Prognostic Significance of Serum and Urinary Neopterin Concentrations in Patients with Rectal Carcinoma Treated with Chemoradiation

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Abstract. *Aim: To analyze the prognostic significance of serum and urinary neopterin concentrations in patients with rectal adenocarcinoma treated with (chemo)radiation. Patients and Methods: Urinary and serum neopterin and peripheral blood cell count were determined in 49 patients with rectal carcinoma before the start of (chemo)radiation. Results: Neopterin concentrations exhibited a significant inverse correlation with hemoglobin and positive correlation with leukocyte count, platelet count and platelet-to-lymphocyte ratio. Increased serum neopterin concentration was associated with significantly inferior relapse-free survival (RFS) and overall survival. However, a significant association was observed only in 28 patients treated in the neoadjuvant setting. Although increased urinary neopterin was also associated with inferior RFS and overall survival, this was not statistically significant. The neutrophil-to-lymphocyte ratio was also associated with poor prognosis. Conclusion: The data presented herein indicate a prognostic significance of serum neopterin concentrations in patients with rectal cancer treated with neoadjuvant chemoradiation.*

Rectal carcinoma is a common tumor associated with a relatively high mortality rate. In addition to surgery, external-beam radiation and systemic chemotherapy play an important role in the management of patients with rectal carcinoma. It has been demonstrated in prospective clinical trials that the concomitant combination of external-beam radiation and chemotherapy improves outcome (1, 2). However, this combined modality approach is associated with relatively high morbidity and serious, sometimes even fatal, adverse events. The identification of parameters that would predict prognosis or complications is therefore of potential importance in patient management. These prognostic and predictive factors may be determined clinically, as well as in the laboratory.

Laboratory biomarkers play an increasingly important role in medical oncology (3). While most attention so far has been focused on biomarkers associated with the activity of the tumor cells, there is mounting evidence that biomarkers that reflect the host response to neoplasia are also of paramount importance. These biomarkers can be assessed either in the tumor tissue (*e.g.* the presence of tumor-infiltrating lymphocytes) (4-6) or in body fluids (7, 8). Neopterin is a pteridine compound produced by macrophages activated by interferon-gamma. Urinary and serum neopterin concentrations have been established as a biomarker of systemic immune and inflammatory response across a range of different disorders (9-11). Notably, increased concentrations of neopterin have been reported in most malignant disorders, including colorectal carcinoma (11). Robust biomarkers of inflammatory response may be obtained by calculating the ratios of lymphocytes to other cellular components of peripheral blood (12). The neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) represent independent prognostic biomarkers across a spectrum of solid tumors, including colorectal carcinoma (13-17).

Although several studies have investigated the prognostic significance of neopterin concentration in patients with colorectal carcinoma, to the best of our knowledge no

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investigations have so far focused on the prognostic significance of neopterin in patients with rectal carcinoma treated with chemoradiation. The aim of the present study was to analyze the prognostic significance of serum and urinary neopterin concentrations in this clinical setting.

Patients and Methods

Forty-nine consecutive patients, 32 males and 17 females, aged (mean±standard deviation) 67±8 (range=54-85) years with histologically verified rectal carcinoma treated at the Department of Oncology, Palacký University Medical School and Teaching Hospital in Olomouc were included in the present study. Forty-seven patients had adenocarcinoma, while neuroendocrine carcinoma and squamous cell carcinoma were present in one case each. Eleven patients had stage IIA rectal carcinoma, four stage IIC, five stage IIIA, 17 stage IIIB and 12 had stage IIIC. All patients were treated with external-beam radiation administered as intensity-modulated radiation therapy or three-dimensional conformal radiotherapy as described before (18). All but five patients were treated with concomitant chemotherapy (infusional 5-fluorouracil or capecitabine). Twenty-eight patients were treated in the neoadjuvant and 21 patients in the adjuvant setting. Urinary neopterin, serum neopterin, serum creatinine and peripheral blood cell count were determined as described earlier (18, 19), and NLR, LMR and PLR were calculated. This was a part of an investigation studying biomarkers of toxicity in this population, and the results on changes of citrulline and neopterin concentrations during chemoradiation were reported separately elsewhere (18). The investigations were approved by our institutional ethical committee and the patients signed informed consent.

