Neopterin in Autoimmune Rheumatic Diseases

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Abstract

Neopterin concentrations in body fluids allow to monitor the activation status of the cellular (=Th1-type) immune system in an easy but also sensitive way. Autoimmune disease result from deterioration of almost all immune system compartments. Rheumatic disorders comprise an important group of diseases featuring several aspects of autoimmune disorders. Already several years ago increased neopterin concentrations were demonstrated in patients with rheumatoid arthritis and later on in patients with systemic lupus erythematosus. Neopterin concentrations were found to correlate with the activity of the diseases and to conveniently indicate effects of therapy. Similar data were obtained in patients with acute rheumatic fever, and more recently, the utility of neopterin determination was also demonstrated in patients with Wegeners granulomatosis and with polymyositis/dermatomyositis. In this article we intend to summarize the current knowledge about the usefulness of neopterin measurements in patients with rheumatic diseases.

Key words : Neopterin, Autoimmune disease, Rheumatic disease

Introduction

Systemic rheumatic diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), polymyositis/dermatomyositis (PM/DM) and Wegeners granulomatosis (WG) are multifactorial auto-immune disorders characterized by a diversity of clinical features which are matched by the many functional defects identified in cells of the immune system. Defects of cytokine secretion which originate in the imbalance within subpopulations of CD4+ T helper (Th) lymphocytes (Th1 and Th2 lymphocytes) seem to play a fundamental role in the immunopathogenesis of autoimmune rheumatic dis-

eases (1). Th1 lymphocytes produce mainly interleukin-(IL)-2, interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α) are primarily responsible for cell mediated immunity, while Th2 lymphocytes produce mainly IL-4, IL-5, IL-13, and IL-10, and are an important component of humoral immune response, supporting the activation of immunoglobulin secreting cells (2). Recent data show different patterns of cytokine production (Th1 and Th2) between patients with autoimmune rheumatic diseases. In particular, based on these differences, RA (3-6) and WG (7) are considered to be "Th1 cytokine driven" diseases, whereas SLE (8,10) and PM/DM (9) are considered to be "Th2-cytokine dependent" diseases. However, patterns of cytokines seem to be controversial in autoimmune rheumatic diseases. In fact, different clinical presentations of diseases are characterized by distinct cytokine secretion

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profiles. For example, the elevated serum concentrations of IFN- γ and TNF- α (11), both Th1-type cytokines, were found in SLE patients with lymphadenopathy, nephrotic syndrome and thrombocytopenia, respectively. Moreover, accurate evaluation of cytokine levels is very difficult due to their short serum half-life (12), rapid uptake/utilization *in vivo* (13) and/or the presence of blocking factors such as soluble receptors on inhibitors in serum. (14,15). An alternative approach is to measure serum concentrations of biologically stable products of immune activation.

From a clinical point of view, neopterin is a particularly useful marker and increased production of neopterin is characterized by the synthesis of a certain pattern of cytokine in human disease states. Increased concentrations of neopterin in serum and urine have been observed in patients with a number of neoplastic, infectious, and immune mediated diseases (16), including autoimmune rheumatic diseases (17). Neopterin, a by-product of the guanosine triphosphate pathway, is produced by activated macrophages upon stimulation with IFN- γ (Th1-type of cytokine) (18). In other cells, only small production rates of neopterin could be demonstrated *in vitro* and it seems that they are of minor relevance as an additional source of increased neopterin concentrations in patients. TNF- α has been found to be a potent costimulus for the production of neopterin (19-21), and *vice versa*, exogenous neopterin increased TNF- α synthesis in human macrophages (22) and vascular smooth muscle cells (23). These findings are particularly important since significant amounts of TNF- α have been documented in the tissue and blood of patients with autoimmune rheumatic diseases, e.g., RA. Serum concentrations of both types of sTNF-Rs correlate to some degree with levels of TNF- α , and elevated serum levels of sTNF-55R and sTNF-75R also correlate with neopterin in patients with autoimmune rheumatic diseases.

