

Clinical Relevance of Nerve Growth Factor Serum Levels in Patients with Atopic Dermatitis and Psoriasis

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Key Words

Atopic dermatitis • Eosinophil cationic protein • IgE • Neurotrophins • Nerve growth factor • Psoriasis

Abstract

Background: Nerve growth factor (NGF) is known to act as a potent mediator in neuroinflammatory processes. Recent studies point to a role of NGF in the skin pathophysiology of atopic dermatitis (AD) and psoriasis. Hereby, NGF was found to interact with the major cellular components (mast cells and eosinophils) of both skin diseases. **Objectives:** In order to investigate NGF serum levels as a possible clinical marker of disease activity and immunological status, we determined serum NGF, eosinophil cationic protein (ECP), total IgE and score of AD (SCORAD) in 57 patients with AD as well as NGF and the psoriasis area and severity index (PASI) in 17 patients with psoriasis. Fifty healthy subjects served as controls. **Methods:** We used a highly sensitive improved fluorometric two-site ELISA system for serum NGF detection. IgE and ECP were measured by CAP-FEIA and radioimmunoassay. **Results:** We did not find a significant correlation between NGF

and either ECP, total IgE, or severity of disease assessed by SCORAD. Also in patients with psoriasis, there was no significant correlation with disease activity determined by PASI. **Conclusion:** Even though there is increasing evidence showing NGF to be involved in the local inflammatory pathophysiology of AD within skin lesions, our findings suggest that NGF systemic serum concentration is not a suitable parameter to estimate the clinical or immunological status of AD or psoriasis patients. This result is inconsistent with some previous studies showing a positive correlation of serum NGF with AD severity, which might be, for example, due to the fact that the present results are corrected for unspecific binding.

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Introduction

Apart from its long known role in neuronal development, survival and homeostasis [1], nerve growth factor (NGF) plays an important role in immunoregulation and inflammation [2, 3]. Especially allergic inflammation has been pointed out to be relevantly modulated by NGF [4–7]. There is now striking evidence that local immune processes in the skin are under control of NGF including

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wound healing, allergic and autoimmune and stress-related skin disorders [8–10].

Atopic dermatitis (AD) is a chronic inflammatory skin disease. Current data point to a crucial role of local immune cell-derived NGF (especially mast cells and eosinophils) in the pathophysiology of AD. It represents a target of mast cell-nerve interaction that is thought to crucially contribute to the development and progression of chronic inflammatory lesions [11] and abnormal skin sensitivity [12] in AD. Also in psoriasis, a second very common chronic inflammatory skin disease, NGF was shown to be relevantly involved in its immunopathophysiology [for a review, see Botchkarev et al. 8]. In patients with AD, increased levels of serum NGF [11, 13] but also of another neurotrophin, the brain-derived neurotrophic factor (BDNF), were reported [14, 15]. However, in a previous study, we did not find a positive relation between NGF serum levels in AD patients compared to healthy controls [16].

Since there is little and conflicting available evidence on NGF as a possible clinical marker of disease activity in allergic skin diseases, this study investigates to what extent serum levels of NGF correlate with the clinical phenotype and severity of AD. In order to distinguish between AD-specific and possibly AD-unspecific inflammation-dependent NGF alterations, we also investigated NGF serum levels in psoriasis, a second common skin disease.

Patients and Methods

Patients and Blood Samples

A total of 57 patients (31 males, 26 females; mean age 33.4 years with a range of 18.7–76.1) with a confirmed diagnosis of AD according to the criteria of Hanifin and Rajka, from the Department of Dermatology, University of Schleswig Holstein, Campus Kiel, Germany, and 17 patients (11 males, 6 females; mean age 54.6 years with a range of 30–81) with a confirmed diagnosis of psoriasis, from the Department of Dermatology, University of Hamburg, Germany, were enrolled in this study. Patients did not receive systemic medication for at least 1 week before NGF testing nor local steroids. All diagnoses were approved by a senior dermatologist. A total of 50 healthy volunteers participated in this study. The volunteers, including 26 male and 24 female subjects (mean age 48.7 years with a range of 26–72), were recruited by newspaper advertisement. Any allergic or psychiatric disorder in their medical history and any history of cardiopulmonary or metabolic diseases led to their exclusion from the study. After complete description of the study to the subjects, written informed consent was obtained. This study was conducted according to the ethical standards of the local ethics committees.

After blood take, the samples were directly centrifuged and stored at -20°C . Since the multicenter approach of the study did

not guarantee a parallel generation of the sera, samples of healthy control subjects were taken repeatedly at time points corresponding to the disease groups to control for possible age-dependent degradation of NGF concentrations. Age/storage of the samples did not have a significant impact on NGF levels during the study.

