RESEARCH LETTER

Long-term prognostic utility of selected acute phase proteins in colorectal cancer

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Introduction Colorectal cancer (CRC) belongs to one of the world's most frequent malignancies. Although 5-year survival rates are increasing, there is still an urgent need for new prognostic markers in CRC.[1] Local inflammation in cancerous and pericancerous tissue is believed to play an important role in disease progression, although the clinical relevance of these phenomena is not well studied.[2] Therefore, we aimed to estimate the prognostic utility of several inflammatory markers, assessed in serum and locally, as well as their relationship with the different clinicopathological characteristics of CRC.

Patients and methods A total of 55 patients with CRC (25 men and 30 women; mean [SD] age, 66 [8] years) were included in the study. All patients underwent surgical treatment according to the current oncological guidelines. The exclusion criteria were as follows: presence of concomitant autoimmune or other chronic diseases, intestinal perforation diagnosed intraoperatively, treatment with steroids or immunosuppressants, and neoadjuvant chemo- or radiotherapy.

Patients' functional status was determined by the Karnofsky Performance Score. For staging, the seventh edition of TNM classification was used. The 5-year survival rates were assessed according to the data from the National Cancer Registry and Central Statistical Office in Poland. For the assessment of clinical prognosis, a Glasgow prognostic score was calculated.[3]

Before surgery, each patient provided a serum sample for the assessment of selected acute phase proteins (APPs). In particular, serum C-reactive protein (CRP), α1-acid glycoprotein (AGP), and α1-antichymotripsin (ACT) concentrations were estimated by the Laurell rocket immunoelectrophoresis method (DakoCytomation, Glostrup, Denmark).

Histological assessment The subtype and grading (G1–G3) of CRC were assessed histopathologically (hemotoxylin and eosin staining). The immunohistochemical expression of Ki-67 (mouse anti–human Ki-67; clone MIB-1, code no. M 7240, Dako, Glostrup, Denmark), AGP (rabbit anti–human orosomucoid, code no. A 0011, Dako), and ACT (rabbit anti–human α1-antichymotrypsin, code no. A0022, Dako) were determined separately for CRC and the pericancerous tissue (inflammatory infiltrates closely adjacent to the cancerous tissue without any suspicion of malignant involvement) in 10 representative areas of each specimen (magnification × 40). The Ki-67 expression was calculated as a mean percentage of positive cells in the field. Cytoplasmatic expression of AGP and ACT was determined using a semiquantitative scale (0, no expression; 1, weak expression; 2, moderate expression; 3, strong expression).

The control group consisted of 48 healthy persons (matched for age and sex), in whom the concentration of serum inflammatory markers was assessed.

Statistical analysis Statistical analyses were performed with the STATISTICA 12.0 PL (StatSoft Polska, Kraków, Poland) and StatXact 11.0 (Cytel Inc., Cambridge, Massachusetts, United States). *P* values of less than 0.05 were considered significant. The Spearman rank correlation analysis or Fisher exact test was used to evaluate the association between the analyzed parameters. The comparison of serum APP concentrations between the study and the control group was performed by the *t* test. The estimates of the cumulative survival distributions were calculated by the Kaplan–Meier method, and the differences between the groups were compared using the log-rank test. The significance of prognostic parameters was evaluated using the univariate and multivariable

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TABLE 1 Univariate and multivariable Cox regression analysis showing the utility of selected parameters in predicting long-term survival in patients with colorectal cancer

Variable		Univariate analysis			Multivariable analysis		
		HR	95% CI	*P* value	HR	95% CI	*P* value
Grading, G1/G2 vs G3		2.376	0.972–5.813	0.06	_	_	_
Disease location, rectosigmoid vs nonrectosigmoid		1.934	0.993–3.769	0.05	_	_	_
AGP expression	Cancerous, 3 vs 1–2	0.378	0.182–0.788	0.009	0.358	0.165–0.774	0.009
	Pericancerous, 3 vs 1–2	2.936	1.478–5.829	0.002	2.826	1.378–5.795	0.005
TNM staging	I/II vs III	3.113	1.429–6.782	0.004	2.732	1.259–5.927	0.01
	I/II vs IV	12.973	4.909–34.282	<0.001	18.074	6.039–54.094	<0.001

A *P* value <0.05 is considered significant.

Abbreviations: AGP, α1-glycoprotein; CI, confidence interval; HR, hazard ratio

Cox proportional hazards regression model. The variables considered to be significant based on the univariate regression model were selected and analyzed by the multivariable Cox regression model (Supplementary material, *Figure S1*).

The Bioethics Committee at the Poznan University of Medical Sciences approved the study (No. 193/2011).

