patients (mean age  $72 \pm 5$  years) and 41 healthy individuals (mean age  $79 \pm 7$  years). In our study population, 42 patients presented positive skin tests, mainly to house dust mites. All patients were clinically stable and presented an average percentage of predicted forced expiratory volume in the first second (FEV1) of  $73.6 \pm 25.3$  and predicted median expiratory flow percentage (MEF50) of  $38.8 \pm 26.7$ .

Blood cell counts showed statistically different mean values of eosinophils between allergic and non-allergic controls ( $5.42 \pm 4.7\%$  versus  $2.8 \pm 2.8\%$ ;  $p < 0.04$ ). IgE values were increased in allergic asthmatic patients when compared with non-allergic asthmatic patients ( $493.2 \pm 549.8$  IU/ml versus  $85.3 \pm 194.4$  IU/ml;  $p = 0.000$ ).

Allergic asthmatic patients presented mean neopterin levels similar to those found in the control group ( $2.4 \pm 2.8$  ng/ml versus  $2.1 \pm 1.9$  ng/ml). In contrast, in non-allergic asthmatic patients these values were higher when compared with the control group ( $4.0 \pm 4.7$  ng/ml versus  $2.1 \pm 1.9$  ng/ml). Neopterin levels were lower in allergic asthmatic patients when compared with non-allergic asthmatic patients ( $2.4 \pm 2.8$  ng/ml versus  $4.0 \pm 4.7$  ng/ml).

Within asthmatic patients, those with higher neopterin values ( $>2.1$  ng/ml) presented lower mean IgE values (IgE  $\leq 336.58$  IU) than those with lower neopterin values ( $\leq 2.1$  ng/ml) who presented mean IgE values of 402.70 IU.

Our initial findings may lead to a better understanding of the immunoinflammatory pathways in asthma. Further studies will probably show that serum neopterin could become a useful marker for asthma classification including in elderly patients with long lasting disease.

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**Keywords:** Neopterin; Asthma; Aging; IgE; Allergy

### 1. Introduction

Neopterin is synthesized by human monocyte-derived macrophages upon stimulation with interferon- $\gamma$  during the

Th1-type immune response [1–3]. Measurement of neopterin concentration is useful to monitor cell-mediated (Th1-type) immune activation.

Asthma is a chronic inflammatory disorder of the airways [4], affecting all ages [5] with a progressive decline of pulmonary function that is correlated with age, sex disease duration and severity [6,7]. Asthma is often under diagnosed in the elderly and is sometimes associated with allergic triggers. In allergic asthma, environmental control measures can improve the disease prognosis [8].

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Cell-mediated immune response depends on activation of Th1 cells, which typically produce and release interferon- $\gamma$  and interleukin-2, whereas the activation of Th2 cells is characterized by the release of cytokines such as interleukin-4, interleukin-5, and interleukin-10. Th2 cytokines are involved in humoral immune response and trigger immunoglobulin production like IgE that is associated with allergic disorders. There is a cross-regulatory interplay between Th1-type and Th2-type immune responses associated with an inverse relationship between IgE and neopterin concentrations [1].

The studies in chronic asthma, that includes long disease evolution, can give important information since the changes observed are a result of continuous exposure to several triggers and are often irreversible.

Neopterin concentrations may also be increased in the elderly, which is associated with the fact that healthy elders develop several immune changes including activation, deregulation and impaired cell function [9,10].

The aim of this study is to analyze the level of neopterin in long-lasting asthma considering its role as a marker of Th1 environment, as well as to evaluate allergic parameters such as IgE and blood cell counts.

## 2. Material and methods

### 2.1. Population

A group of 105 elderly individuals non-currently or former smokers, which included 64 asthmatics ( $72 \pm 5$  years/42 allergic), and 41 controls ( $79 \pm 7$  years) were selected (Table 1). In this study the group of allergic asthmatics included 27 females and 15 males, while the non-allergic ones included 16 females and 6 males. Informed consent was obtained from all participants. Patients were observed in an outpatient lung disease department for intermittent chest tightness, wheezing or shortness of breath and reversible airways obstruction for more than 30 years, consistent with the diagnosis of mild persistent asthma according to Global Initiative for Asthma criteria (GINA). They were controlled using beclometasone dipropionate, 250–500  $\mu$ g, daily and all other anti-asthmatic drugs were suspended at least 4 weeks prior to this study. The control group was recruited from elderly residences.

