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Fecal Galectin-1 as a potential marker for colorectal cancer and disease severity Фецесни галектин-1- потенцијални маркер колоректалног карцинома и тежине болести

Milan Jovanovic1, Nevena Gajovic2, Natasa Zdravkovic3, Marina Jovanovic3, Milena Jurisevic4, Danilo Vojvodic5, Darko Mirkovic1, Bosko Milev1, Veljko Maric6, Nebojsa Arsenijevic2

1 Clinic for General Surgery, Military Medical Academy, Belgrade, Serbia
2 Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia
3 Department of Internal medicine, Faculty of Medical Sciences, University of Kragujevac, Serbia
4 Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Serbia
5 Institute for medical research, Military Medical Academy, Belgrade, Serbia
6 Department of Surgery, Faculty of Medicine Foca, University of East Sarajevo, Bosnia and Herzegovina

Corresponding author:
Nevena Gajovic, MD
Center for Molecular Medicine and Stem Cell Research
Faculty of Medical Sciences University of Kragujevac
Svetozara Markovica 69, 34000 Kragujevac, Serbia
Tel +38134306800, Fax. +38134306800112
E-mail: gajovicnevena@yahoo.com
ABSTRACT

**Background/Aim:** Colorectal cancer (CRC) represents one of the most common cancers worldwide. CRC is frequently diagnosed at advanced stages with poor prognosis, indicating a need for new diagnostic and prognostic markers. The aim of this study was to determine systemic and fecal values of galectin-1 (gal-1) and ratios between gal-1 and proinflammatory cytokines TNF-α, IL-1β and IFN-γ in patients with CRC and the relationship with clinicopathological aspects of disease.

**Methods:** The blood samples and feces liquid fraction of 58 patients with CRC were analyzed. Serum and fecal levels of TNF-α, IL-1β and IFN-γ and galectin-1 were measured using sensitive enzyme-linked immunosorbent assay (ELISA) kits.

**Results:** Fecal level of gal-1 was increased in CRC patients with higher nuclear grade and poor tumor tissue differentiation. Gal-1/TNF-α ratio in serum and feces had a higher trend in patients with advanced TNM stage as well as detectable lymphatic and blood vessel invasion. Gal-1/TNF-α and Gal-1/IFN-γ ratios were increased in serum of patients with presence of lung/liver metastasis or peritoneal carcinomatosis, respectively, while enhanced Gal-1/IL-1 ratio was detected only in serum of patients with lung metastasis. Positive correlation between Gal-1 value in feces and histological differentiation of tumor and biomarkers AFP and CA 19-9, respectively, was also observed. Fecal values of gal-1 higher than 13708.29 pg/g present a highly sensitive and specific marker for histological differentiation of tumor tissue.

**Conclusion:** We believe that predomination of gal-1 over pro-inflammatory cytokines TNF-α, IL-1β and IFN-γ in patients with advanced and progressive disease may implicate an immunomodulatory role of gal-1 in limiting ongoing proinflammatory processes. The fecal values of gal-1 can be used as a valuable marker for CRC severity.

**Key words:** Colorectal carcinoma, galectin-1, feces
SAŽETAK

_Uvod/Cilj:_ Kolorektalni karcinom (engl. Colorectal carcinoma- CRC) je jedan od najučestalijih karcinoma na svetu. CRC se često dijagnostikuje u uznapreodavalim stadijumima sa lošom prognozom, ukazujući na potrebu za novim dijagnostičkim i prognostičkim markerima. Cilj ove studije bio je utvrđivanje sistemskih i fekalnih vrednosti galektina-1 (gal-1) i odnosa između gal-1 i proinflamacijskih citokina TNF-α, IL-1β i IFN-γ kod pacijenata sa CRC i odnosa sa kliničko-patološkim aspektima bolesti.