Correlations were analyzed using Spearman's rank correlation coefficient. Relapse-free survival (RFS) was defined as interval between the start of therapy and the diagnosis of relapse or death. Overall survival (OS) was defined as the interval between the diagnosis and death. Patients without a RFS or OS event were censored at the last visit. RFS and OS were evaluated with the Kaplan–Meier method, and the differences between subgroups of patients defined by clinical parameters or dichotomization of neopterin concentrations were studied by log-rank test. Cutoffs for dichotomization were selected based on prior reports (11, 13, 14, 18, 20) or upper limits of normal values. Multivariate analysis was performed using Cox regression. All dichotomized clinical and pathological variables were entered into a hierarchical forward and switching model and the results were expressed as hazard ratio (HR). The decision on statistical significance was based on $p=0.05$ level. The analyses were performed using NCSS software (Number Cruncher Statistical Systems, Kaysville, UT, USA).

Results

Apart from higher LMR and a trend for lower hemoglobin concentration in patients treated in the adjuvant setting, no difference was observed in serum neopterin, urinary neopterin, peripheral blood leukocyte counts and peripheral blood platelet counts and ratios between patient treated with preoperative (neoadjuvant) and postoperative (adjuvant) radiation (Table I). No significant difference in the investigated parameters was observed between patients with stage II and those with stage III tumors.

Serum and urinary neopterin concentrations exhibited a significant inverse correlation with hemoglobin and a positive correlation with age and platelet count (Table II). In addition, serum neopterin correlated significantly with the leukocyte count. A significant positive correlation was observed for urinary and serum neopterin/creatinine ratios with PLR, but not with NLR or LMR.

A trend for inferior RFS (median=17.4 months *vs.* not reached; $p=0.20$) and significantly inferior OS (29.7 months *vs.* not reached; $p=0.006$) was observed in patients treated in the neoadjuvant setting compared to patients treated with adjuvant chemoradiation. The staging of patients treated in the neoadjuvant and adjuvant setting was not comparable since the stage was determined based on clinical criteria in patients treated with neoadjuvant therapy, while pathological staging was used in the adjuvant setting.

In the entire cohort, a serum neopterin concentration of 3 μg/l (11.86 nmol/l) or more was associated with significantly inferior RFS (median 5.5 months *vs.* not reached; $p=0.049$; Figure 1) and OS (median 17.4 months *vs.* not reached; $p=0.009$; Figure 2). Serum neopterin 3 μg/l or more was a significant predictor of poor RFS (median 2.8 months *vs.* not reached; $p=0.001$; Figure 3) and OS (median 9.3 months *vs.* not reached; $p=0.0006$; Figure 4) in patients treated with neoadjuvant chemoradiation, but not among patients treated in the adjuvant setting (data not shown). In fact, none of the patients treated with neoadjuvant chemoradiation with neopterin concentrations 3 μg/l or more survived. A serum neopterin/creatinine value of 146 μmol/mol creatinine or more was associated with also significantly inferior OS (median=16.9 months *vs.* not reached; $p=0.016$), but the difference in RFS did not reach statistical significance (median 8 months *vs.* not reached; $p=0.093$). A serum neopterin/creatinine value of 146 μmol/mol creatinine or more was associated with significantly inferior RFS (median 3 months *vs.* not reached; $p=0.025$) and OS (median 9.3 months *vs.* not reached; $p=0.009$) in patients treated with neoadjuvant chemoradiation. Although increased (≥214 μmol/mol creatinine) urinary neopterin was also associated with inferior RFS (median 16.3 months *vs.* not reached) and OS (medians not reached), this was not statistically significant ($p=0.248$ and $p=0.337$, respectively). Age (<65 *vs.* ≥65 years), stage (stage II *vs.* III), grade (≤2 *vs.* 3), hemoglobin concentration (<120 g/l *vs.* ≥120 g/l), leukocyte ($<10.0\times10^9$/l *vs.* $\geq10.0\times10^9$/l) and platelet counts ($<400\times10^9$/l *vs.* $\geq 400\times10^9$/l) were not associated with the outcome (data not shown). NLR of 3 or more was also associated with worse OS (median 29.7 months *vs.* not reached; $p=0.034$), but not RFS (medians not reached; $p=0.867$). LMR of 3 or more, and PLR of 150 or more were not predictive of RFS or OS (data not shown). When