In this article our findings in patients with autoimmune rheumatic diseases are reviewed and extended.

Pnts and Methods

The general characteristics of patients with

autoimmune rheumatic disease which are summarized in this study are shown in Table 1. The diagnosis of SLE was based on the revised criteria of American Rheumatism Association (1982) (24). The Index of European Consensus Lupus Activity Measurement (ECLAM) was used for calculating disease activity (25).

Patients with RA met American College of Rheumatology criteria for classical RA (26). The relationship between clinical variables and serum neopterin levels was investigated. Disease activity and disease severity indices were determined as described by W. Wilkey, *et al.* for more accurately assessment (27). Disease activity index includes the following 4 parameters: pain (none=score 0, intermittent=score 1, constant=score 2), duration (min) of early morning stiffness (<15=score 0, 15-30=score 1, >30=score 2), total count of swollen and/or tender joints (0-2=score 0, 3-5=score 1, >5=score 3); ESR (mm/h): <30=score 0, 30-45=score 1, >45=score 2). Total maximum score is 8. Severity disease index was computed by adding the prognostic factors to disease activity score. Prognostic factors include the following parameters: deteriorating functional capacity during previous 6 months - score 1; sustained (>6 months) elevated ESR (>30 mm/h) - score 1; RF titer (>1:640 latex) - score 1; extraarticular disease (rheumatoid nodules, inflammatory eye disease, sicca complex, vasculitis, and pleural or pulmonary disease - score 1. Total score is 5. Articular damage was assessed radiologically by modified Sharps method.

Patients with PM/DM fulfilled the definite criteria of Bohan and Peter (28) for PM and DM. Fifteen patients with primary PM, 13 patients with primary DM and 16 patients with myositis and systemic sclerosis in overlap were investigated. The global clinical assessment was based on a physical examination, measurement of muscle strength using the MRC scale (neuromuscular score) and activities of daily living questionnaire (ADL).

Patients with WG fulfilled the American College of Rheumatology criteria for WG (29). Among 26 patients, 17 were considered to have generalized WG and 9 were classified as having a limited form of disease. The Birmingham Vasculitis Activity Score (BVAS) was used to evaluate disease activity (30).

Table 1. Characteristic of patients with autoimmune rheumatic diseases

	Nr of patients	Sex Female/male	Age Mean(range)
Systemic lupus erythematosus	52	47/5	31
Rheumatoid arthritis	67	57/10	45
Polymyositis/dermatomyositis	44	33/11	38
Wegener's granulomatosis	26	15/11	42.2

Serum neopterin was measured by radioimmunoassay or ELISA (BRAHMS, Berlin, Germany). Concentrations of 55 kDa sTNF-R and sIL-2R were also measured by commercially available ELISA. All tests were performed according to the manufacturers instructions. The upper normal limit of all immune markers was taken as the mean of an internal control group ± 2 S.D.: neopterin 8.8 nmol/l (5.2 ± 1.8 nmol/l, $n=31$); sTNF-55R 4.2 ng/ml (2.55 ± 0.78 ng/ml); sIL-2R 913 U/ml (509 ± 202 U/ml).

Results

As shown in Table 2, serum neopterin concentrations were found to be raised in 73% of patients with SLE, in 47% of patients with RA, in 80% of patients with PM/DM and in 50% patients with WG. Mean values of neopterin in patients with autoimmune rheumatic diseases were significantly higher as compared with healthy volunteers ($p < 0.0001$ in all cases). Mean values or frequency of neopterin elevations above normal did not significantly

Table 2. Serum concentrations of neopterin in patients with autoimmune rheumatic diseases and in normal controls (mean $\pm$ S.D., percent with levels above the 95th percentile of healthy controls)

Disease	Neopterin (nmol/l)
Sme lupus erythematosus n=52	10.5 ± 6.1 (73%)
Rheumatoid arthritis n=55	10.5 ± 6.1 (47%)
Polymyositis/dermatomyositis n=44	20.0 ± 14.6 (80%)
Wegener's granulomatosis n=27	10.0 ± 11.0 (50%)
Controls n=31	5.2 ± 1.8 (5%)

Table 3. Spearman rank coefficients (rs) of correlation between serum neopterin concentrations and the main clinical and laboratory parameters in patients with rheumatoid arthritis

	rs	p
Ritchie articular index	0.409	0.0001
Lansbury articular index	0.402	0.001
Activity index	0.374	0.002
Severity index	0.441	0.0001
C-reactive protein	0.335	0.02
ESR	0.458	0.0001

cantly differ between groups of patients with different diseases.

