

## CLINICAL SIGNIFICANCE OF INCREASED SERUM NEOPTERIN IN CHRONIC KIDNEY FAILURE AS A BIOMARKER OF CELL-MEDIATED IMMUNITY

### KLINIČKI ZNAČAJ POVEĆANOG SERUMŠKOG NEOPTERINA U HRONIČNOM OŠTEĆENJU BUBREGA KAO BIOMARKER ČELIJSKI POSREDOVANOG IMUNITETA

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#### Summary

**Background:** Neopterin is a pyrazino-pyrimidine compound which is used as a marker of cell-mediated immunity in a variety of diseases. It is known that neopterin levels increase in diseases where interferon-gamma (IFN- $\gamma$ ) stimulation is present, and also as a result of deficiencies in renal function, given that the primary means of elimination of neopterin is through the kidneys. In this study, we aimed to investigate the role of increased neopterin levels as a prognostic biomarker in patients with impaired renal function.

**Methods:** A total of 90 individuals including 63 patients with chronic kidney failure (CKF) and 27 healthy volunteers were included in the study. Serum neopterin concentrations were measured using the enzyme-linked immunosorbent assay. A Mann-Whitney U test and a Pearson Correlation Test were used in the statistical analysis, with a  $p$  value of  $<0.05$  being considered statistically significant.

**Results:** The mean age was  $52.21 \pm 0.16$  years in the patient group and  $56.55 \pm 0.32$  years in the control group. In the CKF patients, serum neopterin levels increased to a significantly greater degree than in the control group ( $p < 0.001$ ), while no statistically significant correlation was identified between serum neopterin levels and age ( $p > 0.05$ ).

**Conclusions:** A significant increase was found in the serum neopterin levels in the CKF patients, due to both the triggering of the disease and the reduction of neopterin elimination.

**Keywords:** cell-mediated immunity, chronic kidney failure, neopterin, renal diseases

#### Kratak sadržaj

**Uvod:** Neopterin je pirazino-pirimidinsko jedinjenje koje se koristi kao marker ćelijski posredovanog imuniteta kod različitih oboljenja. Poznato je da se nivoi neopterina povećavaju kod oboljenja gde je prisutna intreferon-gama stimulacija (IFN- $\gamma$ ) i kao rezultat deficijencije kod bubrežne funkcije, tako što se primarno neopterin eliminiše kroz bubrege. U ovom izučavanju svrha je bila da se prouči uloga povećanih nivoa neopterina kao prognostičkog biomarkera u pacijenata sa oštećenom bubrežnom funkcijom.

**Metode:** Proučavanje je obuhvatilo ukupno 90 osoba uključujući 63 pacijenata sa hroničnim bubrežnim oštećenjem (CKF) i 27 zdravih volontera. Koncentracije serumskog neopterina merene su primenom enzimskog-imunoodređivanja. Za statističku analizu korišćeni su Mann-Whitney U test i Pearson korelacioni test, sa  $p$  vrednošću  $< 0,05$ , kao statistički značajnom.

**Rezultati:** Pacijenti su imali srednju prosečnu starost od  $52,21 \pm 0,16$  godina, a kontrolna grupa  $56,55 \pm 0,32$ . U CKF pacijenata serumski neopterin nivoi povećavali su se značajno više nego u kontrolnoj grupi ( $p < 0,001$ ), dok nije bilo statistički značajne korelacije koja je identifikovana između nivoa serumskog neopterina i godina starosti ( $p > 0,05$ ).

**Zaključak:** Nađeno je značajno povećanje nivoa serumskog neopterina kod CKF pacijenata, kako zbog bolesti tako i zbog smanjenog izlučivanja neopterina.

