

Serum neopterin, soluble interleukin 2- receptor (sIL-2R α) and lung computed tomography in differentiation between pulmonary tuberculosis and bronchogenic carcinoma

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ABSTRACT

In this study, serum levels of neopterin and soluble interleukin 2- receptor alpha (sIL-2R α) were determined in patients with pulmonary tuberculosis (PTB) and lung cancer (bronchogenic carcinoma stage I and II) in order to differentiate between active pulmonary TB, inactive pulmonary TB and lung cancer, and in order to determine the relationship between those findings with the corresponding Computed Tomographic scanning (CT chest) findings. The study included 15 patients with active pulmonary TB, 15 patients with inactive pulmonary TB, 15 with lung cancer and 15 healthy subjects served as control group. History taking, clinical examination, estimation of ESR, serum neopterin, serum sIL-2R α and CT chest were performed for each subject. Serum Levels of neopterin and sIL-2R α were significantly higher in TB patients with active disease compared to patients with inactive TB, lung cancer patients and controls ($P < 0.001$). Levels of neopterin and sIL-2R α in inactive TB and lung cancer patients were significantly higher compared to controls ($P < 0.001$); with significantly higher levels in TB patients with inactive disease than lung cancer patients ($P < 0.05$). Serum neopterin and sIL-2R α in moderate/advanced TB lesions (according to CT findings) were significantly higher than in mild TB. In addition, serum neopterin and sIL-2R α in bronchogenic carcinoma stage I and II with larger tumors (according to CT findings) were significantly higher than those with smaller tumors. Serum Levels of neopterin and sIL-2R α significantly positively correlated to each other in all studied groups. In conclusion, serum neopterin and sIL-2R α levels might be of value in differentiating between pulmonary TB and bronchogenic carcinoma stages I and II; significantly higher levels are in favor of active pulmonary TB. Serum neopterin and sIL-2R α levels also seem to be sensitive markers of TB disease activity. Since CT may be, in few cases, non conclusive in diagnosis of TB activity, therefore combining CT chest with serum neopterin and sIL-2R α may increase the accuracy of diagnosis of pulmonary TB activity.

INTRODUCTION

Tuberculosis is the single most frequent infectious cause of death worldwide (1). Key components of the immune response in tuberculosis include alveolar macrophages and T lymphocytes, particularly CD4+ (so-called T-helper) cells (1). Macrophages are the primary phagocytic cells in defense against Mycobacterium Tuberculosis, and T cells are able to stimulate a variety of macrophage effector functions by secreting cytokines (2). Plasma activation markers, like neopterin, tumor necrosis factor alpha (TNF- α), interleukins etc., are products of cytokine activity and represent immunologic changes throughout the body (3).

Neopterin is 2-amino-4-hydroxy-6-(D-erythro-1',2',3'-trihydroxypropyl)-pteridine derived from guanosine triphosphate and is produced by stimulated macrophages under the influence of interferon-gamma (IFN- γ). Significant associations between enhanced neopterin and IFN- γ production have been obtained in patients. It has been suggested that it is an excellent marker for the activation of the monocyte/macrophage axis in some clinical situations (4). Increased concentrations of neopterin in serum has been found in viral infections including human immunodeficiency virus (HIV) type 1, bacterial infections, various malignant disorders, autoimmune diseases and du-

ring allograft rejection episodes (5). Besides, macrophages also release reactive oxygen species (ROS) and TNF- α (3). The amounts of neopterin produced by activated macrophages correlate with their capacity to release ROS (3). In addition, neopterin was found to enhance the toxic effects induced by ROS. With this background, neopterin concentration can be regarded as an indirect estimate of the degree of oxidative stress emerging during cell-mediated immune response (6).

As a result of mycobacterial infection and the subsequent T-lymphocyte activation, sIL-2R α molecules are released into the circulation (7). Although sIL-2R α is also expressed on B-cells and monocytes, the majority of cells bearing sIL-2R α are T lymphocytes (8). Therefore, the serum levels of sIL-2R α may be used as a serological indicator of T-lymphocyte activation.

