Infiltration of Colorectal Carcinoma by S100+ Dendritic Cells and CD57+ Lymphocytes as Independent Prognostic Factors after Radical Surgical Treatment

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Abstract. Background: S100+ dendritic cells and CD57+ lymphocytes are factors reflecting the immune system’s ability to suppress the progress of tumor growth. CD57+ cells include natural killer cells and late stages of T-effector lymphocytes. We evaluated the relationship between the known clinical and histological factors and tumor markers as well as the presence of S100+ and CD57+ cells in the tissue of colorectal carcinoma with the aim of detecting patients at high risk of short overall survival (OS) or short disease-free interval (DFI) after radical surgical treatment and we further analyzed whether S100+ and CD57+ positivity could bring on new information regarding the treatment regimen. Materials and Methods: Data of 150 patients (97 males and 53 females) that underwent an elective radical surgical procedure for colorectal cancer were studied. The influence on DFI and on OS of the following parameters was evaluated: grading, staging and positivity for S100 and CD57 by immunohistochemical staining. We also analyzed the relation of preoperative serum levels of the tumor markers Carcinoembryonic Antigen (CEA), Cancer Antigen 19-9 (CA19-9), Cancer Antigen 72-4 (CA72-4), Thymidine kinase (TK), Tissue-Specific Polypeptide Antigen (TPS) and Tissue Polypeptide Antigen (TPA) in relation to S100 and CD57 positivity/negativity for the same patients. Results: OS at 1, 3 and 5 years was 92.2%, 76.5% and 70.2%; the DFI at 1, 3 and 5 years was 85.3%, 64.3% and 49.4%. CD57 positivity in the tumor mass was proven as a positive prognostic factor for OS. Risk of short OS was 2.5-fold higher in patients with low tumor infiltration by CD57+ lymphocytes. The combination of N2 stage for lymph nodes and the absence of CD57+ cells was proven to be the strongest negative prognostic factor for OS. No significant influence of CD57 positivity on DFI appeared. There was no significant influence of S100 positivity on OS or DFI; nor was there any statistical dependence of CD57 and S100 positivity or negativity on preoperative serum levels of CEA, CA19-9, CA72-4, TK, TPS or TPA. Both studied factors were shown to be statistically independent factors. Conclusion: The present study showed infiltration of colorectal cancer tissue by CD57+ cells as being an important independent positive prognostic factor for OS.

S100+ dendritic cells (DC) and CD57+ lymphocytes are factors reflecting the immune system’s ability to suppress the progress of tumor growth. Atreya and Neurath demonstrated their role in suppression of the progress of colorectal carcinoma (1). CD57 is a glycoprotein with cell adhesion function also called human natural killer-1 or LEU7 (2). CD57+ cells include natural killer (NK) and late stages of T-effector lymphocytes. NK cells as a part of the cellular innate immunity are able to destroy malignant cells (2). NK cells mediate lysis of malignant cells but the mechanism of detection of cancer cells is different from that of the CD8+ T-lymphocytes (3); NK cells do not recognize specific tumor-associated antigens of cancer cells as CD8+ T-lymphocytes do. NK cells lyse cancer cells that are opsonized by surface antibodies or after stimulation by other signals such as cytokines, produced by antigen-presenting cells (4). A lower preoperative number of NK cells was associated with increased postoperative recurrence of colorectal cancer (5). The S100 antigen protein is present in many types of cells: cells of neural crest, chondrocytes, adipocytes, myoepithelial cells, macrophages, Langerhans cells and dendritic cells. In particular dendritic cells (DC) (antigen-presenting cells) play a key role in activating naïve T-lymphocytes and NK cells. DC gather antigens in their surrounding tissues, process them and migrate to the secondary lymphoid organs to present antigens on major histocompatibility complexes class I or class II to CD8+ cytotoxic lymphocytes or CD4+ helper T-lymphocytes (2, 6). In the majority of the available studies, increased
numbers of immune cells infiltrating the tumors correlate with an improved prognosis for cancer patients (7), but the role of certain lymphocytes remains unclear (8). Here we evaluated the relationship between the known clinical, histological factors and tumor markers that are used in standard clinical follow-up of patients and the presence of S100+ and CD57+ cells in the tissue of colorectal carcinoma (CRC) with the aim of detecting patients with a high risk of short overall survival (OS) or short disease-free interval (DFI), after radical surgical treatment and to analyze whether S100+ and CD57+ positivity could bring new information on the treatment regimen, independently of standard clinical examinations.

Materials and Methods

Data from 150 patients (97 males and 53 females) in a patient cohort that underwent an elective radical surgical procedure at the Department of Surgery of the Teaching Hospital and Medical School in Pilsen between 2004-2007. The influence on DFI and on OS of following parameters was evaluated: grading, staging and positivity of S100 and CD57 by immunohistochemical staining.

Histological and immunohistochemical analysis. Tissue for light microscopy was fixed in 4% formaldehyde and was embedded in paraffin. Five micrometer-thick sections were cut from the tissue blocks and stained with hematoxylin-eosin. Three different sections of each tumor were examined.

