Predictive Value of Interleukin-6 and Neopterin in Patients with Multiple Myeloma

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ABSTRACT

Concentrations of interleukin-6 and neopterin were measured in sera from 44 patients with multiple myeloma. To judge the relative prognostic value of these analyses, other clinical and laboratory variables were concomitantly determined. The patients were followed up to 9 years, and the abilities of all variables to predict outcome were assessed. Both neopterin (P = 0.0008) and interleukin-6 (P = 0.033) were significantly higher in patients with higher stages of the disease. The correlation between interleukin-6 and neopterin was weak but significant (Spearman's rank correlation coefficient, 0.38; P = 0.019). By univariate survival analysis using the product-limit approach, both neopterin (P = 0.0001) and interleukin-6 (P = 0.025) were identified as significant predictors of survival. Multivariate survival analyses by the proportional hazards technique demonstrated that either stage and neopterin or neopterin and interleukin-6 are useful combinations of predictor variables. Thus, interleukin-6, which is supposed to influence progression of multiple myeloma in an autocrine or paracrine manner, failed to contribute to prediction if stage was included in a model. In contrast, neopterin remained significant in all multivariate models.

INTRODUCTION

Interleukin-6 plays a pivotal role in mediating host response to tissue injury. Elevated levels of the cytokine are detectable in body fluids during the course of microbial infections, autoimmune diseases, and neoplasia (1, 2). In particular, interleukin-6 is a potent growth factor for hybridoma/plasmacytoma in mice (3, 4) and was found to be a strong in vitro myeloma growth factor in humans (5-7). Its presentation as an autocrine (5, 8) or paracrine (6, 7) growth factor remains controversial. Serum levels of interleukin-6 reflect disease severity in human plasma cell dyscrasias (9, 10).

Neopterin, which belongs to the class of pteridines, is a pyrazino[2,3-d]-pyrimidine compound which is biosynthesized from GTP. Human monocytes/macrophages, after stimulation by T cell-derived γ-interferon, produce and release increased amounts of neopterin (11). Recently, details of the cytokine-induced biosynthesis of pteridines by human cells and cell lines have been elucidated (12): γ-interferon stimulates only the key enzyme of pteridine biosynthesis, GTP-cyclohydrolase I, whereas the subsequent enzymes (6-pyruvoyl-tetrahydropterin synthase and sepiapterin reductase) are constitutively present. To date, only one human myelomonocytic cell line (THP-1) has been identified which behaves similarly to human monocytes/macrophages with respect to cytokine-induced pteridine biosynthesis (13).

During the last decade, neopterin has been recognized to be a useful in vivo indicator of the activation state of the cellular immune system (14). In particular, concentrations of the compound in serum or urine from patients with various malignancies have been demonstrated to be predictive for the course and progression of the disease (15-20).

The present study was undertaken with the aim to investigate the predictive significance of interleukin-6 and neopterin in patients suffering from multiple myeloma. Univariate and multivariate analyses including other potential predictor variables were done in order to define the relative value of interleukin-6 and neopterin to aid in the prognosis of multiple myeloma.

MATERIALS AND METHODS

Patients. Forty-four patients with multiple myeloma were included in the study. All investigations reported here were performed before therapy was initiated. There were 12 men and 32 women; the median age was 71 years, and the interquartile range of age was from 63 to 75 years. Thirteen of the patients had stage I disease, 12 had stage II disease, and 16 had stage III disease. To characterize patients further, performance status (0, 1, 2, 3) was assessed according to the criteria of the World Health Organization in 33 of the subjects with the following results: performance status 0, 4 patients; status 1, 12 patients; status 2, 5 patients; status 3, 12 patients. The patients were uniformly treated with vincristine, melphalan, cyclophosphamide, plus prednisone.

Laboratory Examinations. Interleukin-6 concentrations were determined by a bioassay using the interleukin-6-dependent 7TD1 hybridoma cell line (4): sera were sterile filtered and decomplemented at 56°C for 30 min. After 4 days of culture in the presence of serial dilutions of the sera and recombinant human interleukin-6 (Genzyme Biochemicals Ltd., Cambridge, Massachusetts) as standard, proliferation of 7TD1 cells was measured using a rapid standard colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (21). Half-maximal proliferation of 7TD1 cells was achieved at 3-5 pg/ml.

Neopterin concentrations in serum were measured using a commercially available radioimmunoassay system (Henning-Berlin, Berlin, Germany). By comparison with high pressure liquid chromatography, this assay has previously been shown to yield very satisfactory results (22). In this previous work, the 95th percentile of neopterin concentrations in sera from 359 healthy adults aged from 18 to 75 years was 8.7 nmol/liter; a sex dependence was not found.