Table I. *Values of biomarkers in patients treated in the neoadjuvant and adjuvant setting.*

Parameter	Setting		*p*-Value
	Neoadjuvant	Adjuvant	
Serum neopterin (μg/l)	3.2±4.2	2.3±1.2	0.518
Serum neopterin/creatinine ratio (μmol/mol creatinine)	170±237	120±59	0.739
Urinary neopterin (μmol/mol creatinine)	197±106	196±98	0.909
Age (years)	68±8	66±7	0.479
Hemoglobin (g/l)	129±21	119±14	0.055
Leukocyte count (10^9/l)	8.4±3.5	7.1±2.3	0.132
Platelet count (10^9/l)	278±91	277±89	0.906
NLR	4.0±2.8	3.1±1.4	0.193
LMR	2.5±0.8	3.2±0.9	**0.027**
PLR	188±85	183±73	0.897

NLR: Neutrophil-to-lymphocyte ratio; LMR: lymphocyte-to-monocyte ratio; PLR: platelet-to-lymphocyte ratio. Values shown represent the mean±standard deviation. Significant differences are highlighted by bold type.

Table II. *Spearman's rank correlation coefficients (corresponding p-value) between neopterin concentration and other biomarkers.*

	Serum neopterin (μg/l)	Serum neopterin/ creatinine ratio (μmol/mol creatinine)	Urinary neopterin (μmol/mol creatinine)
Age (years)	0.429	0.262	0.289
	(0.002)	(0.069)	**(0.046)**
	n=49	n=49	n=48
Hemoglobin (g/l)	–0.416	–0.497	–0.397
	(0.004)	**(0.0004)**	**(0.007)**
	n=47	n=47	n=46
Leukocyte count (10^9/l)	0.306	0.179	0.106
	(0.036)	(0.230)	(0.483)
	n=47	n=47	n=46
Platelet count (10^9/l)	0.379	0.413	0.406
	(0.009)	**(0.004)**	**(0.005)**
	n=47	n=47	n=46
NLR	0.151	0.182	0.085
	(0.311)	(0.220)	(0.576)
	n=47	n=47	n=46
LMR	–0.046	–0.131	–0.084
	(0.756)	(0.380)	(0.579)
	n=47	n=47	n=46
PLR	0.237	0.346	0.355
	(0.108)	**(0.017)**	**(0.015)**
	n=47	n=47	n=46

NLR: Neutrophil-to-lymphocyte ratio; LMR: lymphocyte-to-monocyte ratio; PLR: platelet-to-lymphocyte ratio. Significant correlations are highlighted by bold type.

evaluated separately in patients treated in neoadjuvant or adjuvant settings, age, stage, grade, hemoglobin concentration, leukocyte and platelet counts, NLR, LMR and PLR were not predictive of RFS nor OS (data not shown).

Because of significantly different RFS and OS in patients treated in the adjuvant and neoadjuvant setting, these cohorts of patients were evaluated separately. In multivariate analysis that included age, stage, grade, serum and urinary neopterin, hemoglobin concentration, leukocyte and platelet counts, NLR, LMR and PLR, a serum neopterin level of <3 μg/l was the only independent predictor of RFS (HR=0.14, 95% confidence interval=0.04-0.45; p=0.001) and OS (HR=0.12, 95% confidence interval=0.03-0.56; p=0.007) in patients treated in the neoadjuvant setting, but no independent predictor of OS was identified in patients treated with adjuvant chemoradiation.

Discussion

The data presented herein demonstrate a prognostic significance of neopterin concentrations in patients with rectal carcinoma treated with chemoradiation. In earlier studies, prognostic significance was demonstrated for patients with colorectal carcinoma presenting at different stages (21), as well as in patients with metastatic colorectal carcinoma (11). Urinary neopterin concentrations were studied in these previous reports (11, 21). Interestingly, prognostic significance was evident for serum, but not for urinary concentrations of neopterin in the present study. Moreover, serum neopterin predicted prognosis only in patients treated in the neoadjuvant setting. The absence of an association between neopterin concentrations and outcome in patients treated with adjuvant radiation could be explained by a confounding effect of inflammatory reaction induced by previous surgery. It is well known that surgical intervention results in a marked increase of neopterin concentrations (22, 23). On the other hand, in patients scheduled to be treated by neoadjuvant therapy, increased neopterin concentrations are predominantly the result of the host–tumor interaction that is associated with impaired immune response. Moreover, in a previous investigation in the present cohort of patients, we have reported an association between higher baseline serum neopterin/creatinine ratio and toxicity of therapy (18).