**Ključne reči:** ćelijski posredovani imunitet, hronično bubrežno oštećenje, neopterin, bubrežno oboljenje

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## Introduction

Neopterin is a pyrazino-pyrimidine compound that is synthesized by monocytes and macrophages as a result of stimulation with endotoxin and IFN- $\gamma$  by activated T cells. Neopterin levels in body fluids are elevated in a variety of diseases where T cells or macrophages are activated (1–4), and has been identified as a marker of disease in a variety of conditions, such as viral infections, inflammatory diseases, auto-immune diseases, neurodegenerative diseases and some types of cancer (1, 5). Accordingly, neopterin levels are used in evaluations of disease activity and prognosis in various pathologies (5–7).

In a previous study, increased urinary neopterin levels were reported to be associated with an increase in serum creatinine levels in nephrotic syndrome, chronic renal failure, and end-stage renal disease (6). Although it is suggested in the aforementioned study that neopterin is not specific in the evaluation of disease prognosis, there are many studies suggesting that neopterin is useful as a biological marker when following-up the clinical course of the disease or assess prognosis. Several studies have reported that serum or urine neopterin levels increase in cases of idiopathic or primary nephrotic syndrome, chronic and acute glomerulonephritis, and different stages of chronic kidney disease (7–10), and it has been also shown that neopterin levels are elevated in patients with end-stage renal disease (ESRD) undergoing long-term hemodialysis, and that this increase is correlated with the duration of hemodialysis. Also in the aforementioned study it is demonstrated that in cases of human immunodeficiency virus (HIV) infection, the risk of developing acquired immune deficiency syndrome (AIDS) may increase in patients undergoing dialysis for more than one year. This increase has been also associated with various interactions between blood coagulation molecules and hemodialysis membranes and activated cellular immune response as a result of exposure to the dialysis membrane (11).

Chronic kidney disease is defined as the progressive loss of renal function over a period of months or years. There are no specific signs of impairment in renal function although the presence of diabetes and/or high blood pressure greatly increases the chance of kidney disease (12).

In the present study we investigate whether serum neopterin levels increase more in patients with CKF than in healthy individuals, and evaluate the correlation between serum neopterin levels and age.

## Materials and Methods

### Participants

A total of 90 individuals including 63 CKF patients and 27 healthy volunteers were included in the study. The health status of all individuals in the

control group was inquired in detail, and those with no health problems were included in the control group. The study group consisted of patients undergoing dialysis treatment and diagnosed with CKF. The study was approved by the local Ethics Committee of Inonu University, Faculty of Medicine and was carried out in accordance with the principles of the Declaration of Helsinki.

### Blood sampling

Serum neopterin levels were measured using the following method: Blood samples were taken prior to dialysis in CKF patients. Serum was obtained from coagulated blood samples and collected in a shaded tube, as neopterin is sensitive to light. The blood samples were separated to sera by centrifugation at 4 °C for 10 min at 3,000 rpm and the samples were stored at -20 °C until analysis.

### Neopterin measurement

Neopterin was measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (DRG Diagnostics GmbH, Marburg, Germany). Optical density was measured at 450 nm, with serum neopterin levels expressed in nmol/L.

### Statistical analysis

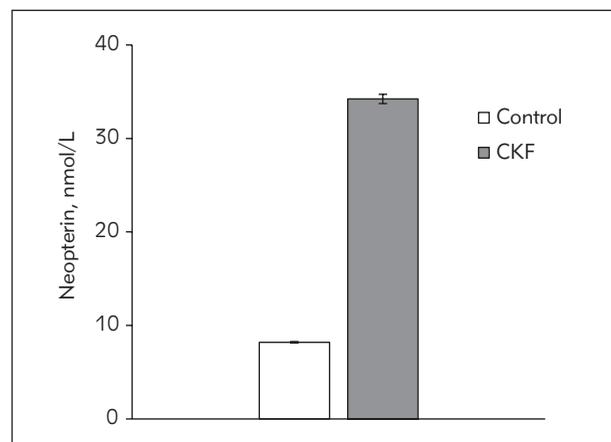
The statistical analysis was carried out using SPSS version 11.5 software (SPSS Inc., Chicago, IL, USA), with continuous variables expressed in mean  $\pm$  standard error (SEM). A Mann-Whitney U test was used to compare the patient and control groups. The relationship between serum neopterin levels and age was determined using a Pearson Correlation Test and the Linear Regression method, and a  $p$  value  $<0.05$  was considered statistically significant.