_Metode:_ Analizirani su uzorci krvi i tečne frakcije fecesa 58 pacijenata sa CRC-om. Serumski i fekalni nivoi TNF-α, IL-1β, IFN-γ i galektina-1 su mereni korišćenjem senzitivnog ELISA (enzyme-linked immunosorbent assay) testa.


Takođe je primećena pozitivna korelacija između vrednosti Gal-1 u fecesu i histološkog tipa tumora i biomarkera AFP i CA 19-9. Vrednosti Gal-1 u fecesu veće od 13708,29 pg/g predstavljaju visoko osetljiv i specifičan marker za histološku diferencijaciju tumorskog tkiva.

_Zaključak:_ Naši rezultati ukazuju da predominacija Gal-1 nad proinflamacijskim citokinima TNF-α, IL-1β, IFN-γ kod pacijenata sa uznapreodavalom i progresivnom bolešću ističe imunomodulatornu ulogu Gal-1 u ograničavanju proinflamacijskih procesa. Vrednosti Gal-1 u fecesu mogu se koristiti kao marker procene težine kolorektalnog karcinoma.

_Ključne reči:_ kolorektalni karcinom, galektin-1, feces
INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers and the fourth cause of cancer-related deaths. Despite the constant achievements in the understanding of cancer biology, the morbidity and mortality rates of CRC continue to increase. In most cases CRC is diagnosed at advanced stages with poor prognosis. This phenomenon highlights the need for new diagnostic and prognostic markers. There has been a sustained interest in the identification of bio-markers for the prognosis and progression of CRC. New markers should contribute to the prediction of prognosis or relapse after therapy. Today, serum markers for CRC are preferred over tissue- or stool-based assays, especially for screening and monitoring purposes, which require repeat testing. Novel studies point to the significance of fecal markers measurement in the detection and prediction of disease severity.

A large body of evidence indicates that galectins participate in a variety of normal cellular functions, and are disregulated in CRC. Among all the known galectins, galectin-1 (gal-1) is well characterized. Galectin-1(Gal-1) is a multifunctional β-galactoside-binding lectin produced by a variety of vascular, interstitial, epithelial, immune cells as well as neoplastic cells. It can be located either inside the cells in nucleus and cytosol or in the extracellular space. It is shown that gal-1 is involved in several biological processes and in various phases of tumorogenesis such as regulation of cell growth and migration, cell-extracellular matrix and cell-cell interactions, angiogenesis, tumor–immune escape. Elevated expression of gal-1 was observed in tissues of various solid malignant tumors, whereas low or no expression was found in normal tissues. The immunomodulatory role of Gal-1 is also familiar, and it’s strong influence on inflammation is well established.

The aim of this study was to evaluate systemic and fecal values of gal-1 and ratios between gal-1 and proinflammatory cytokines in patients with CRC and the relationship with clinicopathological aspects of disease. In this study we demonstrate enhanced fecal concentration of gal-1 in CRC patients with higher nuclear grade and poor tumor tissue differentiation, while predomination of gal-1 over proinflammatory cytokines in patients with advanced TNM stage and metastatic disease.
METHODS

**Ethical Approvals.** The study was conducted at the Clinical center, Kragujevac, Serbia, and Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia, after the study protocol was approved by relevant Ethics Committees of the Clinical center, Kragujevac, Serbia, and Faculty of Medical Sciences, University of Kragujevac, Serbia. All patients gave their informed consent. All research procedures were made according to the Principle of Good Clinical Practice and the Declaration of Helsinki.

**Subjects.** Fifty eight patients with CRC were enrolled in the study. All patients received surgical resection for CRC. Diagnosis was based on endoscopic and histological criteria. Exclusion criteria included no well-defined pathology, no adequate clinical document available and previous treatment with radiation and chemotherapy. Clinical data about age, gender, size of cancer, metastasis, and pathologic reports (vascular invasion, lymph node invasion, nuclear grade and well and moderate + poor differentiation) and clinical stage by TNM (tumor, nodes, and metastasis) were recorded and analyzed in study. Blood and stool samples were taken before the surgery and stored at -80°C until ELISA.