None of the individuals had chronic sputum or had had any respiratory infection in the month previous to the study inclusion. No other clinically relevant diseases were reported.

The following were considered exclusion criteria, both for patients and controls: cancer, autoimmunity, infection,

diabetes, heart failure, renal failure, chronic hepatic and exposure the environmental risk factors.

### 2.2. Methods

All 105 subjects in this study were submitted to skin prick tests to 20 common aeroallergens ALK-ABELLO (1 mm Prick Lancetter-tames Hollister Stier): Dermatophagoides pteronyssinus, Dermatophagoides farinae, Lepidoglyphus destructor, Tyrophagus putrescenciae, Cladosporium herbarum, Alternaria tenuis, Blatella germanica, cat, dog, Dactylis glomerata, Phleum pratense, Poa pratensis, Plantago lanceolata, Taraxacum officinale, Parietaria judaica, Artemisia vulgaris, Chenopodium album, Platanus, Quercus suber e Olea europaea. Histamine dihydrochloride was used as a positive control (10 mg/ml) and saline solution used as negative control. Allergy was defined by positive skin prick test, with wheal and flare diameter 3 mm greater than the negative control to at least one aeroallergen tested.

All patients underwent clinical evaluation to fulfil the clinical exclusion criteria and performed a spirometric test using the same equipment (Vitalograph Compact) at least 6 h after the last dose of any bronchodilator. Predicted values were measured according to Knudson et al. [11]. Their spirometric performances were assessed using a computerized program according to ATS'94 criteria. The approval for analysis was determined using the ATS'94 criteria; accuracy was achieved if, within the same evaluation, three curves were acceptable and reproducible.

Thirty to 50 ml of peripheral blood was withdrawn from all participants.

Total serum IgG, IgA and IgM were measured by nephelometry and total serum IgE was measured using a commercial Kit (Coat-A-Count<sup>®</sup> Total IgE IRMA, DPC<sup>®</sup>, CA, USA) based on an Immuno-radiometric assay (IRMA) of solid phase. This kit uses monoclonal antibodies anti-IgE I<sup>125</sup>-labelled in the liquid phase and polyclonal antibodies anti-IgE attached to the wall of the polystyrene tube. Therefore, the IgE present in the tested sample is retained between these two antibodies. These tubes were measured using a gamma counter (Gamma-C-12, DPC<sup>®</sup>, CA, USA) for 1 min. Total IgE concentration in the sample is directly proportional to the counts per minute. This concentration was determined by comparison with provided calibrators. Sensitivity of this kit is 0.5 IU/ml.

Neopterin levels were measured using an enzymatic immunoassay (ImmuChem<sup>™</sup>, ICN Pharmaceuticals Inc., CA, USA) with sensitivity between 0 ng/ml and 100 ng/ml, in serum samples. The absorbance was read at 450 nm in a microplate reader. The absorbance is inversely proportional to the amount of neopterin in the sample. All samples were collected and stored protected from light and all assays were performed avoiding direct sunlight exposure [12].

All the individuals enrolled in this study were thoroughly examined, including a complete laboratory work-up, and checked for potential exclusion criteria.

Table 1  
Control group vs. asmathics group, sex and age distribution

Population	n	Male/female	Mean age	Range
Control	41	12/29	79.2 $\pm$ 7.0	65/94
Asmathics	64	21/43	72.4 $\pm$ 5.1	65/83

### 2.3. Statistical analysis

Statistical calculations were performed using SPSS 12.0 software package. Kolmogorov–Smirnov test was used to check if variables were normally distributed. For those who had a normal distribution it was used the parametric *t*-test for two independent parameters. Variables that were not distributed normally were evaluated using Mann–Whitney non-parametric test. Significance is defined as a *p*-value < 0.05. Data are expressed as the mean ± standard deviation (S.D.).

### 3. Results

All patients were clinically stable and presented an average percentage of predict forced expiratory volume in the first second (FEV1) of  $73.6 \pm 25.3$  and median expiratory flow percentage of predict (MEF50) of  $38.8 \pm 26.7$  and a tiffeneau index of  $82.8 \pm 13.1$ . The bronchial hyperreactivity studies done in all individuals with FEV1 lower than 85% of the predicted, showed a median increase of  $10.7 \pm 5.4\%$  in FEV1 after 200 µg of inhaled albuterol.

