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Neopterin and Prognosis in Patients with Adenocarcinoma of the Colon

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ABSTRACT

Concentrations of neopterin, a sensitive indicator for the activation of cellular immunity, were measured in urine samples of 44 patients with adenocarcinoma of the colon at diagnosis. To judge the relative predictive value of neopterin concentrations, other routine clinical and laboratory variables were concomitantly determined. The patients were then followed up to 10 yr, and the abilities of all variables to predict death from cancer were assessed. Neopterin concentrations were not correlated with either tumor stage or Dukes' stage. In univariate analyses using the product-limit approach, four variables were significant indicators of poor prognosis: presence of distant metastases (P = 0.0001); high Dukes' stage (P = 0.0009); high urinary neopterin concentration (P = 0.0034); and advanced stage (P = 0.030). Presence *versus* absence of lymph node metastases was not associated with prognosis. Multivariate survival analyses by the proportional hazards technique demonstrated that neopterin provided statistically independent predictive information in addition to either presence *versus* absence of distant metastases or Dukes' stage. When neopterin and tumor stage were investigated for joint prediction, stage failed to be included in the model. Thus, neopterin concentrations provide valuable and statistically independent prognostic information in patients with adenocarcinoma of the colon.

INTRODUCTION

Neopterin belongs to the class of pteridines which are pyrazino-[2,3-*d*]pyrimidine compounds biosynthesized from GTP. In hepatic and neurological tissue the key enzyme of pteridine biosynthesis, GTP cyclohydrolase I (EC 3.5.4.16), is constitutively present, whereas in other tissues, including macrophages and fibroblasts but also in several tumor cell lines, the enzyme exists in a cytokine-inducible form (1). In human monocytes/macrophages, γ-interferon is the most potent inducer of this enzyme (2), and its effects can be potentiated by tumor necrosis factor α and lipopolysaccharide (3). In contrast to all other cellular systems investigated hitherto, human monocytes/macrophages are unique because they produce by far the highest amounts of neopterin after cytokine induction, whereas all other cell types produce equal or higher amounts of biopterin derivatives. This peculiarity of human monocytes/macrophages is due to the fact that, in these cells, the activities of the constitutively present subsequent enzymes of the pteridine pathway, 6-pyruvoyl-tetrahydropterin synthase and sepiapterin reductase (EC 1.1.1.153), are much lower than the activity of the γ-interferon-induced GTP cyclohydrolase I (1). Thus, the 7,8-dihydroneopterin triphosphate being formed from GTP by GTP cyclohydrolase I accumulates, and aromatic neopterin is formed by the action of omnipresent phosphatases and by nonenzymatic oxidation.

Based on these biochemical aspects of pteridine synthesis in human cells, neopterin has been recognized to be a useful *in vivo* indicator of the activation state of the cellular immune system (4–6). For a broad spectrum of diseases, all of which are characterized by involvement of activation of T-lymphocytes and macrophages, interesting applications of neopterin measurements have been reported by a multitude of research groups (for a comprehensive review, see Ref. 6). In malignant diseases, increased neopterin concentrations have been observed in various tumor types. Frequently, a positive correlation was found between high neopterin levels and advanced tumor stage, and in all cancer types investigated so far with respect to long-term prognosis, high neopterin levels were significantly pointing to a poor outcome of the disease (reviewed in Ref. 7).

In a small pilot study, only about 50% of patients with colorectal carcinomas showed pretherapeutically raised neopterin levels, and no dependence on tumor stage was apparent (8). Thus, it was particularly interesting to study the potential predictive value of pretherapeutically measured neopterin concentration in this patient group because several earlier investigations have pointed out that neopterin as a predictor of death from cancer is statistically independent of other clinically important predictors such as tumor stage.