Results The most frequent CRC location was the sigmoid colon and rectum (n = 34; 61.8%). The most frequent histological type and grading was tubular adenocarcinoma (n = 31; 56.4%) and G2 (n = 42; 76.4%), respectively. According to the TNM classification, patients were distributed as follows: stage I, 10 patients (18.2%); stage II, 23 patients (41.8%); stage III, 13 patients (23.6%); and stage IV, 9 patients (16.4%). Serum APP concentrations differed between the study and control groups: median (IQR) CRP, 6 (0–22) and 0 (0–0) mg/l; mean (SD) AGP, 1157.9 (613.6) and 866.7 (288.8) mg/l; mean (SD) ACT, 497.7 (222.7) and 403.3 (162.2) mg/l, respectively ($P < 0.05$). There were no significant associations of serum APPs, cancerous and pericancerous APP immunoexpression with patients' age, cancerous Ki-67 immunoexpression, as well as histological type, grading, and staging scores.

The median survival time was 27 months (95% CI, 16–54 months). The 5-year survival rate was 34.5% (n = 19).

Statistical analysis (the log-rank test) revealed that the survival time was significantly shorter in patients with a low-grade type of CRC (G3 and G1/G2; $P < 0.05$) and more advanced disease, according to the TNM classification (stage IV vs stage II/III vs stage I; $P = 0.001$). Moreover, the rectosigmoid CRC location was associated with better outcomes ($P = 0.04$). The histological type did not differentiate the survival time in the study group ($P = 0.6$).

Correlation analyses including CRP, serum AGP, serum ACT concentrations, cancerous and pericancerous ACT and AGP expression, as well as cancerous Ki-67 expression and Glasgow prognostic score, revealed that only AGP cancerous ($P = 0.02$, $r = 0.35$) and pericancerous ($P = 0.01$, $r = 0.36$) expression was associated with patients' survival (the Fisher exact test). Additional data are described in Supplementary material, *Table S1*.

Finally, considering all significant prognostic parameters, we performed further, confirmatory univariate and multivariable Cox regression analyses (TABLE 1). The univariate model showed that cancerous and pericancerous AGP expression, as well as disease staging, influenced patients' survival. The multivariable Cox regression analysis confirmed that all these variables were independent prognostic factors for overall survival. The Cox proportional hazard regression model obtained was significant ($P < 0.001$) with a Nagelkerke R^2 equal to 68%.

Discussion Colorectal cancer belongs to the most frequent malignant diseases, with significant mortality rates.[1] This is why much effort is being made to identify patients with the highest risk of shorter survival. It is well known that a less advanced disease is connected with better outcomes, which was also confirmed by our study. Nevertheless, there is still an urgent need for additional prognostic markers. Since multiple studies have shown the reflexive relationship between oncogenesis and inflammation, there is a growing body of evidence that several inflammatory parameters can be used as prognostic markers in malignancies.[2] For example, an elevated baseline CRP is associated with the shorter overall survival in CRC and predicts worse progression-free survival in patients undergoing chemotherapy.[4] However, little is known about the prognostic value of other APPs, especially in relation to the 5-year survival rates.

In our study, we showed that the serum concentration of all APPs was significantly higher in patients with CRC when compared with healthy controls. This is not surprising, since there are many data on the induction of a proinflammatory response in cancer, which is believed to play a role in tissue invasion and metastasis.[5] Moreover, it was suggested that elevated APPs can alter the anticancer effects of chemotherapy by binding to therapeutic molecules.[6] On the other

hand, there is evidence to suggest that immune recognition of tumor antigens may play a role in the inhibition of cancer development.[2]

In accordance with data concerning other malignancies, we also found that measured APPs were not related to the histological type of CRC, its grading, or staging scores.[7,8] Interestingly, however, we were able to demonstrate that the assessment of AGP tissue expression had a prognostic utility. Namely, long-term survival rates were significantly lower in patients with a high cancerous expression of AGP and in the case of a low expression of AGP in pericancerous cellular infiltrates. Thus, one can speculate that the acute phase response in CRC tissue can be at least partly responsible for tumor invasiveness, which is in line with data for other malignancies.[9] One of the possible molecular mechanisms explaining this phenomenon is that APPs, especially AGP, can promote migration of microvascular cells and locally stimulate angiogenesis.[10] On the other hand, immune activation in pericancerous histological milieu, reflected in our study by the expression of AGP, seems to have a beneficial effect on patients' survival. This is in accordance with data showing that the intensity of inflammatory infiltration at the tumor margin is related to increased overall survival rates and relapse free survival after surgery in CRC.[11] Mechanisms underlying these phenomena are poorly understood; however, it is believed that the acute phase response at the invasive border of the tumor inhibits the proliferation of malignant cells.[11]

In conclusion, to the best of our knowledge, we showed for the first time that the assessment of the expression of AGP in CRC and in pericancerous tissue could be helpful in prognosticating long-term outcomes after surgery. Our study is in line with the current personalized diagnostic approach to a precise molecular characterization of malignant disease.[12] It is well documented that the natural history of CRC can be different, which seems to be significantly determined by the biology of the tumor.[12] Thus, identification of new prognostic tissue markers could be very helpful in choosing more optimal therapy, adjusted to the individual characteristics of the patient. Nevertheless, whether the assessment of the AGP expression, or other prognostic markers, could become a diagnostic standard in CRC needs to be clarified in larger prospective trials.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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