Among the asthmatic individuals, 42 subjects (65.6%) were considered allergic because they presented positive skin prick tests to common aeroallergens. Allergic patients presented positive skin prick tests to common aeroallergens and most of them were polysensitized. The non-allergic group (22 patients) did not react to common aeroallergens, though in 3 cases drug hypersensitivity had previously been reported (2 to non-steroidal anti-inflammatory drugs and 1 to penicillin).

We found a significant difference in eosinophil counts between allergic and non-allergic patients (mean values  $5.42 \pm 4.7\%$  versus  $2.8 \pm 2.8\%$  and total values  $375.6 \pm 108.2$  cells/mm<sup>3</sup> versus  $187.3 \pm 185.4$  cells/mm<sup>3</sup>; *p* < 0.04).

IgG mean values were increased in allergic asthmatic patients when compared with non-allergic asthmatic patients ( $11.9 \pm 2.5$  g/l versus  $10.4 \pm 2.2$  g/l; *p* = 0.03).

IgA and IgM mean values of allergic asthmatic patients were compared to non-allergic asthmatic patients ( $3.2 \pm 1.4$  g/l versus  $2.6 \pm 1.3$  g/l;  $1.1 \pm 0.5$  g/l versus  $1.2 \pm 0.7$  g/l, respectively) and there were no significant differences.

IgE mean levels were significantly increased in allergic asthmatic patients when compared with non-allergic asthmatic patients ( $493.2 \pm 549.8$  IU/ml versus  $85.3 \pm 194.4$  IU/ml; *p* = 0.000) (Table 2).

Neopterin was decreased in allergic asthmatic patients when compared with non-allergic asthmatic patients ( $2.4 \pm 2.8$  ng/ml versus  $4.0 \pm 4.7$  ng/ml).

Allergic asthmatic patients presented mean neopterin levels similar to those found in the control group ( $2.4 \pm 2.8$  ng/ml versus  $2.1 \pm 1.9$  ng/ml). In contrast, in non-allergic asthmatic patients these values were increased when compared with the control group ( $4.0 \pm 4.7$  ng/ml versus  $2.1 \pm 1.9$  ng/ml) (Table 3).

Table 2

Eosinophil and IgE values in asthmatics allergic group and asthmatics non-allergic group

Population	Asthmatics allergic group (mean values)	Asthmatics non-allergic group (mean values)
Eosinophil counts (%)	$5.42 \pm 4.7$	$2.8 \pm 2.8^*$
Eosinophil total (cells/mm <sup>3</sup> )	$375.6 \pm 408.2$	$187.3 \pm 185.4^*$
IgE values (IU/ml)	$493.2 \pm 549.8$	$85.3 \pm 194.4^{**}$

*t*-Parametric test—statistical significance asthmatics allergic group vs. asthmatics non-allergic group (\**p* = 0.04; \*\**p* = 0.000).

Table 3

Neopterin values in control group, asthmatics allergic group and asthmatics non-allergic group

Population	Neopterin values (ng/ml)
Control group ( <i>n</i> = 41)	$2.1 \pm 1.9$
Asthmatics allergic group ( <i>n</i> = 42)	$2.4 \pm 2.8$
Asthmatics non-allergic group ( <i>n</i> = 22)	$4.0 \pm 4.7$

Fifty-eight percent of non-allergic patients presented serum neopterin levels above normal (>2.1 ng/ml) while only 34% of allergic asthmatic had serum neopterin levels above that value (*p* < 0.05) (Table 4). Within asthmatic patients, those with increased neopterin values (>2.1 ng/ml) presented lower mean IgE values (IgE ≤ 336.58 IU) than those with decreased neopterin values (≤2.1 ng/ml), which presented mean IgE values of 402.70 IU.

Moreover, if the three non-allergic patients (without sensitivity to common aeroallergens) with drug hypersensitivity were withdrawn from the study, because they could present a shift to Th2 cytokine environment, the remaining group (*n* = 19) presented even higher neopterin levels when compared with the control group ( $4.7 \pm 4.8$  ng/ml versus  $2.1 \pm 1.9$  ng/ml; *p* < 0.05) (Table 5).