MATERIALS AND METHODS

Patients. Forty-four patients with adenocarcinoma of the colon were included in the study. All investigations reported here were performed before initiation of therapy. There were 25 men and 19 women; the ages ranged from 45 to 84 yr with a median age of 71 yr and an interquartile range from 59 to 76 yr. There were 3 patients with tumor Stage T_1, 7 with Stage T_2, 28 with Stage T_3, and 6 with Stage T_4. The distribution of patients according to Dukes' classification was: 6 patients with Dukes' Stage A; 14 with Dukes' Stage B; 15 with Dukes' Stage C; and 9 with Dukes' Stage D. There were 15 patients with highly differentiated tumors, 26 with moderately differentiated tumors, and 3 patients with poorly differentiated tumors. The patients were uniformly treated by surgery. In addition, one patient received radiation therapy, and one other patient received chemotherapy. The possible effects of chemotherapy and radiation therapy were not analyzable because of the small number of patients receiving such treatment. During follow-up, four patients experienced a recurrence of their disease; these four plus one additional patient were operated on again. Two patients were diagnosed later to have a secondary malignancy. Because of the small number of these complications, no statistical analyses were performed to investigate their effects on survival.

Laboratory Examinations. Neopterin concentrations in first morning urine specimens were determined by an optimized and fully automated high-pressure liquid chromatography technique without oxidative pretreatment. The procedure is described in detail elsewhere (6). In brief, we used an LC 5500 chromatograph (Varian, Palo Alto, CA) controlled by a Vista 402 data system (Varian), Fluorichrom florescence detector (Varian), UV 200 UV detector (Varian), and a 10-μl injection valve (Rheodyne, Berkeley, CA). A ready-to-use C_{18} reversed-phase column (4 x 125 mm) was used, with 7-μm-diameter packing (Merck, Darmstadt, Germany) together with a guard column (4 x 4 mm) with the same material (Merck). The mobile phase was potassium phosphate buffer (15 mM, pH 6.4), and the flow rate was 0.8 ml per min. One hundred μl of urine were diluted in 1 ml of mobile phase, and 10 μl were injected. By this reverse-phase technique, urinary creatinine is simultaneously determined in the same chromatographic run. To compensate for physiological variations of urine concentrations, neopterin levels are related to these creatinine values and are expressed as μmol of neopterin/mol of creatinine. The native fluorescence of neopterin at 353-nm excitation wavelength and 438-nm emission wavelength is used for detection; creatinine is quantified based on the UV absorption at 235 nm.

The performance characteristics of this technique were reported previously (6). Analytical sensitivity is 120 fmol of neopterin per injection and 36 pmol of creatinine per injection at a peak:noise ratio of 5:1. Thus, the detection limit is 72 nmol of neopterin per 1 liter of urine which is one order of magnitude

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below the lowest concentrations occurring in human urine. Within-run precision is 4.7%, and day-to-day precision is 5.8% for the neopterin:creatinine ratio. A mean recovery of 99.3% was obtained for this ratio. Neither other studied pterins nor urinary components are known to interfere with the method presented.

Several attempts have been made previously to compare the urinary neopterin results obtained by the presented technique with serum neopterin concentrations obtained by radioimmunoassay. In healthy individuals (9) as well as in patients infected by the human immunodeficiency virus type 1 (10), excellent correlations between urinary and serum neopterin levels were observed. Moreover, the frequency of abnormally raised neopterin concentrations was nearly the same for both methods. Thus, it is the current view that the clinical information that can be extracted from both techniques is essentially the same. (This statement does not apply to patients with renal failure; in these, also serum neopterin concentrations must be related to serum creatinine values in order to separate the immunologically induced neopterin elevation from the increase which is due only to impaired renal excretion of the compound).

WBC, hemoglobin level, serum protein concentration, serum urea concentration, and serum concentrations of alanine transaminase, aspartate transaminase, γ-glutamyl transferase, and alkaline phosphatase were also determined at diagnosis and were compared with neopterin.

Pretherapeutic variables were not always available for all patients. Therefore, the numbers of patients are not equal for all variables.

