



The diagnostic value of serum Neopterin levels in patients with newly diagnosed pulmonary/extra-pulmonary Tuberculosis

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ABSTRACT

Background: Tuberculosis (TB) is still a primary public health concern worldwide, the most common of which is pulmonary involvement. The official diagnosis of tuberculosis involves the culture of a tissue sample, which in turn takes time. Neopterin, as one of the indicators of cellular immune activity, is secreted by macrophages and T lymphocytes in different inflammatory phenomena due to the stimulation of interferon-gamma. Therefore, it may be possible to measure the serum level of Neopterin in the diagnosis and follow-up of the response to pulmonary and extra-pulmonary tuberculosis treatment. **Objective:** This study aimed to evaluate the serum level of Neopterin in the diagnosis of newly diagnosed pulmonary/extra-pulmonary tuberculosis. **Method:** Six groups of 20 individuals, including pulmonary TB, extra-pulmonary TB, healthy, proven rheumatologic diseases, patients with pulmonary malignancies, and finally, those with lung infections, entered the study. A serum sample was taken to assess the level of Neopterin. The samples were transferred to the laboratory in less than 30 minutes. **Results:** The level of Neopterin is significantly higher in patients with pulmonary TB into patients with extra-pulmonary TB, healthy and lung infections, and then in extra-pulmonary TB was significantly higher than in healthy individuals, patients with lung cancer and lung infection. **Conclusion:** According to the significant relation between serum Neopterin level in patients with pulmonary and extra-pulmonary TB compared to healthy people without any underlying disease, it may be possible to initiate specific pulmonary tuberculosis treatment in a shorter period. However, it is necessary to follow the tissue culture sample.

Key Words: Neopterin, Tuberculosis, pulmonary TB, extra-pulmonary TB.

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INTRODUCTION

Tuberculosis is one of the ten leading causes of death worldwide and the leading cause of death from an infectious agent. Millions of people are infected by TB each year. In 2018, 1.5 million people died of tuberculosis, 300,000 of whom had pulmonary TB. About 10 million people became infected with tuberculosis this year, including 5.7 million men, 3.2 million women, and 1.1 million children. There have been cases in all countries and age groups, but tuberculosis is treatable and preventable [1]. In addition to pulmonary tuberculosis, extra-

pulmonary types including lymph node TB, pericarditis TB, myocardial TB, laryngitis TB, meningitis TB, the involvement of the digestive system, and the involvement of the genital administration system have also been reported [2, 3]. One of the goals of Sustained Development Goals (SDGs) for countries, developed by the United Nations in 2015, is to end the TB epidemic by 2030. The WHO End TB approach adopted by the World Health Organization in 2015, is a 90% reduction in TB deaths and an 80% reduction in the incidence of the disease by 2030 [4, 5]. Still, the standard diagnostic method in TB patients is direct observation of bacilli in sputum smears, and this

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method is also used to monitor and follow up the response to treatment. Diagnosis of extra-pulmonary tuberculosis is more complicated than pulmonary tuberculosis and requires the use of invasive diagnostic methods such as biopsy and tissue culture. Therefore, using methods that can help faster diagnosis, without using invasive techniques, will be very useful [6].

Neopterin (NPT) (6-D-erythro-trihydroxypropyl pteridin) is one of the indicators of the activity of the cellular immune system in the body and its level of serum increases in infections that stimulate the immune system [7]. Neopterin was first isolated from bee larvae and royal jelly in 1963, and then in 1967 separated from humans [8]. It is produced from the conversion of guanosine triphosphate to neopterin is involved in T lymphocytes stimulated by interferon-gamma and to a lesser extent by interferon-alpha and beta, as well as in macrophages, dendritic and endothelial cells, and to a lesser extent in fibroblasts, smooth vascular wall muscles, and renal epithelial cell [9, 10]. In the case of non-mycobacterial bacterial infections, studies have shown that the urinary excretion of neopterin is lower than that of viral infections [11, 12]. However, in pulmonary infections caused by Mycobacterium tuberculosis, the amount of urinary neopterin is higher than that of bacterial pneumonia and cancer [13]. According to the above mentioned, the availability of a non-invasive method for the diagnosis of extra-pulmonary tuberculosis is essential. This study aimed to investigate the diagnostic value of serum neopterin as a method for diagnosing extra-pulmonary tuberculosis to diagnose the disease by spending less time and money without using invasive techniques.