In some cases it is difficult to differentiate between pulmonary TB and early stages of bronchogenic carcinoma. Tuberculous patients and patients with bronchogenic carcinoma may develop pleural effusion (1). Differential diagnosis between tuberculous and non-tuberculous pleural effusions represents a critical clinical problem (1). Conventional methods for diagnosis of pleural tuberculosis have proven insufficient. Yilmaz et al. (9) reported that presence of fluid within a calcified fibrothorax at CT should

raise concern for active disease. They added that TB effusions are typically loculated and may be stable in size for years.

Most of the studies done on neopterin and sIL-2R α in TB and lung cancer measured these parameters in bronchoalveolar fluid (4) or pleural fluid (10). Both of which are invasive techniques. Also not all patients with pulmonary TB or bronchogenic carcinoma present with pleural effusion especially in stage I and stage II.

Thus the aim of the present work was to estimate the serum levels of neopterin and sIL-2R α in patients with pulmonary tuberculosis and lung cancer (bronchogenic carcinoma stage I and II) in an attempt to: (a) diagnose TB activity; (b) differentiate between pulmonary TB and bronchogenic carcinoma; and (c) determine the relationship between those findings and the corresponding CT findings.

SUBJECTS AND METHODS

The study included forty five patients. They were recruited at the Department of Internal Medicine, Kasr El Aini Hospital, Cairo University. They were divided into: (1) Fifteen patients with active pulmonary TB (subdivided by CT findings to 7 patients with mild lesions and 8 with moderate/advanced lesions); (2) Fifteen patients with inactive TB (on antituberculous treatment for 6 months), (3) Fifteen patients with bronchogenic carcinoma stage I and stage II according to Tumor Node Metastasis (TNM) classification of CT findings (11). Fifteen age and sex matched healthy subjects participated as control group.

Diagnosis was based on clinical, radiological assessment (including chest X-ray, CT chest) and laboratory assessment (including ESR, C-reactive protein (CRP), complete blood count (CBC), tuberculin testing and examination of sputum for *Mycobacterium tubercle bacilli* by Ziehl- Neelsen stained smear).

Antituberculous chemotherapy given: patients with inactive TB were having anti TB treatment for 6 months. The 6-month regimen consisted of isoniazid, rifampin, and pyrazinamide given for 2 months followed by isoniazid and rifampin for 4 months (12). Patients with active TB and patients with bronchogenic carcinoma were not under therapy at the time of sampling.

All patients and control were subjected to: History taking and clinical examination; estimation of serum neop-

terin using enzyme immunoassay (13) (DRG International, Inc. USA); estimation of serum sIL-2R α using enzyme immunoassay (7) (provided by R&D Systems, USA); measurement of erythrocyte sedimentation rate (ESR); radiological assessment: in the form of plain chest Xray (posteroanterior and lateral views) and computed tomographic scanning (CT chest). Patients were considered active based on previously reported CT signs (9).

The patients with active TB were further subdivided into 2 groups; first with mild lesions and second with moderate / advanced lesions as described by Mohamed et al. (4).

Also patients with bronchogenic carcinoma were subdivided into 2 groups by CT scanning according to the tumor size; small (T1 < 3cm) and larger tumors (T2 > 3cm) (8 and 7 patients respectively).

Exclusion criteria: Patients with diseases affecting the level of neopterin as diabetes mellitus, chronic liver diseases, chronic renal failure, and HIV infection.

Statistics: Values were expressed as means \pm standard deviation (SD). For comparison of means between groups one way analysis of variance (ANOVA test) with post, hoc test was performed. To study the relation between two variables, Pearson's correlation coefficient (r) was calculated (14). Student's t - test was performed to compare differences in means between two studied groups (mild and moderate/ advanced TB and between small and larger tumors).