Determination of the Severity of Skin Diseases

The severity of AD was assessed by means of the score of AD (SCORAD) scale [17] taking into account the extent and the severity of erythema, edema/papulation, oozing/crust, excoriation, lichenification and dryness. The score also includes the evaluation of pruritus and sleep loss. The dermatological state of patients with psoriasis was assessed by the psoriasis area and severity index (PASI) described in detail in Fredriksson and Pettersson [18].

Determination of NGF

NGF levels in the AD patient sera were quantified by fluorometric enzyme-linked immunoabsorbent assay (ELISA) with a detection limit of 50 fg NGF/assay as described elsewhere in detail [19, 20]. The previous protocol for this two-site ELISA was simplified in part and these modifications proved not to impair the accuracy and sensitivity of the assay [21]. The measured levels of NGF were corrected for mean recoveries of added mouse 2.5S NGF (125 pg ml^{-1}), which were determined in each assay. Determination of recovery (defined as the quotient of detected exogenous NGF which has been added to each sample prior to NGF determination), specific and unspecific NGF binding (the latter against mouse IgG1 obtained from MOPC 21) involved quadruplicate fluorescence determinations for each serum sample [21].

Immunological Analyses

The concentrations of total IgE and eosinophil cationic protein (ECP) were measured by CAP-FEIA and radioimmunoassay commercial kits, respectively, according to the manufacturer's instructions (Pharmacia, Freiburg, Germany). All assay measurements were performed in duplicate.

Statistics

Data are presented as median and 25th and 75th percentile in box plots and correlation including the regression line. Means were compared using the Kruskal-Wallis one-way analysis of variance by ranks. For correlation analyses, the Spearman rank order correlation with SigmaStat was used.

Results

In the serum of the 57 patients with AD, NGF was detected at a median of 7.67 pg/ml (25th percentile 4.56 pg/ml, 75th percentile 29.123 pg/ml; fig. 1). NGF serum concentrations did not differ ($p = 0.059$) from those of healthy subjects ($n = 50$, median NGF concentration 10.82 pg/ml, 25th percentile 9.88 pg/ml, 75th percentile 11.66 pg/ml). There was no significant correlation between NGF levels and clinical severity of AD assessed by SCORAD (scores ranging from 7 to 87; $r = -0.014$, $p = 0.919$; fig. 2). To investigate a possible relation between NGF levels and sin-

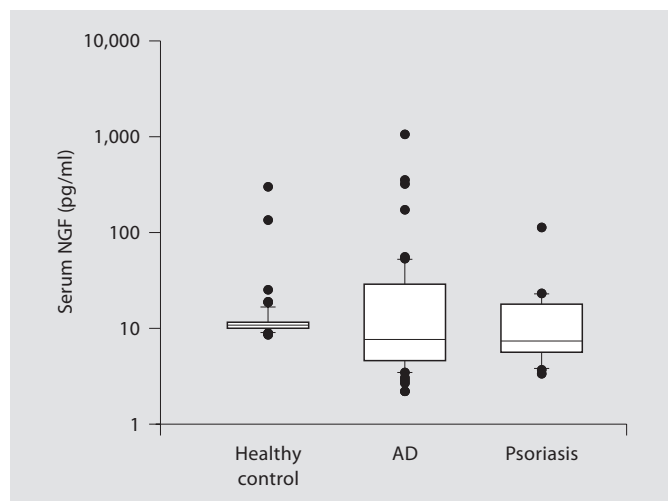


Fig. 1. Box plots showing NGF serum concentrations (in logarithmic scale) of healthy subjects ($n = 50$), patients with AD ($n = 57$) and patients with psoriasis ($n = 17$) measured by highly sensitive fluorometric two-site ELISA.

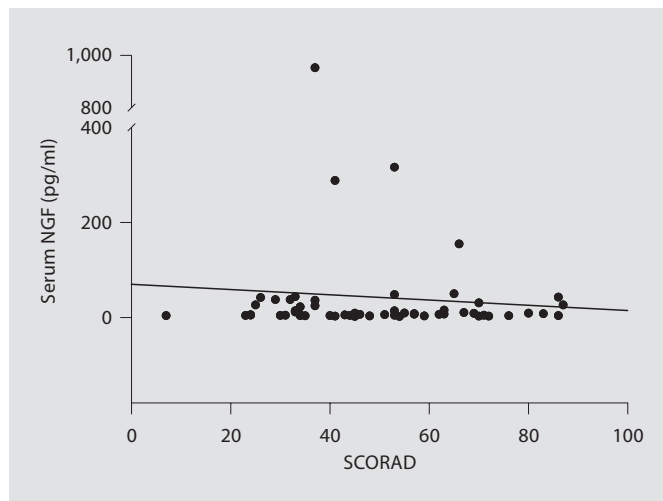


Fig. 2. Correlation including the regression line of NGF with clinical severity (assessed by SCORAD) of 57 subjects suffering from AD. Each dot represents one individual. Correlation was calculated with SigmaStat using Spearman's correlation coefficient.

