Pteridines
Vol. 2, 1990, pp. 107–109

Short Communication

Urinary Neopterin in Monoclonal Gammopathies

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(Received March 1990)

Introduction

Multiple myeloma (MM) is a hematological disorder caused by the emergence of a malignant clone of plasma cells, a mature form of B lymphocytes. The disease is frequently preceded by a phase of monoclonal gammopathy characterized by the presence of a paraprotein in the serum of the patients, but without signs or symptoms of illness. Since 1–2%/year of the patients with paraproteinemia develop MM, most authors use the term "monoclonal gammopathy of unknown significance" (MGUS).

In order to get clinically relevant indicators for the prognosis and treatment of myeloma patients, Durie and Salmon established a staging system which correlates well with the estimated total number of malignant cells (1). Additional factors were found to correlate with the prognosis, e.g., the labeling index indicating the percentage of proliferating plasma cells in the bone marrow (2), and the serum concentration of beta-2-microglobulin, which has been shown to be produced by myeloma cultures in vitro (3, 4).

D-erythro-neopterin is secreted by monocytes/macrophages upon stimulation with interferon gamma (5). This effect can be synergistically enhanced by tumor necrosis factor alpha (6). Neopterin has been shown to be of prognostic value in Hodgkin's disease, non-Hodgkin's lymphoma and chronic myelogenous leukemia (7, 8).

The aim of this study was to analyse the diagnostic value of urinary neopterin in patients with MGUS and multiple myeloma.

Patients and Methods

Patients

51 patients were included in this study. All of them had monoclonal gammopathy confirmed by immunoelectrophoresis of serum and/or urine. 41 patients (80%) suffered from MM, 10 patients had MGUS. MM was excluded in these patients by bone marrow examination (< 10% plasma cells), bone radiography (lack of osteolytic lesions) and a stable course of the myeloma-protein. Staging of MM was performed according to the system of Salmon and Durie (1).

Characteristics of patients are summarized in Table 1. None of the patients with MGUS was treated. Out of the patients with MM, 10/41 were evaluated at the time of diagnosis, 8/41 had no treatment because of remission or stable disease. The other 23 were under

Table 1. Characteristics of patients

Patients	n = 51
Mean age (years	65 (range 37–86)
Male: Female	29:22
MGUS	10 (19.6%)
Myeloma	41 (80.4%)
Stage: I	11
II	6
III	24

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chemotherapy for active disease and determination of neopterin was usually performed four weeks after last therapy.

Analytical procedures

Hemoglobin and leukocytes were routinely evaluated from fresh blood samples using a cell counter (Coulter, Miami, FL, USA). Differential counts were done by examination of stained blood smears. Beta-2-microglobulin was measured using a commercially available radioimmunoassay (Pharmacia, Uppsala, Sweden). Neopterin was determined by an optimized and fully automated high pressure liquid chromatography technique without previous oxidative treatment as described elsewhere (9). By this reverse-phase technique, urinary creatinine is simultaneously determined in the same urine specimen. To account for variations of urine concentrations, neopterin levels are expressed as µmol/mol creatinine.

Statistics

For the evaluation of differences between various groups of patients we used Kruskal-Wallis rank sum test. To define the strength and significance of correlations we computed Spearman's rank correlation coefficients.

Results

Neopterin, beta-2-microglobulin and hematological values (Table 2)

Neopterin and beta-2-microglobulin

In MGUS patients the mean neopterin value was below the upper normal limit. In contrast, in MM patients the mean value was clearly elevated. Due to the high standard deviations, the difference between MGUS and MM did not reach statistical significance ($p = 0.119$). Comparing patients with MGUS and MM stage I (mean value 199 $\pm$ 86) with patients showing MM stage II/III (362 $\pm$ 249) the difference was statistically significant ($p = 0.006$). Beta-2-microglobulin concentrations in the serum were higher in patients with MM stage II/III than in patients with MGUS and MM stage I ($p < 0.05$).

Hemoglobin, monocytes and peripheral lymphocytes

Hemoglobin concentrations and total number of peripheral lymphocytes were significantly lower in MM of advanced stage than in MGUS and stage I MM ($p < 0.001$ and $p = 0.035$, respectively), whereas the number of peripheral monocytes was not different ($p = 0.388$).

Correlation between neopterin, beta-2-microglobulin and hematological parameters

Correlating neopterin with the other values we found a significant negative correlation between neopterin and the hemoglobin concentrations ($r = -0.542$, $p = <0.05$). A positive correlation was seen between neopterin and the number of peripheral monocytes ($r = 0.38$, $p < 0.05$), whereas a correlation with lymphocytes was not found. Beta-2-microglobulin correlated with hemoglobin values in a similar manner as neopterin ($r = -0.702$, $p = <0.01$). Finally we observed a significant positive correlation between neopterin and beta-2-microglobulin ($r = 0.53$, $p < 0.05$).

Discussion

Our data show elevated concentrations of urinary neopterin and beta-2-microglobulin in patients with MM, especially in patients with more advanced dis-

Table 2. Urinary neopterin, beta-2-microglobulin and hematological parameters in patients with MGUS and multiple myeloma

	MGUS	Myeloma	p
Neopterin (µmol/mol creat.)	200 $\pm$ 107	318 $\pm$ 227	0.119
Beta-2-microgl. (mg/L)	3.1 $\pm$ 2.8	8.0 $\pm$ 9.8	<0.050
Hemoglobin (G/100 ML)	14.7 $\pm$ 1.1	11.8 $\pm$ 2.5	0.001
Lymphocytes (n/µl)	2139 $\pm$ 575	1363 $\pm$ 675	0.002
Monocytes (n/µl)	660 $\pm$ 322	477 $\pm$ 339	0.132

ease. Similar results have been described by others (10–12). From our data the question remains open, if neopterin is of special value in the differential diagnosis between MGUS and MM, since we have found similar values in MGUS and MM stage I. Nevertheless, increased values reflect a significant activity of the disease and may represent an additional parameter, e. g., for the decision if therapy is necessary or not.

The close correlation between neopterin and beta-2-microglobulin argues in favor of a similar clinical relevance of both markers, additionally neopterin is not dependent on the renal function, since neopterin values are related to urinary creatinine. Thomas (11) found that urinary neopterin concentrations correlate even stronger with the calculated tumor cell mass than beta-2-microglobulin.

Previous results have shown a negative correlation between hemoglobin and neopterin values (13) pointing to a potential role of immunological factors in the pathogenesis of anemia in hematological disorders. These data have been confirmed also in the present analysis of patients with MGUS and MM. Anemia in these patients might be not only due to bone marrow replacement, but mediated by a number of cytokines, e. g., interferon gamma or tumor necrosis factor, although we found a correlation between the degree of bone marrow infiltration by plasma cells and that of anemia (data not shown).

Elevated neopterin values are a sign of activation of cell mediated immunity. Therefore our data raise the question, whether the monocyte/macrophage system is activated in the framework of an – ineffective – immune reaction against the tumor or whether elevated neopterin concentrations result from macrophage activation by cytokines produced by the malignant cells themselves.

These two aspects, the clinical relevance on the one hand and the expression of an activation status of monocytes/macrophages on the other hand, make neopterin an interesting marker in monoclonal gammopathies.

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