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Urinary Neopterin in Patients with upper Gastrointestinal, Biliary and Pancreatic Carcinomas

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Abstract

Increased urinary neopterin has been associated with advanced disease and poor prognosis in patients with colorectal carcinoma, but less is known about urinary neopterin in other gastrointestinal tumors. We have investigated urinary neopterin in 53 patients with carcinomas of the upper gastrointestinal tract, pancreas and biliary tract. Compared to controls, urinary neopterin was increased in patients with gastric carcinoma, pancreatic carcinoma, cholangiocarcinoma and gallbladder carcinoma. Urinary neopterin was a significant prognostic indicator of survival in univariate as well as multivariate analyses. No significant change in urinary neopterin concentrations was observed during the short follow-up, but increased urinary neopterin concentration at the end of follow-up was associated with poor prognosis. In conclusion, significantly increased urinary neopterin concentrations were observed in the present cohort of patients. Urinary neopterin is an independent prognostic indicator in patients with carcinomas of the upper gastrointestinal tract, biliary tree and pancreas.

Key words: gallbladder carcinoma, gastric carcinoma, cholangiocarcinoma, neopterin, pancreatic carcinoma

Introduction

Malignant tumors are known to elicit host response mediated by both adaptive and innate immune systems. Among other laboratory parameters, advanced tumors are associated with increased concentrations of neopterin in the urine or in serum (1, 2). Neopterin is produced by macrophages activated by interferon- γ (IFN- γ), and elevated neopterin concentrations have been observed in patients with different primary tumors, including gastrointestinal carcinomas. Urinary or serum neopterin concentrations have been studied in patients with colorectal carcinoma (3-5), and increased urinary neopterin has been associated with advanced disease (3) or poor prognosis (5). Much less is known about neopterin concentrations in patients with gastrointestinal tumors other than colorectal carcinoma (6).

Gastric carcinoma, pancreatic carcinoma and biliary tract carcinomas (gallbladder carcinoma and cholangiocarcinoma) occur in Europe with significantly lower frequency compared with colorectal carcinoma

(7). Compared to colorectal carcinoma, the prognosis of these tumors is poor, and most patients die of the disease within one or two years of diagnosis. In the present study, we have evaluated the urinary neopterin in patients with esophageal, gastric, pancreatic and biliary carcinomas.

Material and Methods

A retrospective analysis was performed of urinary neopterin measurements collected between 1997 and 2000 as a part of research project, approved by the institutional ethics committee, evaluating immune system activation in patients with liver tumors. Morning urine samples were collected from 53 patients, 30 males and 23 females, aged 57 ± 13 (range 26 - 79) years, with histologically verified carcinomas of the esophagus, stomach, pancreas, gallbladder or bile ducts, treated at the Department of Oncology and Radiotherapy, Charles University Medical School Teaching Hospital, and 33 healthy controls, 16 males

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Table 1. Urinary neopterin concentrations ($\mu\text{mol/mol}$ creatinine) in patients with cancer and in healthy controls

Primary	n	Mean $\pm$ S.D.	Range	Patients with neopterin ≥ 170 n (%)	Patients with neopterin ≥ 214 n (%)	P (vs. controls)
Gallbladder carcinoma	9	443 $\pm$ 626	83 - 2080	5 (56)	5 (56)	0.002
Cholangiocarcinoma	16	168 $\pm$ 83	70 - 361	5 (31)	4 (25)	0.004
Pancreatic carcinoma	13	204 $\pm$ 116	42 - 481	8 (62)	6 (46)	0.003
Gastric carcinoma	14	201 $\pm$ 181	47 - 767	6 (43)	4 (29)	0.02
Esophageal carcinoma	1	721	-	1 (100)	1 (100)	-
All patients	53	243 $\pm$ 296	42 - 2080	25 (47)	20 (38)	0.00003
Controls	33	110 $\pm$ 52	34 - 293	3 (9)	2 (6)	-

and 17 females, aged 49 ± 13 (range 28 - 88) years. Thirty-four patients were chemotherapy-naïve, 19 patients had previously received therapy. Twenty-nine patients had metastatic disease (including liver metastases) at the time of investigation.

Urinary neopterin was measured by high-performance liquid chromatography. Creatinine was determined by Jaffé reaction, and the results were expressed as neopterin/creatinine ratio ($\mu\text{mol/mol}$ creatinine) as described earlier (8). Peripheral blood cell count was performed as described (9). Hemoglobin was measured by a photometric method using sodium lauryl sulfate, leukocytes were detected by flow cytometry and platelets by impedance method using a Sysmex XE-2100 blood analyzer (Sysmex, Kobe, Japan).

The survival of patients was analyzed by the Kaplan-Meier method, and the comparisons of survival of patients with urinary neopterin $<$ or ≥ 170 $\mu\text{mol/mol}$ creatinine and other survival comparisons

were performed by log-rank test. The cutoff value of 170 $\mu\text{mol/mol}$ creatinine was selected based on our previous study (10). The survival was measured from the date of neopterin determination. Urinary neopterin concentrations of patients and controls were compared by Mann-Whitney U test, neopterin at the start and at the end of follow-up was compared by Wilcoxon signed rank test, and correlations between urinary neopterin and hematological parameters were investigated by Spearman's rank correlation. Multivariate analysis was performed using the Cox regression analysis with the results expressed as hazard ratio (HR). The decision on statistical significance was based on $p < 0.05$ significance level. The analyses were performed using NCSS 2001 software (Number Cruncher Statistical Systems, Kaysville, Utah, USA).