For the immunohistochemical investigations the following primary antibodies were used: CD57 (clone NK1, ready-to-use; Ventana, Rocklin, CA, USA), and S100 (polyclonal, 1:1000; Dako, Glostrup, Denmark). Microwave pretreatment was used in both cases. The primary antibodies were visualized using the supersensitive streptavidin-biotin-peroxidase complex (Biogenex, San Ramon, CA, USA). The appropriate positive and negative control slides were employed. The number of DCs and NK cells was evaluated in five high power microscopical fields and was expressed as the number of immunopositive cells per high power microscopical field.

Oncomarkers. All the blood samples for assessment of tumor markers were obtained under standard conditions from the cubital vein during the morning hours. The serum for the assessment of routine tumor markers was acquired through centrifugation and was stored at −20°C until laboratory analysis. Tumor markers were assessed at the Department of Nuclear Medicine, Faculty Hospital Pilsen with commercial laboratory kits, in accordance with the manufacturers’ recommendations. The following tumor markers were assessed: CEA (ng/ml, Immunotech, Czech Republic), carbohydrate antigen 19-9 (CA19-9, IU/l, Shering-CIS BioInternational, France), cytokeratins: tissue-specific polypeptide antigen (TPS, kIU/l, IDL, Sweden), tissue polypeptide antigen (TPA, kIU/l, DiaSorin, Italy). Thymidine kinase (TK, IU/L) was measured by radioenzyme analysis (REA) using the Immunotech (Prague, Czech Republic) assay kits.

Statistical analysis. Statistical analysis was processed by the statistical software Statistica 9.0 (StatSoft, CA, USA). The relationships between the variables were described by the Spearman rank correlation coefficients. The analyses of OS and DFI were performed by Kaplan-Meier survival functions. The influence of given covariates (clinical and histopathological factors or tumor markers) was tested by the log-rank test and the Wilcoxon test. The Cox regression the hazard model, hazard ratio (HR) and the 95% confidence interval (CI) for HR were computed for the evaluation of given clinical and histopathological factors and tumor markers to OS or DFI. Multivariate analysis was...
performed by the use of classification and regression trees (CART). The Cox regression hazard model (stepwise regression) was applied in order to find the predictors in CART.

**Results**

There were 93 males (mean age=65.27 years, median=65.94 years) and 57 females (mean=68.07 years median=67.72 years) in studied patients cohort. No statistically significant differences were proven regarding the age between males and females. OS at 1, 3 and 5 years was 92.2%, 76.5% and 70.2%; the resulting DFI at 1, 3 and 5 years was 85.3%, 64.3% and 49.4%. CD57 positivity of cells in the mass of tumor was a statistically significant positive prognostic factor (cut-off 1 cell, \( p \)-value=0.0350, Figure 1) of OS. Risk of short OS was 2.5-fold higher in patients with low tumor infiltration by CD57+ lymphocytes. CART showed that the combination of N2 stage of lymph node disease and the absence of CD57+ cells was the strongest negative prognostic factor of OS (Figure 2). No statistically significant influence of CD57 positivity on DFI appeared (Figure 3).

There was no statistically significant influence of S100 positivity on OS nor on DFI (Figures 4 and 5). There was no statistical dependency or correlation of CD57 and S100 positivity or negativity on preoperative serum levels of CEA, CA19-9, CA72-4, TK, TPS or TPA. Both studied factors were found to be statistically independent factors.

**Discussion**

The role of the immunological response in controlling the growth and relapse of CRC remains controversial and contemporary studies have not answered all the questions about the prognosis of patients after radical surgical treatment of CRC (9, 10, 11, 14). We analyzed a large cohort of patients with CRC aiming at detecting the relation between these types of immune cells and the prognosis of patients after radical CRC surgery. This aim was stimulated by some dilemmas in the decision for
surgical and oncological treatment, when early recurrence depreciates the effort of radical surgery due to a high risk of complications and the long duration of the decreased quality of life of the patients (3, 12). Our results support the hypothesis that the immunological response observed in the tumor tissue can influence the behavior of CRC and subsequently affect the prognosis of patients (9). Tumor infiltration by NK cells seems to be a promising positive prognostic factor reflecting the decreased risk of patients for poor OS. Future work should focus on the molecular-biological background of tumor infiltration by lymphocytes to provide understanding over their pathophysiological functions (1). The tumor markers inform us of the negative prognosis of patients (early recurrence (DFI) or for the poor prognosis for long OS (13). On the other hand CD57+ positivity was demonstrated as being a positive prognostic factor for radically operated patients with CRC. This information is more important for clinicians than a negative factor would be, since it could modify their decision about adjuvant oncological treatment or follow-up regimen. The present study demonstrated the positive influence of tumor-infiltrating CD57+ cells on the prognosis of CRC. The results could lead upon intensifying the follow-up strategy for the patients with higher risk of early CRC recurrence.

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References


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