As expected from previous reports (11), a significant correlation was observed between neopterin concentrations and hemoglobin and platelet count. The pathogenesis of anemia in patients with rectal carcinoma may be complex and may involve chronic blood loss, but the finding of a negative association between hemoglobin and neopterin concentrations supports the hypothesis that systemic immune activation is a major mechanism responsible for the anemia of chronic disease observed in rectal carcinoma. The positive

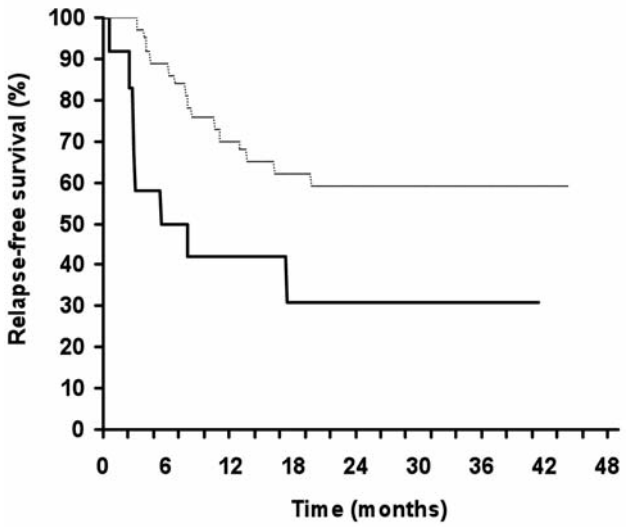

Figure 1. *Kaplan-Meier curve of relapse-free survival of patients with serum neopterin <3 μg/l (dashed line) compared to those with ≥3 μg/l (solid line; whole cohort): median not reached vs. 5.5 months; p=0.049 (n=49).*

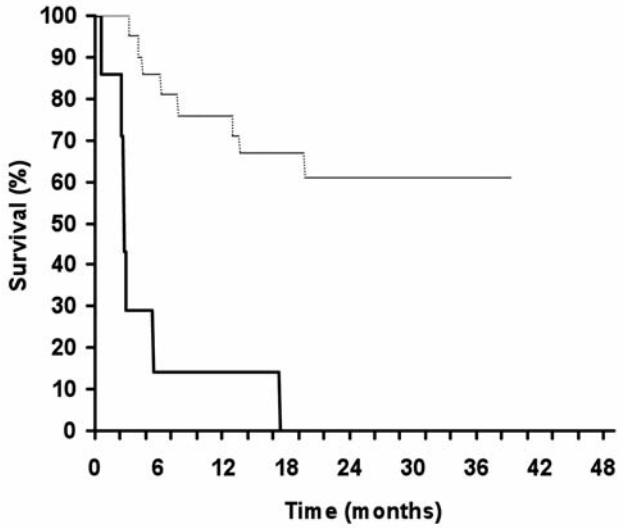

Figure 3. *Kaplan-Meier curve of relapse-free survival of patients with serum neopterin <3 μg/l (dashed line) compared to those with ≥3 μg/l (solid line) treated with neoadjuvant radiation: median not reached vs. 2.8 months; p=0.001 (n=28).*

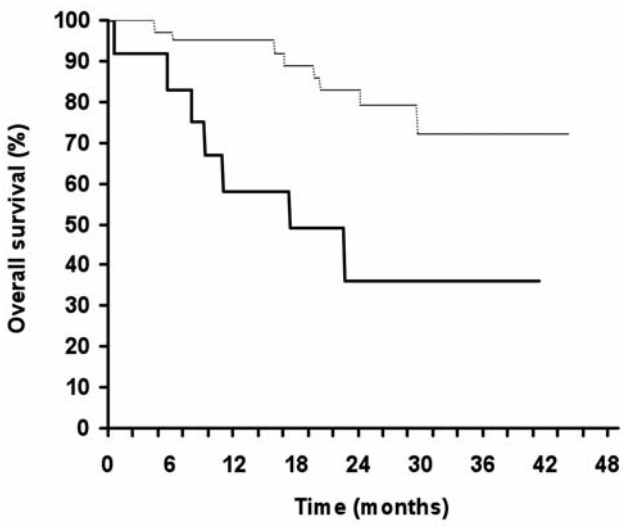

Figure 2. *Kaplan-Meier curve of overall survival of patients with serum neopterin <3 μg/l (dashed line) compared to those with ≥3 μg/l (solid line; whole cohort): median not reached vs. 17.4 months; p=0.009 (n=49).*