METHOD

In the current study, serum levels of neopterin were measured in patients with extrapulmonary TB and compared with serum levels in extra-pulmonary TB, healthy, proven rheumatologic diseases, patients with pulmonary malignancies, and finally those with lung infections. Plasma neopterin levels were determined using high-efficiency fluid chromatography with fluorimetry detection [14]. To determine the sample size, we used a comparison formula of two averages, so the sample size included six groups of 20 individuals and total control of 120 individuals. Inclusion criteria were patients with

pulmonary tuberculosis based on the national protocol with at least two samples of positive sputum smear, or a positive smear sample and clinical symptoms corresponding to tuberculosis, or a positive smear and radiographic evidence of pulmonary tuberculosis, or a positive smear sample with a positive culture of Mycobacterium tuberculosis. It is noteworthy that the condition for entering the recent study is the time range immediately after the diagnosis of tuberculosis and before starting the treatment. Patients with newly diagnosed extracellular tuberculosis entered the study using one of the following methods and before receiving any medication that affected tuberculosis; Pathological evidence for tuberculosis, such as observations of cohesive granuloma, a positive PCR test on the patient's tissue sample, and direct view of the bacillus tuberculosis on the tissue sample before starting anti-tuberculosis drug therapy. Exclusion criteria were patients who had started anti-tuberculosis treatment before sampling, with extrapulmonary tuberculosis as well as tuberculosis, cancer, rheumatoid diseases, bacterial pneumonia, hepatitis or HIV, patients with defective immune system any reason, and consumers of drugs that weaken the immune system. In the present study, participants were divided into three age groups of less than 35 years, 35 to 50 years, and over 50 years.

Statistic

Data are expressed as the mean \pm standard deviation. Significance was established with the SPSS 21.0 statistical (IBM Corp., Armonk, NY, USA). Turkey conducted comparisons between two groups. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

The overall results illustrated some of the main characteristics of the study participants. It can be seen from the data in Table 1 that six study groups consisted of both men and women each included 20 people. The number of participants in each group of the male and female, mean and standard deviation of age, and the mean and standard deviation of the serum level of neopterin are listed in Table 1. As can be seen, the population of men and women in this study is generally equal, and the average age of the participants in different groups did not differ significantly.

Table 1: Characteristics of the study participants

Groups/value		Pulmonary TB	Extrapulmonary TB	Rheumatic Diseases	Pulmonary Malignancies	Pulmonary Infections	Healthy
Number of Participants		20	20	20	20	20	20
Gender	Male	12	13	11	10	10	6

	Female	8	7	9	10	10	14
Age Mean±SD		60.9±18.01	39.65±18.82	48.05±14.69	50.30±14.74	63.05±21.66	42.50±12.44
Less than 35 years %		5%	50%	20%	10%	15%	35%
35 – 50 years %		25%	10%	35%	45%	20%	35%
Over 50 years %		70%	40%	45%	45%	65%	30%
Neopterin nmol/L mean±SD		35.55±23.21	15.75±7.29	16.6±16.77	27.24±23.36	31.47±26.77	6.7±1.75

The gender, age, and serum level of neopterin in six study groups. Data are expressed as mean±SD, P<0.05 was considered to indicate a statistically significant difference.

As can be seen in Table 2, serum neopterin variation in different disease groups is diverse, but none of them has a reduced level. All cases with pulmonary tuberculosis had elevated levels of neopterin and all healthy people had normal values of neopterin. The number of rheumatoid arthritis people with normal and increased levels of neopterin was equal, and in the other three groups, most participants exhibited increased levels of neopterin (Table 2).