Table 4

Percentage of allergic and non-allergic asthmatics presenting increased neopterin values

Neopterin values (ng/ml)	>2.1
Asthmatics allergic group (%)	34
Asthmatics non-allergic group (%)	58*

*t*-Parametric test—statistical significance asthmatics allergic group vs. asthmatics non-allergic group (\**p* < 0.05).

Table 5

Neopterin values in control group, asthmatics allergic group and asthmatics non-allergic group

Population	Neopterin values (ng/ml)
Control group ( <i>n</i> = 41)	$2.1 \pm 1.9$
Asthmatics allergic group ( <i>n</i> = 42)	$2.4 \pm 2.8$
Asthmatics non-allergic group (without drug hypersensitivity) ( <i>n</i> = 19)	$4.7 \pm 4.8^*$

*t*-Parametric test—statistical significance control group vs. asthmatics non-allergic group (\**p* < 0.05).

#### 4. Discussion

In this study, we investigated the serum levels of neopterin, a macrophage-derived biomarker, in individuals with allergic and non-allergic asthma, and determined differences in average and overall range of its expression. Serum concentrations of neopterin and IgE were compared between both asthmatic groups and controls in order to understand the cross-regulatory interplay between Th1-type and Th2-type immune response.

One difficulty usually faced in asthma management is the proper distinction between patients belonging either to the allergic or the non-allergic population. Skin prick tests (SPT) can provide reliable information on this subject when an appropriate group of allergen extracts is selected. Currently, some medical centres are directing their efforts at obtaining a European Standard Allergen Panel to improve the accuracy of this diagnostic method [13]. There are also patients with a strong evidence of allergy that present negative SPT to common allergens. Often, high IgE levels and blood eosinophilia are observed in allergic disorders although these two parameters may also be elevated in other pathological conditions. In contrast, neopterin levels tend to decrease in allergy and to increase in other pathological disorders such as pulmonary infections. Asthma, often starts during childhood but can affect all ages and cause an important decrease in lung function parameters [8]. The individuals selected for this study, without exposure to specific inhalatory risks sources, were predominantly women. They were carefully evaluated to exclude the presence of heart disease or chronic obstructive pulmonary disease (COPD). The difficulties associated with the ageing process, such as chest muscle weakness, arthritis or decline in vital capacity should be considered in the evaluation of these patients. Although asthma is defined as a reversible disease, long lasting asthma may lead to a persistent airflow obstruction and asymptomatic patients may maintain some functional pulmonary impairment due to chronic airway inflammation. According to some authors, the narrowing of the airways and the level of bronchial responsiveness are, in adults over 65, associated with atopy and allergy diagnosis should not be neglected [14]. Although IgE levels tend to decrease with age, an IgE-mediated allergy can be present in 75% of elderly asthmatic patients [15,16]. Proper assessment to accurately diagnose allergic individuals will allow better therapeutic approaches. In fact, in allergic patients, allergen avoidance is an important goal to keep disease under control in any age group [17]. Higher neopterin levels were detected in non-allergic asthmatic patients. This change is significant if patients with drug hypersensitivity were excluded from the study group, suggesting that in these cases, a Th2 pathway or a down regulation of immunoinflammatory response could be present [18].

This finding reinforces the current theory that in humans the activation of Th1 and Th2 cell-mediated immune responses down-regulate each other [19]. Th1 cells pro-

duce IFN- $\gamma$  and IL-2, thereby stimulating human monocytes/macrophages to produce and release large amounts of neopterin, which in turn could down-regulate the humoral IgE-mediated immune response.

In humans, increased serum concentrations of neopterin have also been found in autoimmune diseases and in viral infections. Furthermore, the respiratory infections are considered main triggers in non-allergic asthma. Determination of neopterin concentrations has turned out to be a sensitive and useful way of monitoring Th1-type immune response once significant association between enhanced blood concentrations of neopterin and IFN- $\gamma$  have been observed [1].

These preliminary results could anticipate neopterin and macrophages involvement in asthma, at least in the elderly population with long lasting asthma. Accordingly, histamine, an important mediator of Th2 cell-driven allergic reaction released from mast cells, significantly suppresses neopterin formation [20].

#### 5. Conclusion

Our initial findings may lead to a better understanding of the immunoinflammatory pathways in asthma. Serum neopterin may prove a useful marker for classifying asthmatic patients, including those in the elderly population.