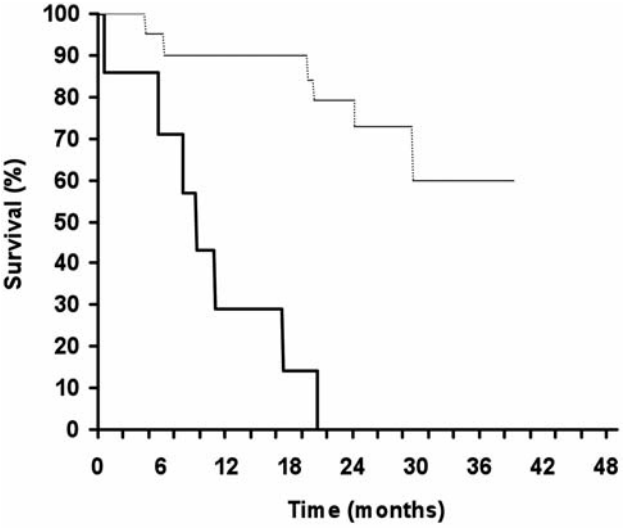

Figure 4. *Kaplan-Meier curve of overall survival of patients with serum neopterin <3 μg/l (dashed line) and ≥3 μg/l (solid line) treated with neoadjuvant radiation (median not reached vs. 9.3 months; p=0.0006; (n=28).*

association between platelet counts and neopterin may also be explained by the effect of systemic immune activation. Cytokines, *e.g.* interleukin-6, are potent inducers of thrombopoiesis.

The subgroups of patients treated in the neoadjuvant or adjuvant settings were not comparable, and the worse outcome of patients treated with neoadjuvant chemoradiation probably reflects more advanced disease that was understaged by imaging studies. The outcome of patients with increased serum neopterin treated in the neoadjuvant setting seems to be particularly poor. Obviously, these results need to be confirmed in a larger prospective cohort. The

identification of a patient population with poor prognosis is of great importance in the development of new therapeutic approaches. New therapeutic methods should be preferentially studied in patients with poor prognosis. Patients with advanced rectal carcinoma and high serum neopterin concentrations scheduled to receive neoadjuvant chemoradiation may represent one such population.

The pathogenic mechanisms underlying the association between increased neopterin concentrations and the outcome of patients with rectal carcinoma treated by neoadjuvant radiation remain speculative. Neopterin concentrations have been correlated with the changes in the cells of the immune system both locally (24) and systemically (8, 25). The immune system plays a crucial role in both tumor control and in the prevalence and outcome of the complications of therapy. Thus, the impaired immune response associated with high neopterin concentration could result in enhanced tumor growth, and a higher rate and severity of complications, resulting in decreased survival. Moreover, higher neopterin concentrations were shown to be associated with the presence of comorbid conditions in patients with cancer (26), which may also determine the patient outcome.

Among the peripheral blood cell count-derived ratios examined, only increased NLR predicted poor survival. Interestingly only PLR correlated with neopterin concentration, while other peripheral blood cell count-derived ratios and neopterin exhibited no significant correlation, indicating that these biomarkers of inflammatory activation are independent. Surprisingly, no correlation was observed between NLR and neopterin concentration, despite a positive correlation between PLR and neopterin concentration. Although the prognostic significance of NLR has been reported in several studies, to the best of our knowledge the prognostic role of peripheral blood cell count-derived ratios have not yet been investigated in the same cohort of patients along with urinary or serum neopterin concentration. The prognostic significance of serum neopterin and NLR in patients with rectal carcinoma treated with chemoradiation should be further confirmed in a prospective study in a larger cohort of patients. Because NLR and neopterin are independent biomarkers, it would be possible to combine these biomarkers in a more complex index reflecting immune and inflammatory activation.

In conclusion, the present data demonstrate a prognostic significance of serum neopterin concentration in patients with rectal cancer treated with chemoradiation, particularly for those treated in the neoadjuvant setting.

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