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Neopterin in Tuberculous and Neoplastic Pleural Fluids

M. F. Baganha,[1] A. Mota-Pinto,[2] M. A. Pêgo,[1] M. A. T. Marques,[1] M. A. Santos Rosa,[2] and A. J. A. Robalo Cordeiro[1]

[1]Pneumology Service, University Hospital of Coimbra, and [2]Immunology Laboratory, College of Medicine, University of Coimbra, 3000 Coimbra, Portugal

Abstract. Neopterin is derived from guanosine-triphosphate, produced by stimulated macrophages under the influence of gamma interferon of lymphocyte origin. It has been suggested as an excellent marker for activation of the monocyte/macrophage axis in some clinical situations. We evaluated its concentration in the pleural effusions of 25 individuals (10 tuberculous and 15 neoplastic) as well as in the blood of 22 of them (8 tuberculous and 14 neoplastic), comparing these levels with those of a control group in 99 normal individuals. The concentration of neopterin was determined by radioimmunologic assay. This showed a significant increase ($p < 0.001$) of neopterin levels in the tuberculous pleural fluid, compared to the neoplastic group (42 ± 23/ 17 ± 9 nmol/L).

In the blood, values were nearly identical to the pleural fluid (41.3 ± 25/15.8 ± 6.9 nmol/L), although with significant differences between them and in relation to the control group ($p < 0.001$), which had a normal serum value (5.11 ± 1.92 nmol/L).

We emphasize the influence of the neopterin levels in the pleural fluid on the diagnosis of causes of pleurisy and its importance as a marker of immunologic cellular activity.

Key words: Neopterin—Tuberculous pleurisy—Neoplastic pleurisy—Pleural fluid—Cellular immunologic marker.

Neopterin, chemically known as pyrazinopyrimidine, is derived from guanosine-triphosphate and is an intermediary product in the metabolic sequence that leads to biopterin synthesis [24].

It is a product of the activated macrophage by gamma interferon of lympho-

Offprint requests to: M. F. Baganha

cytic origin [10, 22, 43, 45], although other factors, such as alpha interferon, tumor necrosis factor-alpha (TNF-alpha), and lipopolysaccharide (LPS) can, to a lesser degree, contribute to its synthesis [14, 16, 21, 42].

Neopterin is excreted in urine [8, 22], and it has been measured, usually in its oxidized form, in this biological milieu as well as in blood, in cerebrospinal fluid, and in bronchoalveolar lavage fluid [9, 34].

Urinary levels, apparently high in women [45], are significantly increased with age [45], and in clinical cases characterized by an inflammatory or immunologic response. For this reason, neopterin has been suggested as an excellent marker of immunologic activity and used as such in several clinical situations: sarcoidosis [11, 26, 29 ,35, 38, 45], celiac disease [14], multiple sclerosis [13], chronic renal insufficiency [8], sepsis [41], pulmonary tuberculosis [15], bronchial asthma exacerbation [33], monoclonal gammapathies [45], several malignant conditions [7,12, 20, 25, 30, 40, 44, 45], transplantations [9, 19, 23, 27, 31, 32, 36, 39, 44], and acquired immunodeficiency syndrome [16–18, 37]. In the latter, according to some authors, it constitutes a useful way to study the disease's evolution, since elevation of its serum level precedes CD4 cell decrease [1, 6, 16–18, 28]. Given the acknowledged importance of immunologic mechanisms in the pathogenesis of pleural effusions, we began to measure neopterin concentration in that biological milieu to verify its possible role in the diagnosis of pleurisy.

Material and Methods

Twenty-five individuals were analyzed (15 men and 10 women, 57.3 ± 13.9 years old), 10 of whom had tuberculous effusions (7 men and 3 women, 49.5 ± 18 years old) and 15 of whom had neoplastic secondary bronchial carcinoma (8 men and 7 women, 63.6 ± 10 years old).

The diagnosis was established by positive direct examinations and cultures of *Mycobacterium tuberculosis* and/or specific pathologic alterations in pleural biopsy specimens in relation to tuberculous pleurisy, and the existence of malignant pleural histopathologic lesions, (in neoplastic effusions). Neopterin blood concentration was evaluated in 22 individuals (8 tuberculous and 14 neoplastic); a group of 99 healthy individuals was used as a normal control group.

Neopterin concentration, both in serum and in pleural fluid, was measured by a radioimmunologic process, for which a Henning RI Acid was used, with the following analytical methodology:

Preparation of biological samples and their distribution, as well as that of the standard and quality control samples, in polystyrene tubes (50 μl per tube).

Addition of a specific antineopterin antibody.

Incubation (1 hr) at normal temperature protected from daylight.

Addition of [^{125}I]neopterin, 100 μl for all tubes.

Incubation (1 hr) at normal temperature protected from daylight.