In addition to neopterin and interleukin-6, creatinine and hemoglobin concentrations were also determined by routine techniques. Not all variables were available for all patients; therefore, the numbers of subjects are not equal for all variables.

Statistical Procedures. To describe associations between grouped data, the χ² test was used. Correlations between variables were assessed by Spearman's rank correlation technique. Additionally, differences of distributions of biochemical variables between different tumor stages were tested for significance by the nonparametric Kruskal-Wallis test. Univariate analyses of survival were done by the product-limit technique (23); significances of differences between survival curves were assessed by the generalized Savage statistic (Mantel-Cox statistic). Categorization of patients according to continuously coded biochemical variables was based on the quartile points of the observed distributions of the variables. Multivariate survival analyses were performed by the proportional hazards method (24), using a forward-stepping algorithm.
RESULTS

Results of biochemical measurements are reported in Table 1. The variables are shown separately for each stage of multiple myeloma, and also the pooled data are presented. Entries in the table are ordered according to decreasing strength of associations with tumor stage, determined by \( x^2 \) test of the respective contingency table. For interleukin-6 and neopterin, the differences between tumor stages are further shown in Fig. 1. Whereas interleukin-6 shows a slightly stronger association with stage than neopterin (Table 1) by contingency testing, by nonparametric analysis of variance neopterin shows a stronger stage dependence (Kruskal-Wallis test, \( H = 14.15, P = 0.0008 \)) than interleukin-6 (\( H = 6.83, P = 0.033 \)).

Associations between Variables. The association between concentrations of interleukin-6 and neopterin was weak but significant \( (R_x = 0.38, P = 0.019) \). Neopterin was also significantly correlated with hemoglobin \( (R_y = -0.56, P = 0.0006) \) and creatinine concentrations \( (R_z = 0.59, P = 0.0004) \).

Univariate Analysis of Survival. Fig. 2 shows product-limit estimates of cumulative survival probabilities for patients, grouped according to stage or quartiles of interleukin-6 and neopterin. Table 2 shows, in more detail, the results of univariate survival analyses using each clinical or laboratory variable as a single candidate predictor (variables missing in this table did not exhibit any predictive significance; \( P > 0.10 \)).

As suggested by Fig. 2, for subsequent analyses, patients were dichotomized according to stage (I versus II/III), interleukin-6 (below versus above the 75th percentile = 3rd quartile of 7.0 pg/liter), or neopterin (below versus above the median value of 12.3 nmol/liter). The results of statistical analyses, when using two categories for each variable defined in the manner indicated, remained essentially the same: the highest predictive significance was found for neopterin (Mantel-Cox test, \( P < 0.0001 \)), followed by stage \( (P = 0.0002) \) and interleukin-6 \( (P = 0.0029) \).

Multivariate Analysis of Survival. A series of trivariate and bivariate models were studied, including stage, interleukin-6, and neopterin as candidate joint predictors. Other variables were not included because in combination with neopterin and tumor stage they did not contribute any additionally significant predictive information (details not shown). Table 3 shows the results. When all three variables were included, interleukin-6 failed to contribute to joint prediction; neopterin, however, was significant in combination with tumor stage (Table 3, Model 1). When neopterin was removed from the set of candidate predictors, only stage was significant, and interleukin-6 failed again to add significantly to prediction (Table 3, Model 2). Finally, removing stage from the potential predictor variables yielded neopterin and interleukin-6 as joint predictors (Table 3, Model 3). The predictive strength of neopterin in this joint model was superior to that of interleukin-6, as can be concluded from the significance tests as well as from the relative risk factors associated with both variables.

Renal Function and Prognostic Value of Neopterin. In order to exclude the possibility that the predictive significance of neopterin was due solely to impaired renal function (see also the correlation between neopterin and creatinine concentrations), a separate product-limit analysis was performed using the ratio between serum neopterin and serum creatinine instead of untransformed neopterin data; however, the result remained essentially unchanged (Mantel-Cox test, \( P = 0.0004 \), when patients were dichotomized according to the median value of this ratio).