Statistical Procedures. Correlations between continuously coded variables were assessed by Spearman's rank correlation technique, since the distributions of observed values were generally non-Gaussian. Additionally, differences of distributions of biochemical variables with either different tumor stages or different Dukes' stages were tested for significance by the nonparametric Kruskal-Wallis test. Univariate analyses of survival were done by the product-limit technique (11); significance of differences between survival curves were assessed by the generalized Wilcoxon test (Breslow test statistic). Categorization of patients according to continuously coded biochemical variables was invariably based on the quartile points of the observed distributions of the variables. Multivariate survival analyses were performed by the proportional hazards method (12), using a forward-stepping algorithm. For two of the patients, the observation time was less than 1 mo; these were not included in the statistical analyses of survival times. Therefore, the results shown in Tables 3 and 4 are based on 42 patients. Notably, these two patients did not die while under observation. Thus, in accordance with statistical terminology their contribution to the analysis was regarded as "censored"; by this, patients are meant who do not experience the terminating event (here, death due to colon cancer) but were lost to follow-up for other reasons. In fact, the presence of such censored observations is the ultimate reason why normal statistical techniques, such as ordinary regression analysis, are not suitable to analyze survival times. The product-limit technique and the proportional hazards method have been developed in order to overcome the problems posed by the presence of censored observations.

Several different models were computed by the proportional hazards technique. In order to compare the overall goodness of the models, Akaike's information criterion AIC (13) was used. The computation of this criterion is as follows. A pivotal result from the proportional hazards computations is the so-called likelihood function L; this statistic describes how well the model fits the data. Generally, models should fit the data as closely as possible while being parsimonious in terms of the number of p variables included. Thus,

$$\mathrm{AIC} = -2\log L + 2\,(p + 1)$$

where log denotes the natural logarithm. The smaller the value of this criterion, the better is the model.

RESULTS

Tables 1 and 2 report results of biochemical measurements, shown separately for each tumor stage (Table 1) as well as for each Dukes' stage (Table 2). Entries in the tables are ordered according to decreasing strengths of association, determined by the Kruskal-Wallis test. Median neopterin concentrations were higher in patients with higher

Table 1 *Distribution according to tumor stage of biochemical variables (ordered according to decreasing H statistic)*

	Tumor stage				Kruskal-Wallis test[b]	
Variable[a]	I (n = 3)[c]	II (n = 7)	III (n = 28)	IV (n = 6)	H	P
WBC (per μl)	5950	6200	7600	8600	10.74	0.013
ALT[d] (units per liter)	10	17	16	23.5	7.19	0.066
Neopterin (μmol per mol of creatinine)	173	222.5	204.5	270.5	3.62	0.31
Serum protein (g per liter)	76	71	75	78.5	2.98	0.40
Hemoglobin (g per liter)	137	142	137.5	124.5	2.32	0.51
AST (units per liter)	21	21.5	24.5	28	2.02	0.57
Urea (mg per liter)	341	404	353.5	434.5	1.48	0.69
Alkaline phosphatase (units per liter)	125	100	128	137	1.43	0.70
γ-GT (units per liter)	15	14.5	17	37	0.55	0.91

[a] Median values are shown.
[b] H, Kruskal-Wallis test statistic; P, level of statistical significance.
[c] Number of cases.
[d] ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transferase.

Table 2 *Distribution according to Dukes' stage of biochemical variables (ordered according to decreasing H statistic)*

	Duke's stage				Kruskal-Wallis test[b]	
Variable[a]	A (n = 6)[c]	B (n = 14)	C (n = 15)	D (n = 9)	H	P
Hemoglobin (g per liter)	147	127	150.5	135	12.38	0.0064
Alkaline phosphatase (units per liter)	114	175	100	137	9.37	0.025
γ-GT[d] (units per liter)	12	18	12	30.5	6.27	0.099
Neopterin (μmol per mol of creatinine)	217.5	285	171.5	206	4.92	0.18
WBC (per μl)	6000	7200	7750	7400	4.84	0.18
ALT (units per liter)	11	18	16	17	4.01	0.26
AST (units per liter)	23	22	22	27.5	2.89	0.41
Urea (mg per liter)	380	317	412	400	1.98	0.58
Serum protein (g per liter)	73.5	76	75	77	0.61	0.89

[a] Median values are shown.
[b] H, Kruskal-Wallis test statistic; P, level of statistical significance.
[c] Number of cases.
[d] γ-GT, γ-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

tumor stages, but this tendency did not reach statistical significance. For Dukes' classification, no regular pattern of association with neopterin is apparent. Furthermore, neopterin concentrations did not show any significant correlations with the other laboratory variables investigated with the exception of alkaline phosphatase (rank correlation coefficient = 0.46, $P = 0.088$); all other variables produced P values exceeding 0.10 (details not shown).