## Results

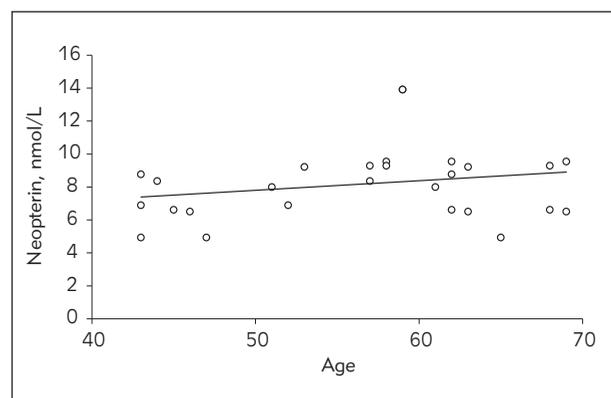
The mean age was  $52.21 \pm 0.16$  (range: 32 to 71) years and  $56.55 \pm 0.32$  (range: 43 to 69) years in the patient and control groups, respectively. Serum neopterin levels were higher in the CKF patients ( $34.22 \pm 0.50$  nmol/L) than in the control group ( $8.19 \pm 0.08$  nmol/L) (Figure 1), to a statistically significant degree ( $p < 0.001$ ), as a result of the disease itself and the decreased disease-related elimination.

As shown in Figure 2, although a positive correlation ( $r = 0.253$ ) was identified between serum neopterin levels and age in the control group, this age-related increase was not statistically significant ( $p = 0.203$ ).

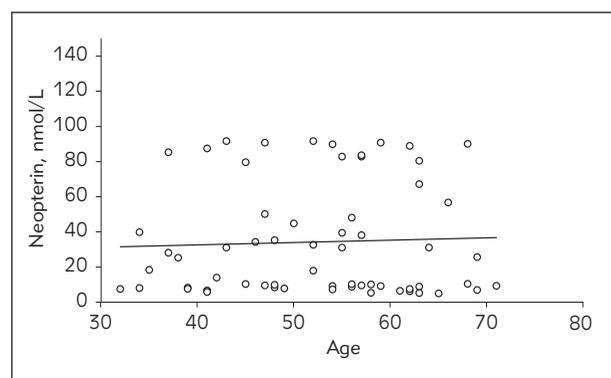
The age-related changes in serum neopterin concentrations in CKF patients are presented in



**Figure 1** Changes in neopterin levels in CKF patients and control group.



**Figure 2** Correlation between neopterin levels and age in the control group ( $p > 0.05$ ; Pearson correlation test).



**Figure 3** Correlation between neopterin levels and age in the CKF group ( $p > 0.05$ ; Pearson correlation test).

Figure 3. Similar to the control group, an increase was noted in serum neopterin levels ( $r=0.043$ ) with increasing age in the patient group although this positive correlation between the age and neopterin was not statistically significant ( $p=0.738$ ).

## Discussion

Neopterin is a biochemical marker of cellular immune response and is a member of a group of compounds known as pteridines (13), which are pyrazino-(2,3-d) pyrimidine derivative compounds synthesized by guanosine triphosphate (GTP). At 253 daltons neopterin is approximately twice the size of creatinine (113 daltons). Urinary neopterin levels were first observed to increase in viral infections and tumors in 1979 (14). In the 1980s, neopterin levels were a popular predictor of postoperative acute rejection reactions and viral infections in transplantation studies, but have since been »forgotten« as a biomarker (15). A significant abnormality has been found in the pteridine metabolism in patients with CKF in subsequent studies (10).