DISCUSSION

This study aimed to define the predictive value of interleukin-6 and neopterin in patients with multiple myeloma. It is important to stress the fundamental difference which supposedly exists between these two analytes with respect to multiple myeloma: interleukin-6, a pluripotent cytokine, very likely is involved in several distinct aspects of the disease. In contrast, neopterin, this result is in excellent agreement with findings obtained in women with cervical (15) or ovarian cancer (17), in men with prostatic carcinoma (16), in patients with hepatocellular carcinoma (18), and in patients with other hematological cancers such as, e.g., non-Hodgkin's lymphoma (19, 20), chronic lymphoblastic and myeloblastic leukemia, and Hodgkin's disease (20). The predictive value of interleukin-6 has also been demonstrated in a preliminary report by our group (25). The findings shown in the present study are compatible with the reported effects of this cytokine on B-cells.

From the data it appears that the predictive strength of neopterin exceeds that of interleukin-6. In particular, the predictive value of interleukin-6 vanishes when, in a multivariate model, tumor stage is included. Thus, the predictive information of interleukin-6 is efficiently represented by the predictor-variable tumor stage, which comprises information concerning several distinct aspects of the disease. In contrast, neopterin, although significantly higher in patients with higher tumor stages, incorporates unique prognostic information and, therefore, remains a significant predictor in all multivariate models. That interleukin-6 and neopterin may indeed represent different aspects of the disease is underlined by the weak, albeit signifi-
Fig. 1. Dependence of interleukin-6 (left) and neopterin concentrations (right) on stage of multiple myeloma. Symbol in the center of each box, median; lower and upper edges of each box, 25th and 75th percentiles (first and third quartiles); vertical lines, range of observations. The numbers in parentheses in the right panel denote extremely high neopterin levels which are out of range of the ordinates.

Fig. 2. Product-limit estimates of cumulative survival probabilities. Left, patients were stratified by quartiles of interleukin concentrations (pg/liter): O, 0–1.3; ●, 1.4–3.0; ▲, 3.1–7.0; ▼, >7.0. Right, patients were stratified by quartiles of neopterin concentrations (nmol/liter): O, ≤7.3; ●, 7.4–12.3; ▲, 12.4–24.9; ▼, >24.9. Small ticks on the survival curves, censored observation.

Table 2 Predictive significance of clinical and biochemical variables in patients with multiple myeloma

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Alive/dead</th>
<th>Value</th>
<th>d.f.</th>
<th>P Value</th>
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</thead>
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<tr>
<td>Stage</td>
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<td>Creatinine</td>
<td>19/22</td>
<td>11.1</td>
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<td>0.011</td>
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<tr>
<td>World Health Organization performance status</td>
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<td>0.014</td>
</tr>
<tr>
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<td>3</td>
<td>0.025</td>
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<tr>
<td>Hemoglobin</td>
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<td>6.5</td>
<td>3</td>
<td>0.089</td>
</tr>
</tbody>
</table>

* Continuously coded variables were grouped into four groups according to the quartile points of observed distributions (see Table 1 for values); categorical variables were used without transformation. For the categories thus defined, product-limit estimates of survival curves were constructed.

Table 2 Predictive significance of clinical and biochemical variables in patients with multiple myeloma

Our data underline the association of interleukin-6 with the disease process in multiple myeloma. Neopterin appears to be significantly correlated with both analytes. The correlation coefficient obtained in the present study ($R_s = 0.38$) agrees well with a previous result ($R_s = 0.33$) found in another cohort of patients (26).

Neopterin is excreted via the kidneys, and high levels in serum are also found in patients with severe renal failure not related to immune activation processes (14). The possibility that renal insufficiency was the cause of high neopterin concentrations in myeloma patients with poor prognosis could be ruled out by analyzing the predictive significance of the neopterin to creatinine ratio: this ratio measures truly the immune activation-associated increase of neopterin, and its predictive significance was essentially the same as that of untransformed neopterin values.

Our data underline the association of interleukin-6 with the disease process in multiple myeloma. Neopterin appears to be
Table 3 Multivariate proportional hazards analysis of prognosis in patients with multiple myeloma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>Relative risk</th>
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<td>Interleukin-6</td>
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<td>Model 2 Stage</td>
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<td>1.07</td>
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<tr>
<td>Interleukin-6</td>
<td>Not included</td>
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<tr>
<td>Model 3 Neopterin</td>
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<td>0.81</td>
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<tr>
<td>Interleukin-6</td>
<td>1.08</td>
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</tr>
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</table>

* Variables were used in dichotomized form: stage I versus stages II/III; neopterin below versus above 12.3 nmol/liter; interleukin-6 below versus above 7.0 pg/liter.
* Relative risk was estimated as the exponential function of the respective regression coefficient.
* Level of statistical significance estimated by computing a chi^2 to remove statistic for the respective variable.

References


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