

CLINICAL REPORT

Serum Neopterin as an Objective Marker of Psoriatic Disease Activity

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Neopterin is a non-specific marker of the activation of cell-mediated immunity. Several studies have demonstrated the crucial role of CD4⁺ T cells in the pathogenesis of psoriasis. We have measured serum and urine neopterin levels and urine neopterin/creatinine ratios by radioimmunoassay in 24 patients with plaque-type psoriasis before and after a course of topical treatment with triamcinolone acetonide 0.1% and coal tar 4%. Results were compared with a group of 20 healthy, non-psoriatic volunteers. Serum neopterin levels were significantly elevated in the psoriatic group compared with the control group ($p=0.001$) and were significantly reduced after treatment ($p=0.01$). There was a correlation between pretreatment serum neopterin levels and psoriasis area and severity scores (PASI) ($r=0.37$, $p=0.03$) and also for pretreatment neopterin/creatinine ratios and PASI scores ($r=0.45$, $p=0.01$). These findings indicate that serum neopterin concentrations reflect disease activity in psoriasis. **Key words:** neopterin; psoriasis; psoriatic activity.

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Psoriasis is a common skin disorder that lacks objective methods for evaluating disease severity, especially when the efficacy of different treatments are compared (1). Since 1978, the psoriasis area and severity index (PASI) has been used in most clinical trials to assess the efficacy of a treatment (2), but there is a high inter-observer variability in the calculation of the PASI score (3) and other assessment techniques (instrumental, histometrical and biochemical) are required.

Neopterin, and the closely related biopterin, are members of a group of compounds known as pteridines, containing a ring which differs in the state of oxidation and may exist in the tetrahydro-, dihydro-, or a fully oxidized form (native neopterin). Neopterin and its reduced derivatives (7,8 dihydroneopterin and 5,6,7,8 tetrahydroneopterin) are synthesized *in vivo* from guanosine triphosphatase (GTP) via a series of reactions; the first is catalysed by GTP-cyclohydrolase-I (4). Neopterin appears to be produced by human monocytes and macrophages in response to stimulation with interferon-gamma (IFN- γ) released from activated T cells (5). Thus, neopterin is a non-specific activation marker of cell-mediated immune system. However, human umbilical vein endothelial cells (6) and B cells (7) are also capable of producing neopterin. Furthermore, cytokines (IL-1, IL-2, IL-6 and α -TNF) (8) and lipopolysaccharides

(9) may generate neopterin by induction of IFN- γ , by synergy with low levels of induced IFN- γ , or by IFN- γ -independent mechanisms.

At present, a definite biochemical and physiological function of neopterin and its derivatives has not been established, but it seems that pteridines play an important role as endogenous antioxidants (10).

The determination of native or oxidized forms of neopterin levels may, to some extent, reflect activity of cellular immune mechanisms (11), and thereby the activity of autoimmune diseases that are mainly mediated by T cells (12,13), malignancy (14,15) viral infections (including HIV) (16) and allograft rejection of transplant recipients (17–19).

Psoriasis is a chronic, inflammatory skin disorder, caused by a persistent T-lymphocyte-mediated immune response to a hitherto unidentified antigen (20). Several authors have studied neopterin levels in psoriasis. Fuchs and co-workers (21–23) reported that only a minority of patients with severe psoriasis presented with increased neopterin levels either in urine or serum. However, improvement during therapy was paralleled by decreasing neopterin concentrations. De Rie et al. (24), found that serum neopterin levels were substantially raised in only 1 of 9 untreated patients. Harland et al. (25), studying 40 psoriatic patients, reported a significant elevation of urine neopterin and neopterin/creatinine ratios, compared with the control group. Urine neopterin levels and neopterin/creatinine ratios were significantly reduced following treatment and there was a good correlation between pretreatment neopterin levels and neopterin/creatinine ratios with PASI scores.

In the present study, we measured serum and urine neopterin levels in patients with psoriasis and studied the correlation between disease activity and neopterin levels.

MATERIALS AND METHODS

Patients and healthy controls

Twenty-four patients with plaque-type psoriasis (12 females, 12 males; median age 48.5 years, range 19–73) were studied. At the time of enrolment, none of the patients had received either local or systemic treatment for at least 4 weeks. Urine and serum neopterin levels were measured 2 weeks before and after topical treatment with triamcinolone acetonide 0.1% and coal tar 4% in a combined cream. The disease severity was assessed in all the patients by means of the PASI score (2) before and after treatment, always by the same dermatologist (M.S.R.).

Twenty healthy, age- and sex-matched volunteers were used as controls (11 females, 9 males; median age 43.5 years, range 19–72).

Patients and controls were excluded from the study if there was evidence of renal or hepatic dysfunction or medication with methotrexate, cyclosporin A, retinoids or folates.

Neopterin measurements

The serum and urine neopterin concentrations were determined by radioimmunoassay (RIA), according to the manufactures instructions (IMMUtest Neopterin, Henning Berlin GMBH, Berlin). Briefly, this RIA is based on the competition of unlabelled neopterin derived from the samples and HPLC (high-performance liquid chromatography)-purified ^{125}I -radiolabelled neopterin for the binding sites of the specific sheep IgG antibody (double antibody technique). There is an excellent correlation between IMMU test neopterin and HPLC procedure, which is considered to be the gold standard.

Urine and serum neopterin levels, urine creatinine levels and neopterin/creatinine ratios were assessed for every subject.

Early morning urine specimens were used. Urine creatinine concentrations and neopterin/creatinine ratios were measured in an attempt to overcome diurnal variability of neopterin excretion.