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#### References

- [1] M. Ledochowski, C. Murr, B. Widner, D. Fuchs, Inverse relationship between neopterin and immunoglobulin E, *Clin. Immunol.* 98 (2001) 104–108.
- [2] J. Kaski, Neopterin—a forgotten biomarker, *J. Am. Colloid Cardiol.* 42 (2003) 1140–1146.
- [3] D. Mildvan, J. Spritzler, S. Grossberg, J. Fahey, D. Johnston, B. Schock, J. Kagan, Serum Neopterin, an immune activation marker, independently predicts disease progression in advanced HIV-1 infection, *Clin. Infect. Dis.* 40 (2005) 853–858.
- [4] New NHLBI Guidelines for the Diagnosis and Management of Asthma, vol. 2, National Heart, Lung and Blood Institute, Lippincott Health Promotion Letters, 1997, pp. 8–9.
- [5] F. Radenne, C. Verkindre, A. Tonnel, Asthma in the elderly, *Rev. Mal. Respir.* 20 (2003) 95–103.
- [6] M. Kupczyk, Long-term deterioration of lung function in asthmatic outpatients, *Respiration* 71 (2004) 233–240.
- [7] A. Vignola, N. Scichilone, J. Bousquet, G. Bonsignore, V. Bellia, Aging and asthma: pathophysiological mechanisms, *Allergy* 58 (2003) 165–175.
- [8] S. Braman, J. Kaemmerlen, S. Davis, Asthma in the elderly, *Am. Rev. Respir. Dis.* 143 (1991) 336–340.

- [9] B. Frick, K. Schroecksnadel, G. Neurauter, F. Leblhuber, D. Fuchsa, Increasing production of homocysteine and neopterin and degradation of tryptophan with older age, *Clin. Biochem.* 37 (2004) 684–687.
- [10] J. Fahey, J. Schnelle, J. Boscardin, J. Thomas, M. Gorre, N. Aziz, H. Sadeghi, P. Nishanian, Distinct categories of immunologic changes in frail elderly, *Mech. Ageing Dev.* 115 (2000) 1–20.
- [11] R. Knudson, R. Slavin, M. Lebowitz, B. Burrows, The maximum expiratory flow volume curve: normal standards, variability and effects of age, *Am. Rev. Respir. Dis.* 113 (1976) 587–600.
- [12] A. Laich, G. Neurauter, B. Wirleitner, D. Fuchs, Degradation of serum neopterin during daylight exposure, *Clin. Chim. Acta* 322 (2002) 175–178.
- [13] L. Heinzerling, A. Frew, C. Bindslev-Jensen, S. Bonini, J. Bousquet, Standard skin prick testing and sensitization to inhalant allergens across Europe—a survey from the GA2LEN network, *Allergy* 60 (2005) 1287–1300.
- [14] D. Renwick, M. Connolly, Persistence of atopic effects on airway calibre and bronchial responsiveness in older adults, *Age Ageing* 26 (1997) 435–440.
- [15] K. Huss, P. Naumann, P. Mason, Asthma severity, atopic status, allergen exposure, and quality of life in elderly persons, *Ann. Allergy Asthma Immunol.* 86 (2001) 524–530.
- [16] N. Scichilone, M. Messina, S. Battaglia, F. Catalano, V. Bellia, Airway hyperresponsiveness in the elderly: prevalence and clinical implications, *Eur. Respir. J.* 25 (2005) 364–375.
- [17] P. Enright, R. McClelland, A. Newman, D. Gottlieb, M. Lebowitz, Underdiagnosis and undertreatment of asthma in the elderly, *Chest* 116 (1999) 603–613.
- [18] G. Menz, J. De Sousa, T. Rothe, M. Schmitz-Schumann, C. Virchow, Neopterin in phases of exacerbation of bronchial asthma and in experimental asthma conditions, *Pneumologie* 44 (1990) 232–233.
- [19] C. Murr, U. Hainz, E. Asch, P. Berger, B. Jenewein, M. Saurwein-Teissl, Association of increased neopterin production with decreased humoral immunity in the elderly, *Exp. Gerontol.* 38 (2003) 583–587.
- [20] A. Gruber, C. Murr, B. Wirleitner, G. Werner-Felmayer, D. Fuchs, Histamine suppresses neopterin production in the human myelomonocytoma cell line THP-1, *Immunol. Lett.* 72 (2000) 133–136.