RESULTS

Results of measurements are summarized in tables 1 and 2. Table 1 shows significant difference between all the studied groups compared to control group and to each other (P < 0.001) concerning mean levels of ESR, serum neopterin and sIL2R α (p < 0.001 with the exception of the difference between sIL2R α in the inactive TB and lung cancer where p < 0.05).

Serum neopterin showed significant positive correlations with sIL2-R α in all TB patients; active and inactive group (n=30), [r=0.9, p<0.001 Fig. (1)]. Serum neopterin showed significant positive correlations with sIL2-R α in all studied groups: (r = 0.83, 0.55, 0.6 and 0.91 p<0.001, p<0.05, p <0.05 and p<0.001) in inactive TB, active TB groups lung cancer [Fig. (2)], as well as in all patients (45 patients) respectively.

Table 1

Sedimentation rate [ESR] (mm) and serum levels of neopterin (nmol/L) and sIL-2R α (pg/ml) in control, inactive TB, active TB and bronchogenic carcinoma groups.

Measurement	Controls Mean \pm SD	Inactive TB Mean \pm SD	Active TB Mean \pm SD	Bronchogenic carcinoma (Mean \pm SD)
ESR	6.2 \pm 2.1 (a)	24.6 \pm 5.4 (b)	70.4 \pm 9.2 (c)	54.3 \pm 11.8 (d)
Serum neopterin	5.8 \pm 1.3 (a)	34.2 \pm 4.2 (b)	52.6 \pm 6.7 (c)	21.6 \pm 4.1 (d)
Serum sIL-2R α	367.6 \pm 81.5 (a)	913.8 \pm 158.7 (b)	1620.4 \pm 110.5 (c)	840.7 \pm 93.5 (d)

Groups having different letters denote that the difference between the corresponding means of these groups is statistically significant i.e. p < 0.05

Table 2

Serum levels of neopterin (nmol/L) and sIL-2Ra (pg/ml) in patients with mild and moderate/advanced TB and patients with bronchogenic carcinoma with small tumors (T1) and with larger tumors (T2) [comparing tumor size]

Measurement	Tuberculosis		Tumor	
	Mild n=7	Moderate/advanced n=8	Small (T1) n=8	Large (T2) n=7
Serum neopterin (nmol/L)	47.4±4.3	57.2±4.8*	18.47±2.57	24.48±3.1*
Serum sIL-Rα (pg/mL)	1554.6±95.5	1678.1±91.8*	791.28±79.82	883.87±86.4*

Values are represented as means±S.D.

*denotes that the difference between the means of the mild and moderate/advanced TB groups and between the the larger tumor size group(T2) and the small tumors (T) group are statistically significant (P < 0.05).

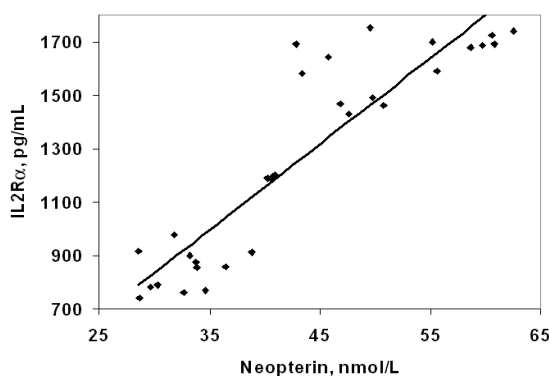


Figure 1

Correlations between serum neopterin (nmol/L) and interleukin-2 receptor (sIL-2Rα) (pg/ml) in all TB patients (Active and Inactive, n=30)

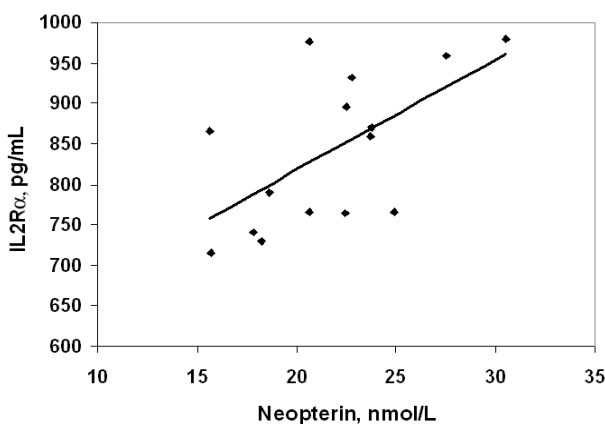


Figure 2

Correlations between serum neopterin (nmol/L) and interleukin-2 receptor (sIL-2Rα) (pg/ml) in lung cancer group (bronchogenic carcinoma n=15).