Table 2. Univariate analysis of prognostic indicators

Parameter	Cutoff for survival analysis	Median survival (months)	P (log-rank test)
Urinary neopterin ($\mu\text{mol/mol}$ creatinine)	< 170 (n=28) vs. ≥ 170 (n=25)	12 vs. 5	0.008
Leukocytes ($10^9/\text{l}$)	< 8 (n=21) vs. ≥ 8 (n=32)	11 vs. 6	0.06
Hemoglobin (g/l)	< 125 (n=33) vs. ≥ 125 (n=20)	6 vs. 13	0.03
Mean erythrocyte volume (fl)	< 84 (n=13) vs. ≥ 84 (n=40)	4 vs. 8	0.10
Platelets ($10^9/\text{l}$)	< 270 (n=23) vs. ≥ 270 (n=30)	10 vs. 7	0.13
Previous therapy	yes (=19) vs. no (n=34)	7 vs. 7	0.68
Distant metastases	no (n=24) vs. yes (n=29)	11 vs. 6	0.05
Primary	non-biliary (n=28) vs. biliary (n=25)	7 vs. 11	0.03

Figure 1. Survival of patients with urinary neopterin <170 µmol/mol creatinine (bold line) and ≥ 170 µmol/mol creatinine (dashed line)

Results

Urinary neopterin was significantly increased compared to controls in the whole patient group as well as in patients with gallbladder carcinoma, cholangiocarcinoma, pancreatic carcinoma or gastric carcinoma (Tab. 1). A significant percentage of patients had urinary neopterin equal or above 170 µmol/mol creatinine and equal or above 214 µmol/mol creatinine (mean + 2 standard deviations of controls). No statistically significant difference was observed in urinary neopterin levels among patients with different primary tumors, in patients with or without metastases (192 ± 145 µmol/mol creatinine vs. 305 ± 406 µmol/mol creatinine) as well as in chemotherapy-naïve patients and patients previously treated with chemotherapy (194 ± 118 µmol/mol creatinine vs. 330 ± 463 µmol/mol creatinine). A significant correlation was observed between urinary neopterin and the age of the patients ($r_s = 0.44$, $p = 0.001$) and peripheral blood leukocyte count ($r_s = 0.27$, $p = 0.05$), but not with hemoglobin, mean erythrocyte volume and peripheral blood platelet count.

Median survival of all patients was 7 months (7 months in patients with gastric carcinoma, 8 months in pancreatic carcinoma and 11 months in patients with biliary tract carcinoma). In univariate analysis, urinary neopterin below 170 µmol/mol creatinine, hemoglobin concentration, biliary primary and presence of distant metastases (including liver metastases) were significant indicators of survival, and peripheral blood leukocyte count was of borderline significance (Table 2). In multivariate analysis, only urinary neopterin below 170 µmol/mol creatinine (HR 0.39, $p = 0.002$) and absence of distant metastases (HR 0.49, $p = 0.02$) were significant indicators of survival.

In 26 patients, urinary neopterin was determined on more than one occasion. The mean ($\pm$ standard deviation) number of neopterin measurements was 5 ± 3 (range 2 - 16), and mean time between the earliest and latest measurement was 3.5 ± 3.2 (range 0.1 - 13) months. No significant change in urinary neopterin was observed between the earliest (234 ± 285 µmol/mol creatinine) and latest (214 ± 154 µmol/mol creatinine) measurements. In patients with urinary neopterin at the time of the latest measurement equal or above 170 µmol/mol creatinine significantly shorter survival was observed (median 4 vs. 12 months, $p = 0.05$), but in some patients specimens were collected during therapy, in others rather shortly before death.

Discussion

In the present study significantly increased urinary neopterin concentrations were observed, compared to controls, in the whole group of patients as well as in patients with gallbladder carcinoma, cholangiocarcinoma, pancreatic carcinoma, or gastric carcinoma. This observation was not unexpected as elevated urinary or serum neopterin concentrations have been reported in patients with wide range of tumors (2). Similarly to other cancers, increased neopterin in the present study was significant indicator of poor prognosis on univariate analysis both at the start and at the end of follow-up, as well as on the multivariate analysis. Both chemotherapy-naïve patients as well as patients undergoing chemotherapy were included in the present study (with the sample collected before the next chemotherapy cycle). In another study (Melichar *et al.* manuscript in preparation), we have demonstrated that the chemotherapy regimens used in gastrointestinal cancer (usually based on 5-fluorouracil) do not lead to a significant change in urinary neopterin, we therefore included in the present analysis also the patients who have already been treated with chemotherapy.