gle symptoms and chronicity of AD, a correlation analysis between NGF and the severity of erythema, excoriation, lichenification and oozing/crusting was performed. There was no significant correlation between the severity of any of these symptoms and the serum NGF concentration (erythema $r = 0.007$, $p = 0.960$; excoriation $r = 0.025$, $p = 0.860$; lichenification $r = 0.003$, $p = 0.982$; oozing/crusting $r = 0.063$, $p = 0.654$). Furthermore, neither total specific IgE (median 1,072.50 kU/l, 25th percentile 123.50 kU/l, 75th percentile 6,100.00 kU/l) nor ECP (median 27.10 $\mu\text{g/l}$, 25th percentile 14.45 $\mu\text{g/l}$, 75th percentile 58.90 $\mu\text{g/l}$) showed a significant correlation with NGF levels (IgE, $r = -0.043$, $p = 0.752$; ECP, $r = 0.074$, $p = 0.612$). Additional analyses of a possible relation between the age of the outbreak of the disease (mean: at the age of 6 years, range 1–36) and NGF plasma levels did not reach significance ($r = 0.031$, $p = 0.817$).

In patients with psoriasis ($n = 17$), serum NGF showed a median of 7.16 pg/ml (25th percentile 5.13 pg/ml, 75th percentile 13.16 pg/ml). There was no significant difference between NGF serum concentrations in our healthy controls and NGF levels in patients suffering from psoriasis ($p = 0.059$) (fig. 1). PASI as an indicator of clinical activity ranged from 5.7 to 51. There was no significant correlation between NGF serum levels and PASI score ($r = 0.18$, $p = 0.48$) (fig. 3).

Determination of NGF recovery revealed a median recovery in AD patients of 105.14% (25th percentile 97.18%,

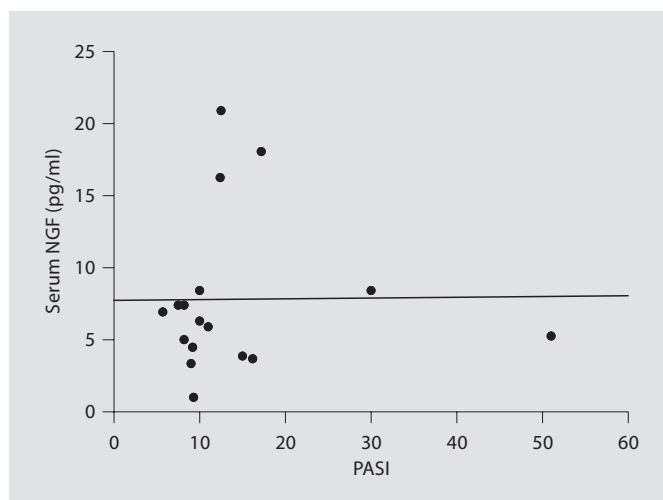


Fig. 3. Correlation including the regression line of NGF with clinical severity (assessed by PASI) of 17 subjects suffering from psoriasis. Each dot represents one individual. Correlation was calculated with SigmaStat using Spearman's correlation coefficient.

75th percentile 121.20%), in psoriasis patients of 91.46% (25th percentile 77.69%, 75th percentile 110.19%) and in healthy controls of 100.28% (25th percentile 79.64%, 75th percentile 115.07%). The recoveries between the various groups did not differ significantly ($p = 0.08$).

Discussion

In this study, we provide evidence that NGF serum concentrations do not significantly differ from our healthy controls. In a previous study, we could demonstrate that the repetitive measurements of NGF serum concentrations over 4 weeks are very stable. It has thus been shown that this neurotrophin is an intraindividually solid marker [20]. Moreover, we show that at least in our clinically and immunologically well-characterized patients with AD, serum NGF is not significantly correlated with clinical severity of the disease nor with serum IgE levels. Hence, both results of an independent and larger sample of 57 patients represent a replication of our previous study including 23 patients with AD [16]. This is the first study on NGF levels that not only considers global severity of AD by SCORAD but specifically analyzes single signs of clinical severity and chronicity. None of these symptoms showed a significant relation to NGF serum concentration. Additionally, we show that there is no relation between ECP or total IgE and serum NGF. In our sample, NGF serum concentrations differ between individuals, and 7.0% of the participants revealed relatively high NGF serum concentrations (>100 pg/ml). In previous studies, we have shown this nonparametric distribution of NGF serum concentrations in healthy volunteers (7.7% >100 pg/ml) [20] and even in aged men and women [21].

