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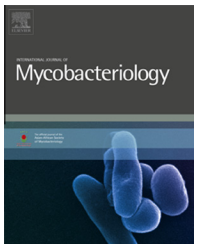


# Clinical importance of serum neopterin level in patients with pulmonary tuberculosis

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ABSTRACT

*Objective:* Neopterin is a sensitive marker for cell-mediated immune response. Because of this, the neopterin levels of body fluids show cell-mediated immune response in different infectious diseases which involve T cells and macrophages.

The aim of this study was to determine the clinical importance of neopterin levels in patients with tuberculosis and compare with those levels of healthy subjects.

*Methods:* Seventy patients with tuberculosis (46 newly diagnosed cases, 15 relapse cases, and 9 multidrug-resistant tuberculosis cases) and 18 healthy adult individuals were included in the study. Neopterin concentrations were measured by the ELISA method according to the protocol of the manufacturer. Chi-square test was used in statistical analysis; $p \leqslant 0.05$ was considered statistically significant.

*Results:* Serum mean neopterin levels were 23.74 ± 21.8 nmol/L (median: 18.3) in newly diagnosed patients with pulmonary tuberculosis; 28.69 ± 21.2 nmol/L (median: 21.2) in relapse patients and 31.28 ± 14 nmol/L (median: 25.4) in multidrug-resistant tuberculosis cases, respectively. Serum mean neopterin levels were 4.03 ± 5.12 nmol/L (median: 5.1) in healthy subjects. The serum neopterin levels were found to be significantly higher in patients with tuberculosis than the control group.

There was a statistically significant correlation between neopterin positivity (neopterin level ⩾10 nmol/L was accepted to be positive) and clinical symptoms of hemoptysis and weight loss. Besides statistically significant correlations between neopterin positivity and hemoglobin level, sedimentation rate, mean leukocyte count and radiological involvement (localized or diffuse) were determined.

*Conclusion:* Serum neopterin levels can be used as a helper laboratory finding for the diagnosis of patients with tuberculosis. For this aim, further controlled studies are needed.



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## Introduction

Neopterin is a pyrazino-pyrimidine compound belonging to the pteridine class, which is only produced by living cells [1]. Neopterin is produced from guanosine triphosphate by stimulated macrophages under the effect of lymphocyte-originated gamma interferon. Neopterin is an important marker which plays a key role in the interaction of monocyte/macrophage activation [2]. Neopterin was demonstrated to be a sensitive marker of cell-mediated immune reactions; therefore, the identification of neopterin levels in various body fluids has diagnostic significance in numerous diseases including the diseases of T-lymphocytes and macrophages [1,3].

*Mycobacterium tuberculosis* (MTB) is an intercellular pathogen with the ability to live for a long time in macrophages. Tuberculosis (TB) bacilli that are phagocyted by macrophages cannot be killed by polymorphonuclear leukocytes. Neopterin is released by the stimulation of intracellular viruses and bacteria, such as TB, and provides information about the status of cell-mediated immunity [1,4]. The aim of this study is to determine the clinical importance of serum neopterin levels and compare them with those levels of a control group.

## Materials and methods

This study was performed in cooperation with the Ankara Training and Research Hospital and Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Chest Diseases Department.

Diagnosis of pulmonary tuberculosis (PTB) was made with clinical symptoms, acid fast bacillus (AFB) smear positivity in sputum microscopy, culture positivity for MTB, and radiologic findings.

Symptom control among the healthy individuals was eliminated with physical examination and PA chest radiography.

The patients were comprised of three groups, including newly diagnosed, relapse and multidrug-resistant (MDR) patients with TB.

Newly diagnosed patients with TB: These are the patients that have not taken TB treatment before or the patients that have taken TB treatment less than one month.

Relapse case of TB: The patient was accepted as a relapse if the patient who had been diagnosed with TB and who had completed the treatment successfully was diagnosed with TB again; in other words, the presence of AFB smear positivity in sputum microscopy.

Multidrug-resistant tuberculosis (MDR-TB) cases: Resistance of a patient to both isoniazid and rifampicine, which are used in the treatment of TB.

Blood samples were taken from all TB patients before the diagnosis and before beginning the treatment.

After separation, the serum samples were stored at −40 °C in a deep freezer. Neopterin levels were determined by ELISA method in accordance with the instructions of the producing company (IBL Medical, Turkey). Data were recorded using SPSS software. Chi-square test was used for statistical evaluation. Patients were evaluated in three groups, including newly diagnosed (Group 1), relapse (Group 2) and MDR patients (Group 3), respectively. The associations of the groups with gender, symptoms (production of sputum, dyspnea, hemoptysis, chest pain, fever, and weight loss), duration of symptoms and degree of radiological involvement (diffuse cavitary, localized cavitary) were statistically evaluated.