Univariate Analysis of Survival. Fig. 1 shows product-limit estimates of cumulative survival probabilities for patients, grouped according to tumor stages, Dukes' stages, presence *versus* absence of distant metastases, and quartiles of neopterin concentrations. Table 3 shows that these four variables were the only univariately significant predictors found in this study; no other clinical and laboratory variables produced significantly different survival curves when used as criteria for grouping the patients.

Some details should be noted from Fig. 1. All patients with Stage I survived. Patients with Stages II and III showed an intermediate cumulative incidence of death, and those with Stage IV had a very unfavorable prognosis. In marked contrast, patients with Dukes' Stage A, B, and C had a very similar life expectancy, and only those with distant metastases (Dukes' Stage D) had a very poor outcome (see also the curves obtained for presence *versus* absence of distant metastases for comparison). Patients with increasing neopterin values had an increasingly worse prognosis, the main difference being noted between those with neopterin below *versus* above the median value of 220 μmol/mol of creatinine. Therefore, for the subsequent multivariate analyses of survival, patients were dichotomized according to neopterin concentrations below *versus* above this limit.

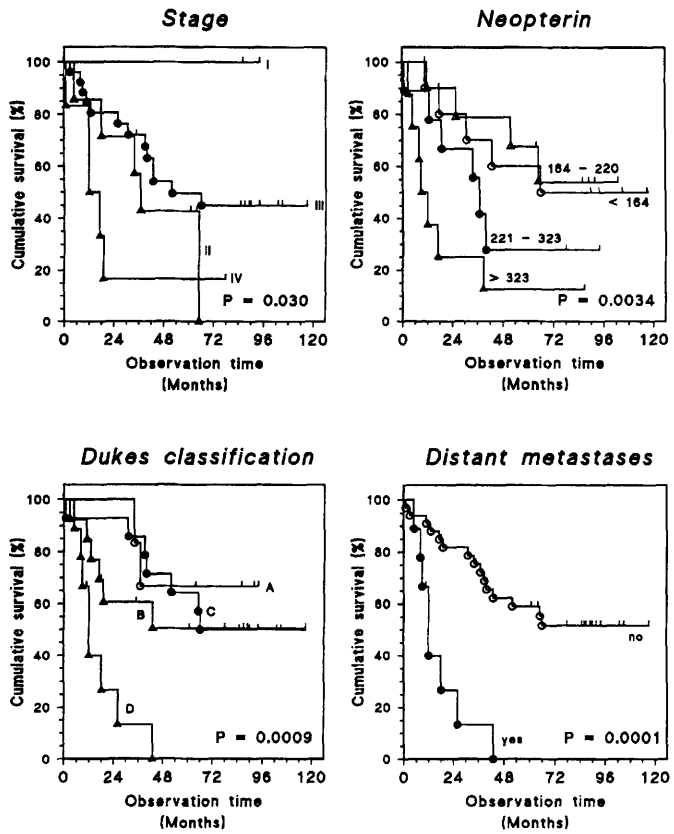

Fig. 1. Product-limit estimates of cumulative survival probabilities. Patients were stratified according to tumor stage (*I*, T_1; *II*, T_2; *III*, T_3; *IV*, T_4), Dukes' stage (*A*, *B*, *C*, *D*), presence of distant metastases (*no*, no distant metastases present; *yes*, distant metastases present), or *quartiles* of urinary neopterin concentrations. P values denote statistical significance of differences between survival curves as assessed by the generalized Wilcoxon test (Breslow test); *small ticks* on the survival curves denote censored observations.