Table 2. Neopterin variation in different groups

Groups	Variation	Frequency	Percent
Pulmonary TB	increased	20	100.0
	normal	0	0.0
Extrapulmonary TB	normal	2	10.0
	increased	18	90.0
Rheumatic Diseases	normal	10	50.0
	increased	10	50.0
Healthy	normal	3	10.0

Pulmonary Malignancies	increased	17	85.0
	normal	7	35.0
Pulmonary Infections	increased	13	65.0
	normal	0	0.0
Healthy	normal	20	100.0

Figure 1 compares the results obtained from the preliminary analysis of neopterin in groups. It is apparent from this figure that there was a significant difference between the Neopterin level among pulmonary tuberculosis patients against healthy individuals and extrapulmonary TB. There is a significant difference between the healthy, pulmonary TB, pulmonary malignancies, and pulmonary infections groups. Further analysis showed that neopterin level in the extrapulmonary TB group is significantly lower than pulmonary TB, pulmonary malignancies, and pulmonary infections groups. No significant differences were found between rheumatoid patients and others (Figure 1).

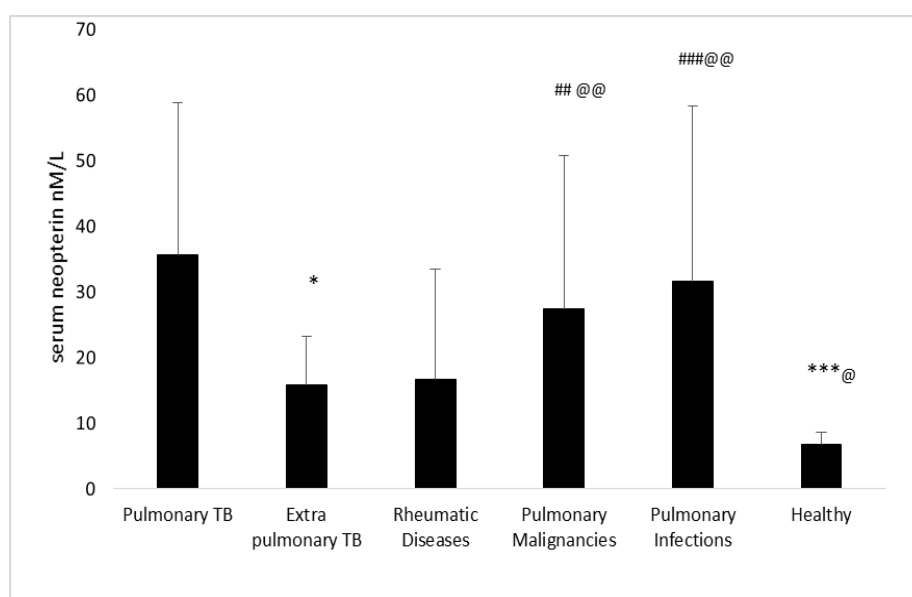


Figure 1. The neopterin level in groups. Data are expressed as mean±SD. P<0.05 was considered to indicate a statistically significant difference. * p<0.05, * p<0.001 the pulmonary TB vs other groups. ## p<0.01, ### p<0.001 healthy vs. other group. @ p<0.05, @ @ p<0.01 the extrapulmonary TB vs other groups.**

DISCUSSION

Tuberculosis is a common infectious disease with tenth-degree global morbidity and, in many cases, it leads to death. It is challenging to diagnose active tuberculosis solely based on signs and symptoms. It is also difficult to diagnose it in patients with immunodeficiency [15, 16].

This study aimed to compare the serum levels of neopterin, as an inflammatory factor, in several groups of patients with common clinical symptoms. The highest amount of neopterin in the serum was in pulmonary TB, pulmonary infections, Pulmonary Malignancies, Rheumatic Diseases, Extrapulmonary TB, and healthy groups, respectively. High levels of neopterin in TB have been well discussed in the literature. Various studies have shown that in response to anti-tuberculosis treatment, the level of this factor becomes comparable to healthy people or non-tuberculosis diseases, such as pneumonia and malignancy [17].