Neopterin is an unconjugated pteridine synthesized from guanosine triphosphate (GTP). GTP is converted to 7,8-dihydroneopterin by the enzyme GTP cyclohydrolase I, and this reaction represents the first step in a pathway leading to the formation of tetrahydrobiopterin (1). GTP cyclohydrolase I is the rate-limiting enzyme in this pathway, and its activity is induced by IFN- γ (11), that is produced by T-lymphocytes and natural killer (NK) cells (12). Human macrophages possess significant GTP cyclohydrolase I activity when stimulated by IFN- γ , while the activities of other enzymes of this pathway are low (13), leading to the accumulation of 7,8-dihydroneopterin-triphosphate which undergoes autooxidation to neopterin.

Among gastrointestinal tumors, neopterin concen-

trations have been studied most extensively in patients with colorectal carcinoma. In patients with colorectal carcinoma neopterin concentrations were associated with advanced stage (3), poor prognosis (5), and correlated with the decrease of serum tryptophan and decreased quality of life (14). Increased serum or urinary neopterin concentrations have also been reported in patients with hepatocellular carcinoma (15, 16). Kawasaki *et al.* (16) observed increased urinary neopterin in 18 patients with hepatocellular carcinoma. A significant correlation was observed between urinary neopterin and tumor size, and survival was significantly shorter in patients with higher urinary neopterin.

Much less is known about the presence and prognostic impact of increased neopterin concentrations in patients with other gastrointestinal tumors. In pilot studies, high urinary neopterin concentrations have been observed in substantial number of patients with gastric carcinoma and most patients with pancreatic carcinoma (6). Manes *et al.* (17) reported markedly increased serum neopterin in patients with pancreatic carcinoma. Increased serum neopterin concentrations were also reported in patients with pancreatic carcinoma by Birk *et al.* (18). Contrary to other reports in patients with malignant tumors as well as to the present report, in the study reported by Birk *et al.* (18) higher serum neopterin has been associated with better prognosis. In another study in patients with gastric and colorectal carcinomas, the urinary neopterin concentrations in patients with gastric carcinoma were similar to patients with colorectal carcinoma (19). Even less is known about neopterin in patients with biliary tract carcinoma (gallbladder carcinoma and cholangiocarcinoma). In one report published so far, increased urinary neopterin has been observed in 8 out of 9 patients examined (6), and present data are in agreement with this report, although the number of patients with increased urinary neopterin was lower in the present series.

Limited number of patients with each primary was evaluated in the present study, and the data were pooled for survival analysis for all patients. In clinical studies, patients with gallbladder carcinoma and cholangiocarcinoma are almost invariably handled together as a single group and, because of a relatively low frequency of these tumors, biliary tract carcinomas are often pooled together with other gastrointestinal carcinomas. Because of the relatively low incidence, carcinomas of the biliary tract and pancreas have been studied together even in prospective clinical trials (20). This approach may be justified as prognosis and chemosensitivity of these tumors is similar. Moreover, in some cases it may be difficult to distinguish cholangiocarcinoma from pancreatic carcinoma, and the diagnosis of cholangiocarcinoma relies on the exclu-

sion of a primary tumor elsewhere in the gastrointestinal tract (21), notably in the pancreas or stomach. Inclusion of patients with upper gastrointestinal of pancreaticobiliary carcinomas in one study may be further justified by the fact that the site of the primary may be determined only after extensive studies, and an information about prognosis could be helpful at the start of patient evaluation. However, future studies on a larger cohort of patients should address the prognostic significance of increased urinary neopterin in patients with individual primary tumors in this group.

Advanced cancer is often accompanied by anemia that may be further aggravated by antitumor therapy. In the majority of cancer patients, the anemia may be categorized as anemia of chronic disease, characterized by low serum iron, low serum iron binding capacity and high ferritin (22). The decline of hemoglobin concentrations in anemia of chronic disease results from the alterations of iron metabolism associated with acute phase response and direct inhibition of hematopoietic progenitors by cytokines responsible for systemic inflammatory and immune response, e.g. IFN- γ or tumor necrosis factor- α (22), and decreased hemoglobin levels are associated with increased neopterin concentrations. A negative correlation between serum neopterin concentrations and hemoglobin has been observed in patients with hematological and gynecological malignancies (23, 24). In contrast to observations made in patients with other primary tumors, no correlations were observed between hemoglobin or mean erythrocyte volume, an indicator of anemia of chronic disease, and urinary neopterin. This lack of correlation may be explained by the possibility that anemia in these patients may be caused or aggravated by factors other than systemic inflammatory or immune response, e.g. chronic gastrointestinal blood loss or, in previously treated patients, by chemotherapy. The correlation between urinary neopterin and peripheral blood leukocyte counts reflects an association between different parameters of systemic inflammatory and immune response. The significant correlation observed between urinary neopterin and the age of the patients is in agreement with our earlier study demonstrating increased concentrations and prognostic significance of urinary neopterin in the elderly (9).

In conclusion, increased urinary neopterin was observed in patients with gastric carcinoma, pancreatic carcinoma, cholangiocarcinoma and gallbladder carcinoma. Urinary neopterin is an independent prognostic indicator in patients with carcinomas of the upper gastrointestinal tract, biliary tree and pancreas.

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