Table 3 *Predictive significance of clinical and biochemical variables in patients with carcinoma of the colon (ordered according to decreasing statistical significance)*

Variable[a]	Alive/dead	Breslow test		
		Value	d.f.	P
Distant metastases	19/23	15.34	1	0.0001
Dukes' stage	19/23	16.46	3	0.0009
Neopterin	16/22	13.64	3	0.0034
Stage	19/23	8.96	3	0.030
γ-GT[b]	17/19	5.47	3	0.14
Histological grading	19/23	3.46	2	0.18
Serum protein	17/22	4.95	3	0.18
Urea	18/22	4.87	3	0.18
Age	19/23	3.46	3	0.33
ALT	16/19	2.87	3	0.41
Alkaline phosphatase	15/20	2.79	3	0.43
Sex	19/23	0.60	1	0.44
Lymph node metastases	19/23	0.60	1	0.44
AST	16/19	2.42	3	0.49
Hemoglobin	18/22	1.31	3	0.73
WBC	18/22	1.10	3	0.78

[a] Continuously coded variables were grouped into four groups according to the quartile points of observed distributions; categorical variables were used without transformation.
[b] γ-GT, γ-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 4 *Multivariate proportional hazards models of prognosis in patients with colon carcinoma*

Variable[a]	Regression coefficient[b]			Log L[c]	AIC[d]
	Value	SE	P		
Distant metastases	2.23	0.558	0.0001	−59.97	125.94
Neopterin	1.51	0.477	0.0012		
Dukes' stage	0.88	0.296	0.0012	−62.09	130.18
Neopterin	1.37	0.455	0.0023		
Neopterin	1.107	0.442	0.011	−67.33	138.66
Stage	Not included[e]				

[a] Neopterin was dichotomized: below *versus* above 220 μmol per mol of creatinine.
[b] The exponential function of the regression coefficient denotes the relative risk associated with a variable; SE, standard estimation error of regression coefficient; P, level of statistical significance estimated from a χ^2-to-remove statistic.
[c] Logarithm of the likelihood associated with a model.
[d] AIC, Akaike's information theoretical criterion.
[e] Not included in the model because of lack of joint significance.

Multivariate Analysis. First, a series of bivariate models were studied, including tumor stage, Dukes' stage, presence *versus* absence of distant metastases, and neopterin below *versus* above the median value of 220 μmol/mol of creatinine, as candidate joint predictors. Other variables were not included because, in combination with the variables indicated above, they did not contribute any statistically significant predictive information (details not shown), which was in good agreement with the outcome of the univariate analyses (see Table 3). Table 4 demonstrates the results. In all models, the contribution of neopterin to joint prediction was significant, but stage failed to add significant information when considered in combination with neopterin. The best model obtained was the combination of the variable presence *versus* absence of distant metastases with the variable neopterin below *versus* above the median of 220 μmol/mol of creatinine. As can be estimated from the regression coefficients for this model, the relative risk of death associated with the presence of distant metastases is exp(2.23) = 9.3, the relative risk associated with neopterin above 220 μmol/mol of creatinine is exp(1.51) = 4.5, and the relative risk associated with both unfavorable indicators is exp(2.23 + 1.51) = 42 times higher than that of patients with absence of distant metastases and neopterin below 220 μmol/mol of creatinine. Fig. 2 was constructed from this model. In both patient groups, without and with distant metastases, patients with neopterin levels above 220 μmol/mol of creatinine had a more unfavorable course of disease; *i.e.*,

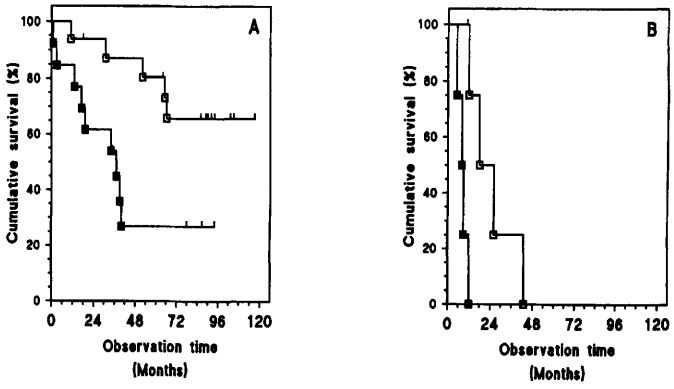

Fig. 2. Survival curves resulting from the best bivariate model from Table 4. *A*, patients without distant metastases; *B*, patients with distant metastases. □, patients with neopterin concentrations below 220 μmol/mol of creatinine; ■, patients with neopterin concentrations above 220 μmol/mol of creatinine. *Small ticks* on the survival curves denote censored observations.

the survivor functions dropped more rapidly than in those with neopterin concentrations below this cut-off value.