Statistical analysis

Statistical analysis was conducted using SPSS for Windows statistical software. All results were expressed by means \pm standard deviation (SD) and analysed by student *t*-test. A stepwise multiple regression procedure was carried out; *p* values of <0.05 were regarded as significant.

RESULTS

The levels of serum neopterin and urine neopterin/creatinine ratios in both psoriatic patients and controls are summarized in Table I. Serum neopterin was significantly elevated in the psoriatic group compared to the controls ($p=0.001$).

In the psoriatic group, a clinical improvement was experienced by all patients (pretreatment PASI 4–40, mean 12.26; post-treatment 1–10, mean 3.22; $p<0.01$) but only serum neopterin levels were significantly reduced following treatment ($p=0.01$) (Table II). In the majority of cases, the

Table I. Serum and urine neopterin levels, and urinary neopterin/creatinine ratios in psoriatic patients and healthy controls

	Controls	<i>p</i>	Psoriatic patients
Number of subjects	20		24
Serum neopterin ($\mu\text{mol/l}$)	4.3 ± 0.7	0.001	6.1 ± 2.7
Neopterin/creatinine ratios in urine ($\mu\text{mol/mol}$)	124 ± 34.9	n.s.	144.6 ± 64.7

Values are means \pm SD
n.s. = not significant

Table II. PASI scores, serum and urine neopterin levels, and neopterin/creatinine ratios in psoriatic patients before and after treatment ($n=24$)

	Before treatment	<i>p</i>	After treatment
PASI scores	12.2 ± 8.4	<0.01	3.2 ± 1.9
Serum neopterin ($\mu\text{mol/l}$)	6.1 ± 2.7	0.01	5.2 ± 1.8
Neopterin/creatinine ratios in urine ($\mu\text{mol/mol}$)	144.7 ± 64.7	n.s.	148.6 ± 60

Values are means \pm SD
n.s. = not significant

neopterin levels decreased in parallel with the clinical improvement.

A significant relationship was observed between the serum neopterin levels and age in untreated patients ($r=0.3$, $p=0.01$) and also between the neopterin/creatinine ratios and age ($p<0.03$).

A correlation was observed between pretreatment serum neopterin levels and PASI scores ($r=0.37$, $p=0.03$) and also for pretreatment neopterin/creatinine ratios and PASI scores ($r=0.45$, $p=0.01$). Since both variables were related to age, they were adjusted for this by means of a multiple regression analysis. As a result an improved correlation was found between serum neopterin levels and PASI scores ($r=0.44$, $p=0.01$).

DISCUSSION

In contrast to previous work, our study indicates that serum neopterin levels are significantly elevated in patients with plaque psoriasis compared with non-psoriatic control subjects and, also, that these values are reduced by successful topical treatment. Furthermore, a strong correlation was found between pretreatment serum neopterin levels and PASI scores. Thus, our results show that serum neopterin levels may be a marker of psoriatic disease activity and therefore useful for evaluating the efficacy of treatment.

In another study, Harland et al. (25) found that urine neopterin levels were significantly elevated in psoriatic patients and reduced by treatment with dithranol, tar plus UVB or PUVA. By contrast, Sepp et al. (21–23) and De Rie et al. (24) have reported that only a minority of patients had elevated neopterin levels either in urine or serum, however in 6 of 7 patients PASI and neopterin levels were closely correlated during treatment with cyclosporin A (23).

There is some controversy concerning the methodology used for neopterin measurements (26). In previous studies (23,25), neopterin was assessed in urine samples by HPLC and serum neopterin levels were measured by radioimmunoassay (23). Harland et al. (23) measured only urine neopterin levels, because the concentration of neopterin is a thousand fold higher in urine than in serum. In our study, serum and urine neopterin were measured by RIA (IMMUtest) and, surprisingly, only serum neopterin levels and not urine concentrations were elevated in psoriatic patients. Due to the excellent correlation between IMMUtest and HPLC, it is confirmed that the RIA method for the determination of neopterin is accurate and specific for research and routine laboratory work.

In our study, we have employed early morning urine samples for urine neopterin measurements, despite the fact that pterin concentrations remain constant throughout the day (27). In an attempt to avoid diurnal variability of urine concentrations, urine neopterin levels were also expressed as a ratio (μmol neopterin/mol creatinine). There was a good correlation between pretreatment neopterin/creatinine ratios and PASI scores.

Neopterin measurements may be increased in the course of diseases where there is activation of cellular immunity, viral infections, or certain autoimmune disorders; it may also be elevated in malignant diseases, renal or hepatic dysfunction or during treatment with methotrexate. These conditions should be excluded when a neopterin determination is performed.

As neopterin is produced by human monocytes and macrophages upon stimulation with $\text{IFN}\gamma$, increased levels indicate T-cell activation and $\text{IFN}\gamma$ production (28). Activated, HLA-DR+, CD4+ T lymphocytes are found in both the epidermis and dermis of active psoriatic plaques (29). The cytokine profile within psoriatic plaques is characterized by predominance of IL-2, IL-12 and $\text{IFN}\gamma$; thus indicating a Th1 profile disease (30). In the study of De Rie et al. (31), the levels of soluble IL-2 receptor (sIL-2R), a T-cell activation product, were well correlated with disease activity in patients with psoriasis and the authors concluded that, like neopterin, quantitation of sIL-2R can be used to monitor therapeutic responses.

Our data and that of others (25) show that patients with psoriasis present with significantly elevated neopterin levels either in urine or serum compared with control subjects. These findings confirm a role for cell-mediated immunity in the pathogenesis of psoriasis.

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