In AD, increasing data point to a role of NGF in cutaneous neurogenic inflammation by the augmentation of the secretion of neuropeptides [22]. Apart from neuron-derived NGF in the central and peripheral system, NGF secretion has been demonstrated in immune cells including T-cells, B-cells, monocytes, eosinophils, mast cells but also in structural cells like fibroblasts, keratinocytes, epithelial cells, and smooth muscle cells [11, 12, 23]. To what extent each of these sources contributes to NGF concentrations in the serum remains unclear. Some authors assume an interaction of these mediators and mast cells in AD. Interestingly, NGF was shown to induce intracellular tryptase activity and histamine content in the human mast cell line 1 [11]. Furthermore, eosinophils were shown to express the specific high-affinity receptor TrkA and low-affinity p75 receptors of NGF suggesting an additional pathway regulating inflammatory responses in allergic reactions [24, 25]. Additionally, a former study shows a positive correlation between major basic protein, disease activity and NGF serum levels [13]. However, in contrast to the latter, we do not find a significant correlation between NGF levels, disease activity and the

eosinophil marker ECP in our 57 patients with AD. This discrepancy might be due to the fact that this is the first study dealing with NGF levels in patients with AD that explicitly considers the rate of spike recovery of exogenous NGF within the serum quantification of endogenous NGF by ELISA. Especially, diseases going along with increased immunoglobulin levels are susceptible to interfere with antibody-linked detection systems. So far, little attention has been paid to endogenous circulating anti-NGF antibodies in the serum. Even though Fabricant and Todaro [26] have shown the existence of those autoantibodies in pooled human sera, few is known about their physiological relevance or their influence on NGF measurement. It has just been revealed that NGF as well as anti-NGF antibodies might not be detectable because of NGF/NGF antibody complexes [27]. In our improved two-site fluorometric ELISA system, we were able to control unspecific binding as well as possible and reduce interference of endogenous autoantibodies by adding an excess of unspecific IgG1 antibodies to the sample [21]. Apart from possible ethnic differences, the fact that our NGF measurement reflects NGF levels without signals from unspecific binding of, for example, endogenous antibodies might also explain why our mean NGF concentration of 42.47 pg/ml differs from serum NGF levels of previous studies in AD patients in Japan (mean serum NGF: $2,314.5 \pm 726.2$ pg/ml [13]). Only in schizophrenic patients with cannabis abuse [28] and patients with acute Behcet's disease [6] have we been able so far to detect similarly high NGF serum concentrations by means of our improved NGF ELISA. Hence, it seems uncertain whether the different study populations of patients with AD are comparable.

The findings of this study are in contrast to the regulation of the neurotrophin BDNF which has repeatedly been shown to be increased in AD [14, 15]. Thus, the present study points to a differential regulation of these structurally related mediators in this disease. In the serum, a differential regulation of BDNF, which is primarily stored in circulating platelets [29], and NGF has also been reported under different conditions such as the menstrual cycle [29, 30] and the perinatal period in women [30, 31].

To our knowledge, this is the first study investigating systemic NGF levels in patients with psoriasis, the second highly prevalent chronic inflammatory skin disease. In contrast to AD, which has been shown to be a T helper cell 2 (Th2)-driven disease, psoriasis rather presents a Th1-driven disease [32]. In psoriasis, locally enhanced NGF was detected by immunohistochemical studies in

keratinocytes in lesional and nonlesional psoriatic tissue compared to controls [33, 34]. In the severe combined immunodeficient mouse-human skin xenograft model of psoriasis, the administration of the high-affinity NGF receptor (TrkA) inhibitor K52a was tested in a double-blinded, placebo-controlled manner. Treatment with K52a led to a significant improvement of psoriatic plaques [35]. TrkA inhibition in NC/nga mice, a well-characterized mouse model of AD with increased NGF in lesional skin [36], significantly inhibited the development of scratching behavior and dermatitis and reduced nerve fibers in the epidermis [37, 38]. Thus, there is first evidence for a relevance of NGF as immunomodulator also in psoriasis. In contrast, our data show serum NGF levels to be neither elevated compared to control subjects nor correlated with disease activity.

In conclusion, the present study on NGF in patients with AD does confirm our pilot study [16] reporting that NGF serum levels are not a clinical marker that is positively correlated with severity of AD or the eosinophil basic protein. In addition, in patients with psoriasis, serum NGF does not suit as a clinical marker of disease activity. Therefore, we draw the conclusion that neither a Th1-activating skin disease (psoriasis) nor the predominantly Th2-driven pathology in AD [32] change NGF concentrations systemically. On the other hand, there are data from previous studies of altered NGF within the inflamed tissue in both diseases pointing to a functional role of NGF on a local rather than on a systemic level, which needs to be elucidated further.

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