DISCUSSION

This study was undertaken with the aim to define the predictive value of pretherapeutically measured urinary neopterin concentrations in patients with adenocarcinoma of the colon. It is important to note that neopterin is clearly not a tumor marker in the usual sense of the word; in contrast, neopterin is a product of human monocytes/macrophages stimulated by γ-interferon and, as such, increased concentrations of neopterin in body fluids mark early activation processes involving the cell-mediated immune system.

Neopterin is strongly associated with the prognosis of adenocarcinoma of the colon; *i.e.*, the higher the concentrations of this analyte in urinary specimens collected at the time of diagnosis, the worse is the survival expectation of the patients. Thus, the results of the present study agree perfectly with a series of similar investigations undertaken in other types of malignant cancers; essentially the same results were obtained in women with cervical (14) or ovarian carcinoma (15), in men with prostatic carcinoma (16), in patients with hepatocellular carcinoma (17), and in patients with various hematological disorders (18–20).

The most important predictor variable in the present study was the presence *versus* absence of distant metastases. Surprisingly, no association at all could be detected between prognosis and presence *versus* absence of lymph node metastases (survival curves not shown in detail). This is also seen from Fig. 1. The survival curve of patients with Dukes' Stage C (limited or extended tumor size, presence of lymph node metastases) was slightly—albeit not significantly—more favorable than that of patients with Dukes' Stage B (only extended tumor size, but absence of lymph node metastases). Additionally, from Table 2 it is evident that the laboratory findings in patients with Dukes' Stage B were more unfavorable than in those with Dukes' Stage C. These results, taken together, point to a quite prominent role of the size of the primary tumor in determining the pathological processes; in contrast, lymph node metastasis seems to play a minor role in this context.

The selection of concomitantly determined laboratory variables in the present study could be criticized. In particular, specific tumor markers were not included for comparison. In fact, carcinoembryonic antigen was determined on 13 patients but was not included in the final statistical evaluation in order to avoid loss of too many data records due to missing data. When the data on carcinoembryonic antigen were analyzed separately (not shown in detail), there was no correlation detectable with stage and with Dukes' stage, and no association with prognosis was found. These findings must not be overinterpreted; the chance to detect significant relationships was too low because of the small amount of data available.

In this respect, however, results obtained in two previous investigations may be of interest. In a recent analysis of the relative merits of neopterin and interleukin 6 as predictive markers in patients with multiple myeloma (20), neopterin was less strongly correlated with tumor stage than interleukin 6, which is known to play a direct role in this disease as a growth factor for the malignant plasma cells. In contrast to this correlation with tumor stage, however, neopterin was found to be a significantly better predictor for long-term prognosis. Similarly, in patients with second-look laparotomy after ovarian carcinoma, the specific tumor marker CA-125 showed a stronger correlation than neopterin to the amount of malignant tissue found during the surgical exploration, but neopterin was a significantly better indicator for long-term prognosis after a second-look operation (21). Thus, one might speculate that also in other cancers specific tumor markers might be more closely related to the instantaneously present tumor mass or tumor stage than neopterin. The latter, however, seems to be better in predicting long-term prognosis, particularly in combination with tumor stage or similar composite variables, because it reflects important tumor-host interactions which are not easily monitored by other variables. The significant predictive value of neopterin for long-term survival found in this study despite the lack of correlation with tumor stage corroborates this expectation.

A comment seems appropriate concerning the fact that there were no survivors with Stage II disease, but there were survivors in the Stage IV group (Fig. 1). The reason for this seemingly surprising fact lies in the details of how cumulative survival probabilities are estimated by the product-limit approach. By this method, the cumulative survival probabilities are computed, in a discrete manner, only at those time points when at least one patient dies. At a specific death time j, the survivor function obtained at the most recent death time $(j-1)$ is multiplied by a factor

$$\frac{n_j - d_j}{n_j},$$

where n_j is the number of subjects at risk immediately before time j and d_j is the number of subjects dying at time j. In Stage II, five of seven patients died, and in Stage IV, five of six patients died. However, while in the Stage IV group the one patient surviving was still alive after more than 6 yr, the two patients with Stage II who survived (and thus contributed censored observations to the analysis) were lost to follow-up already before the last death in this group occurred. Thus, the above-described factor, computed at the time of the fifth death in the Stage II group, was equal to zero. The survivor function dropped to zero, too, as a consequence of the mathematical model used. The important feature, however, is the observation that the deaths in the Stage II group occurred at significantly later times when compared with the deaths of patients with Stage IV, and in fact, the survivor function was very similar to that of Stage III patients.