Cases with neopterin levels $\geqslant$10 nmol/L and <10 nmol/L were determined as positive or negative, respectively. Duration of symptoms between patients with positive and negative Neopterin symptoms (coughing, sputum, dyspnea, hemoptysis, and chest pain), duration of symptoms and laboratory findings (hemoglobin levels, leukocyte count and sedimentation rate) were also compared statistically; $p$ levels $\leqslant$0.05 were accepted to be significant.

Informed consent forms were received from the patients, and approval of the Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital Ethic Committee was obtained for the study.

## Results

A total of 70 inpatients from Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Chest Diseases Department, including 46 newly diagnosed patients with TB (Group 1), 15 relapse patients (Group 2), 9 patients with MDR-TB patients (Group 3) and a total of 18 healthy subjects as the control group were enrolled in the study.

Time to onset of the symptoms was 62 ± 39 days in Group 1, 17 ± 61 days in Group 2, and 58 ± 13 days in Group 3. There was a statistically significant difference between the Groups 1 and 2 ($p < 0.01$) regarding the time to onset of the symptoms, but not between the Groups 1 and 3 ($p > 0.01$).

Neopterin levels were significantly higher in the patient groups versus the control group ($p < 0.05$). Results are presented in Table 1.

Neopterin levels were positive (neopterin level $\geqslant$10 nmol/L) in 45 of 70 (64.3%) patients with TB and 4 of 18 (22%) in the control group. Neopterin positive patients (34 males, 11 females) and neopterin negative patients (16 males and 9 females) did not differ significantly in terms of gender ($p > 0.05$). Comparison of neopterin levels between the patient groups did not reveal any difference ($p > 0.05$).

The associations of neopterin levels with clinical symptoms (for example, time to onset of the symptoms, sputum formation and dyspnea, etc.) and laboratory findings (for example, existence of cavitation, involvement degree of the lungs, microscopic gradient [from 1+ to 4+ positive], sedimentation rates, etc.) were statistically compared between the groups. Results are shown in Table 2.

## Discussion

Detection of neopterin concentrations in body fluids provides information about cell-mediated immune response. Neopterin is a biologically stabilized molecule, thus the messages generated during the immune responses can be detected easily. It is widely used in the follow-up of HIV infection. Neopterin is produced by macrophages as a response to interferon gamma stimulation, and neopterin concentrations show cellular immune activation [2–5].

**Table 1 – Neopterin levels of newly diagnosed, relapse and MDR-TB patients and the control group.**

| Groups | Number | Neopterin levels nmol/L (Mean ± SD*) | Median | Neopterin positivity (⩾10 nmol/L) |
|---|---|---|---|---|
| | | | | n (%) |
| Group 1** | 46 | 23.7 ± 21.8 | 18,3 | 24/45 (53) |
| Group 2*** | 15 | 28.7 ± 21.2 | 21,2 | 12/45 (27) |
| Group 3**** | 9 | 31,3 ± 14 nmol/L | 25,4 | 9/45 (20) |
| Control group | 18 | 4.0 ± 5.1 | 5,1 | 4/18 (22) |
| Total | 88 | | | 49/88 (55,7) |

SD*: Standard deviation.
Group 1**: Newly diagnosed cases; Group 2***: Relapse cases; Group 3****: Multidrug-resistant tuberculosis (MDR-TB) cases.
* *p* value shown statistically significant.

**Table 2 – The associations of neopterin levels with clinical symptoms and laboratory findings.**

| Clinical symptoms | Neopterin positive group (n:45) | Neopterin negative group (n:25) | *p* value |
|---|---|---|---|
| Onset of the symptoms | 34.48 days | 33,02 days | 0.772 |
| Sputum formation and dyspnea | 22/45 (49%) | 6/25 (24%) | 0.169 |
| Hemoptysis | 43/45 (95.5%) | 17/25 (68%) | 0.034* |
| Chest pain, night sweats, cough and fever | 6/45 (13%) | 2/25 (8%) | $p > 0.05$ |
| Weight loss | 32/45 (71.2%) | 8/25 (32%) | 0.000* |
| Laboratory findings | | | |
| Radiological involvement (localized or diffuse involvement) | 24/45 (53%) | 5/25 (%20) | 0.018* |
| Cavity formation | 18/45 (40%) | 10/25 (40) | 0.671 |
| Microscopic gradient (mean rank) | 35.2 | 31.6 | 0.444 |
| Mean Hb levels (gr/dl) | 12.27 ± 1.8 | 13.38 ± 0.8 | 0.001* |
| Mean sedimentation rate (mm/h) | 70.04 ± 18.6 | 57.52 ± 2 | 0.018* |
| Mean leukocyte count ($mm^3$) | 37.9 | 25.9 | 0.018* |

* *p* value shown statistically significant.