In SLE a strong positive correlation between serum concentrations of neopterin and ECLAM score ($rs=0.59$, $p<0.0001$) was established. The associations between other markers of immune activation and ECLAM score were significantly weaker as compared with neopterin (sTNF-55R: $rs=0.30$, $p=0.05$; sIL-2R: $rs=0.37$, $p<0.05$).

In patients with RA the serum concentrations of neopterin significantly correlated with several clinical parameters of disease activity (Table 3). The serum levels of neopterin in patients with highest activity (>6) and severity (>9) scores were significantly elevated as compared with patients with low activity (<5) and low severity (<5) scores (Table 4). During follow-up (12 month), significant decrease of serum neopterin was observed in patients with a $>20\%$ improvement (Table 5) compared with patients without according to the ACR criteria (31).

In patients with PM/DM the medium levels of neopterin were significantly higher in those with myositis overlap (26.8 ± 19.1 nmol/l) and primary DM (20.6 ± 11.3 nmol/l) as compared with primary PM (11.3 ± 4.6 nmol/l) ($p=0.007$ and $p=0.005$, respectively). There were no correlations between

Table 4. Serum concentrations of neopterin according to disease activity and severity indices in patients with rheumatoid arthritis

	Activity index		
	<5 , n=28	5-6, n=22	>6 , n=17
Neopterin (nmol/l)	9.2 ± 4.5	9.6 ± 3.4	14.0 ± 8.9
	<5 versus >6 hp=0.022	<5 versus 5-6 p>0.05	5-6 versus >6 p>0.051
	Sity index		
	<5 , n=17	5-8, n=26	>8 , n=24
Neirin (nmol/l)	7.8 ± 2.0	10.0 ± 4.8	13.1 ± 7.9
	<5 versus >8 p=0.018	<5 versus 5-8 p>0.05	5-8 versus >8 p>0.05

Table 5. Associations between changes of serum of neopterin concentrations and disease activity in patients with rheumatoid arthritis

	Neopterin (nmol/l)	
	Before	After 12 months
>20% improvement (n=23)	11.3 ± 7.7	7.9 ± 2.2*
<20% improvement (n=15)	11.9 ± 5.4	11.7 ± 4.8*

*p=0.04

Table 6. Spearman rank coefficients (rs) of correlation between neopterin and sTNF-55R and sIL-2R concentrations in patients with autoimmune rheumatic diseases

	sTNF-55R	sIL-2R
Smic lupus erythematosus	0.68 p<0.0001	0.35 p<0.01
Rheumatoid arthritis	0.32 p<0.01	0.26 p<0.06
Polymyositis/dermatomyositis	0.46 p<0.001	0.70 p<0.0001
Wegeners granulomatosis	0.80 p<0.001	0.55 p<0.01

neopterin and neuromuscular score, ADL score and serum creatine kinase. Elevated serum concentrations of neopterin were found in a group of patients with pronounced muscle weakness and normal creatine kinase level. Interestingly, all these patients had severe non-muscular (systemic) manifestations, such as arthritis, Raynauds phenomenon and scleroderma-like features.

In patients with WG BVAS index and neopterin correlated strongly ($r_s=0.59$, $p<0.01$). In 9 patients with active WG (BVAS>10) mean neopterin was significantly higher as compared with 13 patients with inactive disease (11.4 ± 4.2 nmol/l versus 6.2 ± 2.4 nmol/l, $p<0.05$).