By several investigations of patients with quite different types of malignant diseases, a coherent view has eventually emerged. High levels of the immune activation marker neopterin at the diagnosis of a malignant disease invariably indicate a poor prognosis, independent of other predictive variables.

In searching for an explanation of these observations, two fields of recent biochemical research may deserve attention. As briefly mentioned in the "Introduction", most cellular systems produce, after

induction of the enzyme GTP cyclohydrolase I, biopterin derivatives, in particular 5,6,7,8-tetrahydrobiopterin (1). Although no biochemical function has been established for neopterin so far, 5,6,7,8-tetrahydrobiopterin has long been known to be an essential cofactor for the hydroxylating enzymes phenylalanine hydroxylase, tyrosin hydroxylase, and tryptophan hydroxylase (22). Most recently, it has been shown to be an essential cofactor for the biosynthesis of nitric oxide (also known as endothelium-derived relaxing factor), which is an important signalling molecule in different systems. Nitric oxide originates from the oxidation of L-arginine to citrulline, and its actions range from vasodilatation to neurotransmitter-like functions in the brain (23, 24). The requirement for 5,6,7,8-tetrahydrobiopterin in the nitric oxide synthase reaction has been demonstrated in endothelial cells (25) and the cerebellum (26, 27) where the enzyme activity is constitutively present, as well as in mouse macrophages (28–30) and in rat liver (31), where the enzyme is inducible by cytokines and/or endotoxin. We have demonstrated a 5,6,7,8-tetrahydrobiopterin-dependent synthesis of nitric oxide in murine dermal fibroblasts, and this study also showed that the pivotally important intracellular concentration of 5,6,7,8-tetrahydrobiopterin in these cells is under the control of cytokines (32). While a direct action of 5,6,7,8-tetrahydroneopterin on biosynthesis of nitric oxide is lacking (33), there might well exist an indirect link between the production of high amounts of neopterin by human macrophages after induction by cytokines and the nitric oxide synthase reaction, because cytokines lead also to increased biosynthesis of the cofactor 5,6,7,8-tetrahydrobiopterin and thus nitric oxide, in, *e.g.*, human endothelial cells (34).

The synthesis of guanine nucleotides is increased in cancer cells, mainly due to an upregulation of the rate-limiting enzyme IMP dehydrogenase (35–38). On the other hand, GTP is the substrate for the starting enzyme of the biosynthetic pathway leading to pteridines, GTP cyclohydrolase I. One might speculate that the increased availability of the substrate GTP in cancer cells was responsible for the increased neopterin concentrations in urine and blood from patients with cancers. In our view, such a link, if existing after all, could by no means establish a satisfactory explanation for the observed neopterin increases. We have provided evidence suggesting that GTP cyclohydrolase I is genuinely stimulated by γ-interferon. It takes 25 h after the addition of γ-interferon to reach its maximum activity, the K_m value remaining constant (1). Under physiological conditions the enzyme characteristics point to substrate saturation, and it is unlikely that an increase in GTP leads to an increased neopterin concentration.

In conclusion, besides the well-established immunosuppressive actions of malignant tumors, there is evidence available suggesting an activation of certain immunological mechanisms in patients with cancer. We are only beginning to gain an understanding for the overwhelmingly complex actions and interactions of the multitude of cytokines, growth factors, and other effector molecules. At present, neopterin measurement in our view is particularly valuable since it provides a means of monitoring, in a more global manner, the combined biological activities of a subset of cytokines the direct determination of which presents major problems, both due to analytical and biological reasons such as short life time, or binding to cells or proteins.

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