TB is one of the leading infectious diseases that cause mortality and morbidity in the world. Cell-mediated immunity (macrophage activation and T lymphocyte proliferation) plays an important role in the pathogenesis of TB. Elevated neopterin levels are reported in patients with PTB [3–5].

Yüksekol et al. [5] reported that neopterin levels in bronchoalveolar lavage (BAL), serum and urine samples of patients with TB were significantly higher than those with lung cancer, pneumonia and the healthy control group. In the same study, higher neopterin levels were determined in urine samples of patients with moderately advanced radiological lesions than those with minimal disease. This study concluded that urine neopterin levels could reflect the activity of lung TB prior to the diagnosis of the disease via culture tests, and that these levels were correlated with radiological involvement.

Immanual et al. [6] reported that serum neopterin levels are found to be increased prior to the treatment in patients with HIV-negative TB; they decrease in the first month of the treatment and return back to the normal levels at the end of the treatment. In this study the decreased rates were found to be 11–56% in patients with restricted lesions and 11–45% in patients with diffuse lesions. The increased neopterin levels following the treatment were found to be associated with bacteriologically-confirmed patients who relapsed. Consequently, they stated that identification of neopterin levels could be a marker which could be used to monitor the response rate.

Baganha et al. [2] reported that neopterin levels in pleural fluid were higher in pleurisy patients than in those with lung cancer.

Chiang et al. [7] reported that neopterin adenosine deaminase (ADA) and soluble interleukin receptor (SIL-2R) levels were significantly higher in the pleural fluid of TB patients with pleurisy compared with those with malignant effusion. The same study revealed pleural neopterin levels to be higher in patients with uremic pleural effusion.

Cok et al. [8] compared neopterin and ADA levels in patients with TB pleurisy and malignant pleurisy. In this study, neopterin levels in patients with TB were determined to be higher than those with malignant pleurisy; however, unlike ADA, neopterin was concluded not to be a significant marker in clinical usage.

Mohammed et al. [9] stated that neopterin levels in BAL fluids could reflect the activity grade in patients with PTB.

In a study performed by Gordeuk et al. [10], in 32 HIV-positive patients among a total of 49 patients with PTB, neopterin levels were found to be increased at the initiation of the treatment and decreased at the end of the treatment. Neopterin levels and IL-2 concentrations of HIV-positive TB patients were reported to be higher than HIV-negative patients.

This study did not reveal a statistically significant difference between newly diagnosed TB cases, relapse cases and MDR-TB cases.

There was a statistically significant association regarding hemoptysis symptoms and between the neopterin positivity. Also, there was a statistically significant association between neopterin positivity and weight loss. The rate of weight loss was higher in patients with positive neopterin levels.

When investigated in terms of laboratory findings, patients with positive neopterin levels had a higher mean leukocyte count and mean sedimentation rate while patients with positive neopterin levels had lower Hb levels compared with those with negative neopterin levels. In addition to these findings, there was a significant correlation between neopterin positivity and radiological involvements (localized or diffuse).

Radiological involvement is more common in the patients with positive neopterin than with the patients with negative neopterin.

In another study performed by Güler et al. [11] on 40 newly diagnosed patients, pretreatment neopterin levels were reported to be higher than normal controls. Moreover, pretreatment neopterin levels were found to be significantly decreased in the second month of the treatment. Serum neopterin levels were higher in diffuse disease compared with the patients with restricted lesions. The study concluded that neopterin levels were safe immunologic markers to evaluate the activation status and treatment response of the TB patients. In this study, pretreatment neopterin levels in TB patients (newly diagnosed, relapse and MDR-TB patients) were found to be significantly higher than the control group. The results of this study are concordant with the study of Güler et al. [11].

Finally, it has been concluded that the neopterin level is a useful marker in the diagnosis and follow-up of TB. Further controlled trials are needed to evaluate the effectiveness of neopterin levels in the follow-up of the disease.

## Conflict of interest

No conflict of interest declared.

## Acknowledgement

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