It is worth mentioning that two of the active TB cases were diagnosed as active on clinical and laboratory back-

grounds but, diagnosis was not definite by CT. They were categorized by CT as mildly active however; the serum

neopterin and sIL-2R α levels were high matching the results of the moderate/advanced active group.

DISCUSSION

Serum Levels of neopterin and sIL-2R α were significantly higher in active TB patients compared with inactive TB, lung cancer patients and controls. Levels in inactive TB and lung cancer patients were significantly higher compared to controls; with significantly higher levels in TB patients with inactive disease than lung cancer patients. This suggests that serum neopterin and sIL-2R α levels may be sensitive markers of pulmonary TB disease activity.

Serum neopterin and sIL-2R α in moderate / advanced TB lesions were significantly higher than in mild TB. These results suggest a probable relationship between each of serum levels of neopterin and sIL-2R α and the severity of the lung lesion. Serum Levels of neopterin and sIL-2R α were correlated to each other in all studied groups.

These results are in accordance with those reported by Horak et al., (15) in the patients with progressive primary tuberculosis and documented excessively high neopterin levels. They concluded that neopterin may be a useful parameter for measuring the degree of disease activity and the response to therapy. Our finding of high serum neopterin level in moderate/advanced TB lesions is in agreement with Yuksekol et al., (16) who reported that moderately advanced pulmonary TB according to radiographic findings had higher levels of neopterin than patients with minimal disease. They concluded that neopterin levels in serum may reflect pulmonary TB activity before exact diagnosis of the disease by culture results. They also found significant correlation between neopterin and radiological findings.

As malignant tumor cells exhibit altered cell surface compared to non-malignant cells, they may cause reactions of the specific cellular immune system and thereby also neopterin production. Thus, the significant elevation of serum neopterin levels in neoplastic patients suggests an activation of cells in the monocytic/macrophage line. This activation could be a result of increased interferon γ synthesis by natural killer cells or greater TNF - α production, with an increase of the T-cell response to antigenic stimulation (17).

Murr et al. (13) reported a significant increase of neopterin levels in the tuberculous pleural fluid and blood compared to the neoplastic group. In the blood, values were nearly identical to the pleural fluid.

The frequency of elevated neopterin concentrations in serum of patients with malignant diseases at the beginning of the disease indicates a worse outcome. The frequency depends not only on the tumor type, but also on the tumor stage (17). Also a correlation between neopterin levels and the estimated total mass of tumor cells was shown (17).

In general, advanced stages show higher neopterin values than earlier ones (17). This might explain the relatively lower levels of neopterin and sIL-2R α in the

bronchogenic carcinoma patients as compared to the inactive TB patients in the present study as all the bronchogenic carcinoma patients were stage I or II. However, in the present study significantly higher serum levels of both neopterin and sIL-2R α were detected in patients with larger tumor masses than those with smaller ones.

Prommegger et al., (18) reported that successful treatment is associated with decline or even normalization of neopterin levels in most of bronchogenic carcinoma cases.

They also reported that neopterin can serve as an immunologically based estimation of malignant outgrowth. In patients who are operable by clinical tumor stage but have a high risk for operation, elevated preoperative neopterin may help in the decision for a nonoperative treatment. Kronberger et al., (19) reported the significantly predictive role of neopterin level as a predictor of survival, with high level indicating short survival.