Contrary to expectations, this study did not find a significant difference between serum neopterin levels among patients with rheumatic diseases and other groups, and also no significant relation was reported between serum levels of neopterin by age and sex of individuals. However, according to some studies, tuberculosis and serum neopterin can be affected by age and gender [18].

In the current study, the mean serum neopterin levels were 15.75 nmol/L and 6.7 nmol/L in extrapulmonary TB patients and healthy controls, respectively. Our findings were consistent with the results of a similar study in which serum neopterin levels were significantly increased in the group with pulmonary tuberculosis and extrapulmonary TB, compared with the healthy control group [19]. Also, serum levels of neopterin in patients with pulmonary tuberculosis in this study were similar to those of the study by Goyal et al. in 2017 [20].

To answer the question of whether a neopterin is a useful marker for the early detection of pulmonary extracellular tuberculosis, we tend to the significant difference of this factor in several groups. The current study revealed that serum levels of neopterin in patients with extrapulmonary tuberculosis were also significantly different than those in the group of pulmonary malignancies, pulmonary infections, and healthy individuals. Eisenhut, in 2016 found a significant association between serum neopterin levels in patients with extrapulmonary tuberculosis compared with viral and bacterial pneumonia as well as healthy individuals. Besides, the average serum level of neopterin in patients with extra-pulmonary tuberculosis was higher than the others [21].

In the current experiment, the amount of neopterin in pulmonary TB and pulmonary infections were similar. However, the findings of the current study do not support the previous research, in which patients diagnosed with bacterial infection with species other than mycobacteria

showed significantly lower levels of urinary neopterin than patients with viral infections but did not differ statistically [22].

CONCLUSION

The main goal of the current study was to determine the rapid differential value for neopterin in pulmonary and extrapulmonary tuberculosis. These findings suggest that in general, the serum level of the neopterin is most likely to be used to diagnose pulmonary and extrapulmonary tuberculosis and to differentiate pulmonary tuberculosis from pulmonary infections and pulmonary malignancies. However, by this study alone, it is not possible to comment definitively on the differentiation of extra-pulmonary tuberculosis from another pulmonary disease, and further studies are needed. Taken together, these results suggest that other immune system markers secreted during TB disease should be evaluated.

REFERENCES

- [1] <https://www.who.int/news-room/factsheets/detail/tuberculosis>. Tuberculosis 2020
- [2] Chen DY, Li JP, Chen YM, Liao TL, Chen HH, Hsieh CW, Yeh YW, Lan JL. Elevated neopterin levels are associated with increased tuberculosis risk in rheumatoid arthritis patients with quantiFERON conversion during biologic therapy. *PLoS One*. 2016 Nov 18;11(11):e0166301.
- [3] Xu JC, Li ZY, Chen XN, Shi CL, Wu MY, Chen H, Zhu XY, Song HF, Wu MJ, Xu P. More significance of TB-IGRA except for the diagnose of tuberculosis. *Journal of Clinical Laboratory Analysis*. 2018 Jan;32(1):e22183.
- [4] Uplekar M, Weil D, Lonnroth K, Jaramillo E, Lienhardt C, Dias HM, Falzon D, Floyd K, Gargioni G, Getahun H, Gilpin C. WHO's new end TB strategy. *The Lancet*. 2015 May 2;385(9979):1799-801.
- [5] Falzon D, Timimi H, Kurosinski P, Migliori GB, Van Gemert W, Denking C, Isaacs C, Story A, Garfein RS, do Valle Bastos LG, Yassin MA. Digital health for the End TB Strategy: developing priority products and making them work. *European Respiratory Journal*. 2016 Jul 1;48(1):29-45.
- [6] Samara I, Bakakos P, Orphanidou D, Latsi P, Katsimpoula S, Papaetis GS, Dimakou K. Sputum adenosine deaminase activity in patients with pulmonary tuberculosis and lung cancer. *Advances in Clinical and Experimental Medicine*. 2007 Jan 1;16(4):533.
- [7] Plata-Nazar K, Luczak G, Liberek A, Dudzinska-Gehrmann J, Sznurkowska K, Landowski P, Kaminska B. Evaluation of clinical usefulness of