Neopterin is produced by monocyte, macrophage and monocyte-derived dendritic cells as a result of  $\text{IFN-}\gamma$  or endotoxin stimulation (1, 5, 16). Activated Th 1 lymphocytes secrete  $\text{IFN-}\gamma$  during cellular immune response. Activation of Th 1 lymphocytes is the main stimulus for neopterin production and secretion (3, 17). The secreted  $\text{IFN-}\gamma$  binds rapidly to the target structures or is neutralized with soluble receptors, although neopterin is biochemically inert and its serum concentrations are closely related to the activity of the cellular immune system (2).

Recent studies have suggested that elevated serum and urine neopterin levels may be used as diagnostic or prognostic markers in a variety of conditions, including neuropsychiatric disorders (18), renal allograft rejection (19), hepatitis and, HIV, in such viral infections as cytomegalovirus, in bacterial infections (1, 20–22), in silicosis patients (23), in such autoimmune diseases as active juvenile idiopathic arthritis (24), in tumors of, for example, the breast carcinoma, colorectal cancer (25, 26), in type-1 and type-2 diabetes patients with diabetic foot syndrome (27) and in coronary and peripheral artery disease, atrial fibrillation (4), left ventricular dysfunction, heart failure (15).

Circulating cytokines and other markers of inflammation are significantly elevated in patients with CKF. In previous studies, neopterin levels have been found to be higher in patients with a lower glomerular filtration rate, which suggests that renal elimination is impaired, that uremia increases or that inflammation has an adverse effect on renal function in such cases (28).

Chronic systemic inflammation is a result of the release of pro-inflammatory molecules (cytokines) from immune-related cells and the chronic activation of the innate immune system. The C-reactive protein (CRP) is a marker of systemic inflammation (29), with a relationship being established with renal functions in the early stages of CKF (30). Serum neopterin levels have been found to be high in patients with CKF

before dialysis, and it has been reported that a correlation exists between inflammation markers and neopterin, including IL-6, hsCRP, TNF- $\alpha$ , IFN- $\gamma$  and hyaluronan (9, 28). It has been suggested that neopterin, as a marker of activation of the cellular immune response due to IFN- $\gamma$  release, can better reflect uremia-related changes in the immune system seen in cases of renal transplantation than inflammatory markers (16).

Concentrations of neopterin increase due to impaired renal neopterin clearance and/or increased production due to systemic inflammation (9), and neopterin levels are also an indicator of oxidative stress, including the production of reactive oxygen species (ROS), in addition to reflecting the degree of cellular immune activation (3, 16, 17). Neopterin plays an effector role in enhancing the effects of reactive oxygen species, and displays pro-oxidant properties (3, 17). Since neopterin is chemically inert, and elimination occurs only through the kidneys, serum neopterin levels are elevated in diseases in which inflammatory activity does not increase, but renal function decreases (31).

Chronic kidney disease is a global health problem, and health expenditures for this group of patients is 1.8 times higher than for patients without chronic kidney disease. The mean cost of a dialysis patient is 10.3 times greater than of a patient without chronic kidney disease (32). Incidences of ESRD increased from 86.8 million to 347 million within a year, and it was reported that the rate increased steadily until 2002, but has since remained stable (33).

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Several studies have been conducted to investigate this biomarker in cases of impaired renal function, and it has been suggested that neopterin levels may also be used in dose adjustment in clinical practice. Accordingly, in addition to the recognition of neopterin as a biological marker, this property should not be overlooked. Some authors have suggested the use of neopterin as a possible marker in evaluating the efficacy of immunosuppressive therapy. There is currently no drug on the market that specifically reduces neopterin levels, although an overall increase in the level of immunosuppression is associated with low serum neopterin levels. In addition, it is still unknown whether a therapeutic strategy that enhances the elimination of neopterin or inhibits neopterin production and release would be efficacious in clinical practice.

In conclusion, the results of our study suggest that serum neopterin may be a useful marker in predicting disease activity and prognosis in patients with CKF although these results should be supported with further prospective long-term studies of a larger group of patients.

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## Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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