In all groups of patients with autoimmune rheumatic diseases serum neopterin concentrations correlated more or less strong with sTNF-55R and in some cases also with sIL-2R (Table 6). Moreover, our preliminary results indicate that serum concentrations of neopterin (but not sIL-2R and sTNF-55R) weakly, but significantly correlate with serum concentrations of soluble intercellular adhesion molecules 3 ($n=34$, $r_s=0.430$, $p=0.02$), IL-13 ($n=41$, $r_s=0.359$, $p=0.02$) and von Willebrand factor antigen ($n=36$, $r_s=0.376$, $p=0.02$).

Discussion

Our studies confirm and extend the observations on increased serum concentrations of neopterin in the majority of patients with autoimmune rheu-

matic diseases. Individual neopterin concentrations correlate with disease activity as well as with serum concentrations of sTNF-55R and sIL-2R (32-34). Results from serial measurements of serum neopterin in RA appear to be especially important. It should be stressed that assessment of disease activity and progression of RA is still an unresolved clinical problem (35). One of the most important outcome measures in RA is radiological progression. A relationship between acute phase protein, especially C-reactive protein (CRP), and disease activity and radiological evidence of joint damage has been demonstrated in several clinical studies. (36). We found a significant correlation between serum concentrations of neopterin and disease activity in RA patients. Similar observation concerning urinary or synovial fluid neopterin have been reported by other investigators (37). In addition, we showed that patients with persistently elevated neopterin concentrations tended to have higher radiological progression rates than patients with low neopterin levels over a one year of follow-up. Moreover, our preliminary results indicate that in a group of patients with RA there was a better correlation between radiological progression and neopterin concentrations than between radiological progression and CRP and ESR. To our knowledge, this is the first report of prognostic significance of neopterin in RA.

RA and WG are considered to be diseases driven by Th1-(IFN- γ and TNF- α)-type cytokines and, in agreement, neopterin concentrations reflect activity and severity of these diseases. On the contrary, similar findings in patients with SLE and PM/DM seem paradoxical, because they are classified as Th2-type cytokine dependent diseases. However, there is also recent evidence that both, Th1-type and Th2-type, cytokines may be involved in their pathogenesis (38, 39). For instance, in murine lupus models, the IgG isotypes, IgG2a, IgG2b and IgG3, which are most relevant for pathogenesis, depend on IFN- γ . Nephritogenic antibodies in lupus are Th1 cell dependent (40,41). Treatment with anti-IFN- γ antibodies (42) or soluble IFN- γ receptors (43) delays disease onset and reduces mortality. IFN- γ concentrations are increased during active SLE and decline after treatment (44). In MRL/lpr model, it was shown that IFN- γ -/- knockout mice had less end-organ disease (45). Moreover, it has been recently demonstrated that decreased production of Th1-type cytokines can occur early in the course of SLE and is not a feature of well-established disease (46). A strong correlation between both neopterin and sTNF-55R concentrations with each other and with disease activity suggests that elevated neopterin concentra-

Table 7. Value of serum neopterin measurement in the clinical assessment of autoimmune rheumatic diseases

Disease	Activity marker	Response to therapy	Prognostic index
Systemic lupus erythematosus	+	?	?
Rheumatoid arthritis	+	+	+
Polymyositis/dermatomyositis	+	?	?
Wegener's granulomatosis	+	?	?

tions reflect the pathogenetic significance of the Th1-dependent immune system activation in SLE. From a clinical point of view it is important that serum concentrations of neopterin more closely correlate with clinical manifestations of SLE activity than other immune markers such as anti-DNA antibodies, sTNF-55R and sIL-2R.

Also in patients with PM/DM highest concentrations of neopterin (and also sTNF-55R and sIL-2R) are associated with non-muscular overlapping involvement, which is characterized by more generalized (Th1 and Th2 type cytokine dependent) activation of the immune system. From a clinical point of view, the determination of neopterin might be especially useful for the evaluation of global disease activity. The clinical significance of neopterin determination in patients with autoimmune rheumatic diseases is summarized in Table 7.

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