Serum sIL-2R α is a useful marker of the clinical state and evolution of patients with pulmonary tuberculosis (10). In accordance with our results, Porcel et al., (10) reported that serum sIL-2R α levels correlated positively with disease activity as assessed by roentgenographic findings. They added that levels of sIL-2R α were high in sera from patients with extensive parenchymal lesions on the roentgenogram and patients with tuberculous pleurisy. They concluded that pleural sIL-2R α appears to be clinically useful for differentiating tuberculous and non-tuberculous pleural effusions.

These studies indicated that determination of serum concentrations of sIL-2R α is a highly sensitive and specific method for monitoring disease activity in terms of T cell activation in pulmonary tuberculosis.

The role of the elevated sIL-2R α in the immunity against TB is uncertain. It could indicate an inappropriate activation of T -lymphocytes and macrophages/ monocytes, which may have harmful consequences (20). On the other hand, a prolonged elevation of sIL-2R α reflect a continued activation of T-lymphocytes and monocytes/macrophages which may have a possible protective role.

Living mycobacteria are typical long-lasting parasites within tuberculous lesions, and their antigenic components could be presented by macrophages to CD4 specific T-lymphocytes (1).

Aviles et al., (21) reported that both the radiological findings and the clinical state of patients showed a good correlation with sIL-2R α . This was in agreement with our results.

Also, they added that the detection of permanently high values beyond 6 months of treatment suggests that additional drugs or prolonged administration would be advisable in order to ensure full recovery. Chen et al., (22) reported that the sIL-2R α levels were persistently raised up to three months after initiation of anti-TB chemotherapy.

They added that a gradual reduction of serum sIL-2R α level from the second month of treatment onwards. At the

sixth month of treatment the level was significantly lower than that before treatment but still higher than control subjects, despite clinical improvement. This may suggest a delayed resolution of the inflammation in patients with pulmonary TB.

This was in accordance with our results of the inactive group which was having anti TB therapy for six months but the levels of both neopterin and sIL-2R α were still higher than control group.

Naumnik et al., (23) reported significantly higher level of sIL-2R α in lung cancer patients than in controls. However, they added that there were no significant differences in serum sIL-2R α levels in relation to clinical stage of lung cancer and response to therapy.

In contrast to our results, Khanna et al., (24) reported a significant decrease in IL-2 and IL-2 R α levels in patients with pulmonary tuberculosis when compared to normal controls. These results suggested that patients with pulmonary tuberculosis had a defect in IL-2 production.

CT was reported to be useful in all stages of the TB, particularly when there is no correlation between clinical and plain X ray findings (25). They also reported that CT can accurately diagnose active and inactive disease in 80% and 89% of cases, respectively. Many studies suggest that although CT evaluation may be helpful in determination of disease activity in some patients, definitive diagnosis still requires isolation and identification of *M. tuberculosis* in clinical specimens (25). Bass et al. (26) reported that CT should be proposed at the end of an effective antituberculous treatment to better subsequently detect fine lesions suggestive of reactivation of TB. They added that lack of radiographic change over a 4- to 6 month interval generally indicates inactive disease (25). However, because even long-term stability of radiographic findings may occasionally be associated with culture-positive disease, Bass et al. (26) emphasize that such findings should be described as "radiographically stable" rather than "inactive."

Surprisingly, in the present study, 2 cases of the active TB group were not definitely diagnosed by CT. By the clinical and laboratory criteria they were suggested to be active TB and categorized as such. Their serum neopterin and sIL-2R α were high matching the levels of the moderate/advanced TB patients, thus confirming the clinical assessment. This finding may indicate that the measuring these biochemical markers may be sensitive adjuvant to CT improving the accuracy of diagnosis of active TB.

In conclusion, serum neopterin and sIL-2R α levels might be of value in differentiating pulmonary TB and bronchogenic carcinoma; higher levels are in favor of active pulmonary TB. Serum neopterin and sIL-2R α levels also seem to be sensitive markers of TB disease activity. Combining CT chest with serum biochemical markers; neopterin and sIL-2R α may increase the accuracy of diagnosis of pulmonary TB activity.

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