- serum neopterin determination in children with bacterial infections. *Acta Biochimica Polonica*. 2015 Feb 6;62(1).
- [8] Hamerlinck FF. Neopterin: a review. *Experimental dermatology*. 1999 Jun;8(3):167-76.
- [9] Hall RJ, Watne LO, Idland AV, Raeder J, Frihagen F, MacLulich AM, Staff AC, Wyller TB, Fekkes D. Cerebrospinal fluid levels of neopterin are elevated in delirium after hip fracture. *Journal of neuroinflammation*. 2016 Dec 1;13(1):170.
- [10] Yadav AK, Sharma V, Jha V. Association between serum neopterin and inflammatory activation in chronic kidney disease. *Mediators of inflammation*. 2012 Jan 1;2012.
- [11] Eisenhut M. Neopterin in diagnosis and monitoring of infectious diseases. *Journal of biomarkers*. 2013;2013.
- [12] Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. *Current drug metabolism*. 2002 Apr 1;3(2):175-87.
- [13] Liu QY, Han F, Pan LP, Jia HY, Li Q, Zhang ZD. Inflammation responses in patients with pulmonary tuberculosis in an intensive care unit. *Experimental and therapeutic medicine*. 2018 Mar 1;15(3):2719-26.
- [14] Yoshiyama T, Sugioka K, Naruko T, Nakagawa M, Shirai N, Ohsawa M, Yoshiyama M, Ueda M. Neopterin and Cardiovascular Events Following Coronary Stent Implantation in Patients with Stable Angina Pectoris. *J Atheroscler Thromb*. 2018;25(11):1105-17.
- [15] Ryu YJ. Diagnosis of pulmonary tuberculosis: recent advances and diagnostic algorithms. *Tuberculosis and respiratory diseases*. 2015 Apr 1;78(2):64-71.
- [16] Lee Y, Raviglione MC, Flahault A. Use of Digital Technology to Enhance Tuberculosis Control: Scoping Review. *Journal of Medical Internet Research*. 2020;22(2):e15727.
- [17] McNerney R, Daley P. Towards a point-of-care test for active tuberculosis: obstacles and opportunities. *Nature Reviews Microbiology*. 2011 Mar;9(3):204-13.
- [18] Spencer ME, Jain A, Matteini A, Beamer BA, Wang NY, Leng SX, Punjabi NM, Walston JD, Fedarko NS. Serum levels of the immune activation marker neopterin change with age and gender and are modified by race, BMI, and percentage of body fat. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*. 2010 Aug 1;65(8):858-65.
- [19] Goyal N, Kashyap B, Singh NP, Kaur IR. Neopterin and oxidative stress markers in the diagnosis of extrapulmonary tuberculosis. *Biomarkers*. 2017 Oct 3;22(7):648-53.
- [20] Goyal N, Kashyap B, Singh NP, Kaur IR. Comparative Diagnostic Utility of Neopterin and IFN- γ /IL-2 in Extrapulmonary Tuberculosis. *Indian Journal of Clinical Biochemistry*. 2017 Oct 1;32(4):453-8.
- [21] Eisenhut M, Hargreaves DS, Scott A, Housley D, Walters A, Mulla R. Determination of Urinary Neopterin/Creatinine Ratio to Distinguish Active Tuberculosis from Latent Mycobacterium tuberculosis Infection. *Journal of biomarkers*. 2016;2016.
- [22] Pourakbari B, Mamishi S, Zafari J, Khairkhah H, Ashtiani MH, Abedini M, Afsharpaiman S, Rad SS. Evaluation of procalcitonin and neopterin level in serum of patients with acute bacterial infection. *The Brazilian Journal of Infectious Diseases*. 2010 May 1;14(3):252-5.