Addition of a polyethylene glycol suspension (2 ml for each tube).

Tube centrifugation at 2000 g for 10 min; the supernatant was then aspirated.

Gamma radiation reading at 60 min.

Results reckoned from the percentage ratio sample radiation/zero standard (B/Bo).

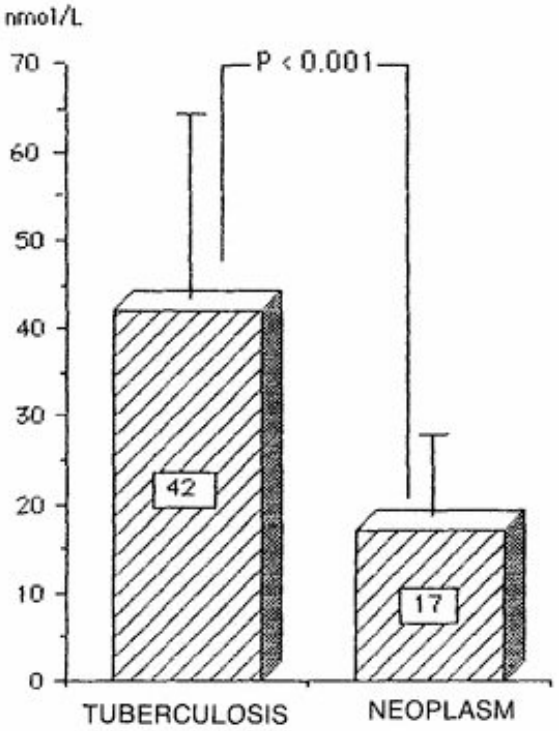

Fig. 1. Median neopterin values in pleural fluid.

The results were expressed in nmol/L. Student's t-test and linear correlation test were used for statistical analysis.

Results

In the pleural effusion (Fig. 1), the neopterin median value of the tuberculous group (42 ± 23 nmol/L) was very high, significantly ($p < 0.001$) higher than in the neoplastic group (17 ± 9 nmol/L). The concentration in the blood of both tuberculous (41.3 ± 25 nmol/L) and neoplastic (15.8 ± 6.9 nmol/L) individuals, was significantly ($p < 0.001$) higher than in the normal control group (Fig. 2).

In each of the analysed pathologic groups, the values in the blood and in the pleural fluid (Figs. 1, 2) are nearly identical (42 ± 23/41.3 ± 25 nmol/L and 17 ± 9/15.8 ± 6.9 nmol/L, respectively), without statistical differences ($p < 0.60$ and < 0.50, respectively).

As for the distribution of neopterin levels, according to sex and age, it was to observe that although women generally showed higher pleural and serum values than men (29.5 ± 21.8/25.8 ± 19.7 nmol/L and 25.2 ± 20.5/24.7 ± 17.9 nmol/L, respectively), no statistically significant differences were found between the sexes ($p < 0.60$ and < 0.50, respectively).

We noticed, however, that neopterin concentration increased significantly with age in the pleural fluid ($r = 0.44$; $p = 0.09$) as well as in the serum ($r = 0.60$; $p < 0.05$) of the neoplastic group (Fig. 3).

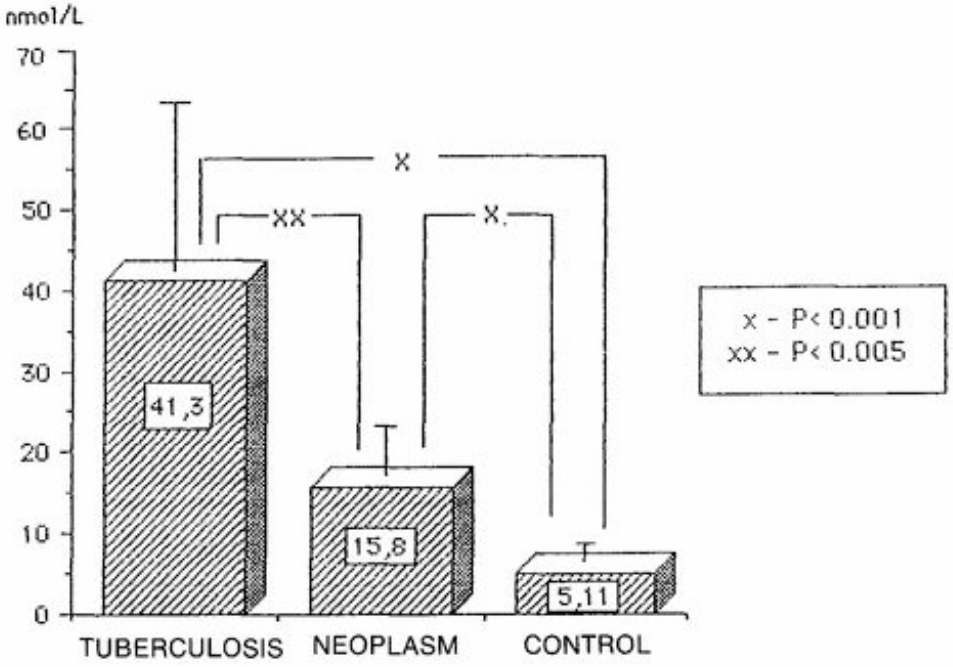

Fig. 2. Median values in blood of 2 groups studied and control group.

Discussion

These results suggest that the study of neopterin in pleural fluid can contribute to an interpretation of the pathogenic mechanisms and diagnosis of causes of the clinical condition, as has been observed in other biological milieux.

The significant increase of neopterin values in tuberculous exudates (in relation to the neoplastic) seems to be confirmed by the cellular immunity abnormalities that follow these pathologic situations, particularly the variations in T lymphocyte subpopulations. Thus, the fact that CD4 cell activation is followed by an increase of neopterin macrophage production [5] agrees with the predominance of CD4 lymphocytes and increased CD4/CD8 ratio in tuberculous effusion (as for the neoplastic group) [3]. Such high neopterin values in tuberculous pleural fluids therefore allow for differentiation of the clinical situations. In addition, the possibility of increasing neopterin production following an inhibition of the T suppressive function [5] seems to prove the importance of the helper T cells.

The significant elevation of serum neopterin levels in the carrier of neoplastic pleural effusion (despite a clearly lower concentration in tuberculous individuals) assumes a stimulation of monocyte/macrophagic line, which, in the presence of a normal or decreased CD4/CD8 ratio in the neoplastic effusion fluids [3], could be a result of increased gamma interferon synthesis by natural killer cells [4] or greater TNF-alpha production, with an increase of the T-cell response to antigenic stimulation [42].

The observation of nearly identical neopterin serum and pleural levels

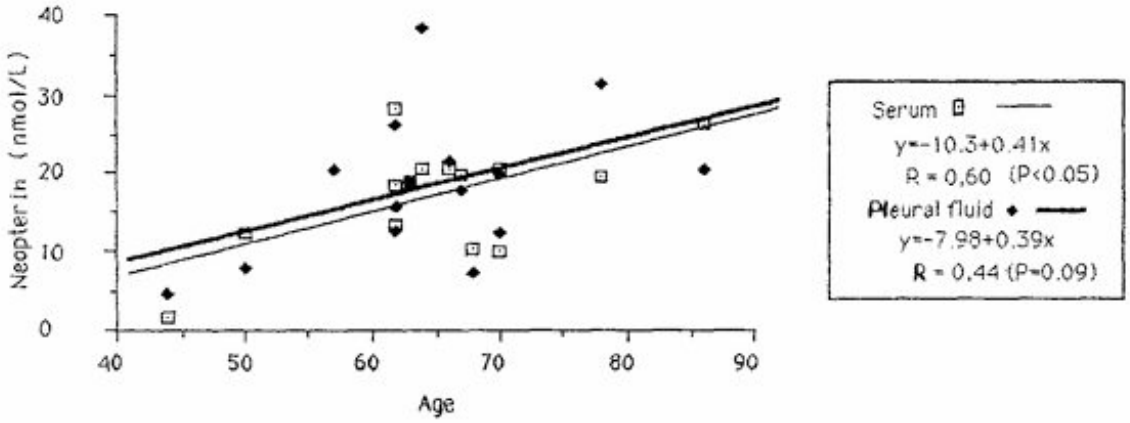

Fig. 3. Correlation between neopterin concentration and age, in pleural fluid and serum of neoplastic group.

in each of the clinical situations examined suggests the absence of a pleural compartment of these phenomena or diffusion of neopterin produced in the pleural cavity into the blood. This speculative interpretation finds important support in the molecular weight of this substance, even considering the difficulties when the pleural serosa is affected by an inflammatory or malignant process.

There were no important differences between the sexes, either in the serum or pleural concentration of neopterin. This would agree with the presence of similar blood levels in both sexes in normal conditions. We found a slight elevation of these values in women (although this was not statistically significant), which is confirmed by other investigations [35] and seems to depend on hormonal and weight differences between men and women [45].

The significant rise with age, observed only in the neoplastic group, supports work done by other researchers [2, 35, 45]. All the neoplastic individuals were in the same state and did have problems with renal function. A possible explanation for these results is the increase in T cells with age. Some authors have shown a correlation between neopterin concentration and the number of lymphocytes in physiological conditions [10].

We also noted the importance of neopterin as a marker of cellular immune activity. Its concentration in the pleural effusion fluid, which reflects macrophage activation, may contribute to the diagnosis of the causes of pleurisy and to a pathogenic interpretation of some clinical situations.

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