**Feces liquid fraction preparation**

Stool samples (1-10 g) were collected in the morning in sterile containers and weighed. One gram of fecal samples was diluted, mixed, homogenised in 5 mL of protease inhibitor cocktail (SIGMA, P83401), and then centrifuged, as previously described. The supernatant fluid was collected and stored at -80°C until ELISA.

**Evaluation of tumor markers in serum.** Serum levels of Alpha-fetoprotein (AFP), Carcino-embryonic antigen (CEA), and Cancer antigen 19-9 (CA19-9) were routinely determined by chemiluminescence enzyme immunoassay (CLIA) in the central biochemical laboratory of the Clinical center Kragujevac.

**Determination of Galectin-1, TNF-α, IL-1β and IFN-γ in serum and feces.** Serum and fecal concentrations of Gal-3 and cytokines were measured, as described, using sensitive enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, for Gal-1, TNF-α, IL-1β and IFN-γ; measurement of cytokines according to the manufacturer’s
instructions). Briefly, the 96-well plates were coated with capture antibody, overnight. The plates were washed with a washing buffer (0.05% Tween-20 in PBS) and incubated with blocking buffer (1% bovine serum albumin in PBS) for 1 hour at room temperature. Serum/faecal samples or standard recombinant Gal-1/TNF-α/IL-1β/IFN-γ were introduced to the plates for 2 hours before the application of biotinylated detection antibody for 1 hour at room temperature. After introduction of streptavidin peroxidase for 1 hour, the plates were developed with substrate reagent for 20 minutes. The reaction was stopped by adding 4mol/L sulfuric acid, and the absorbance was read at 495 nm by a microplate reader. We measured the exact concentration of mentioned biomarkers by intrapolation of a standard curve made by a series of well-known concentrations as per manufacturer’s instruction. Values of measured cytokines are presented as pg/ml of serum and pg/g of feces, respectively.

**Statistical analysis**

The statistical analyses were performed using SPSS 20.0 software. The results were reported as mean and standard error (SE). Determination statistically significant difference between the means of two groups was determined using Student's t-test for independent samples if the data had normal distribution or Mann-Whitney U-test for data without normal distribution. Pearson's or Spearman’s correlation, where appropriate, evaluated the possible relationship between the cytokines and disease severity and progression in patients with CRC. Numerical values were assigned to different histological differentiation stages (well=1; moderate + poor = 2). Strength of correlation was defined as negative or positive weak (-0.3 to -0.1 or 0.1 to 0.3), moderate (-0.5 to -0.3 or 0.3 to 0.5) or strong (-1.0 to -0.5 or 1.0 to 0.5). P-value of 0.05 was considered as statistically significant.

**RESULTS**

Fifty eight patients with CRC were enrolled in the study. There was no significant difference in gender distribution (34 males and 24 females). Patients were similar in age (mean age 66 ± 1). Clinical and pathologic characteristics of these patients are presented in Table 1.
Serum and fecal concentration of Gal-1 and ratio between Gal-1 and pro-inflammatory mediators, regard to histo-pathologic characteristics of CRC

Patients with CRC were categorized into 3 groups according to nuclear grade of tumor tissue: I, II and III, and analyzed for systemic and fecal values of ratio between Gal-1 and pro-inflammatory mediators (TNF-α, IL-1β and IFN-γ). As shown in Figure 1A, CRC patients with higher nuclear grade appear to have higher fecal level of Gal-1 (III vs. II: 21936,14 ± 3601,19 vs. 13286,97 ± 782,97 pg/ml; p=0.020; III vs. I: 21936,14 ± 3601,19 vs.15724.30±1903.49 pg/ml; p=0.047), systemic value of Gal-1/TNF-α ratio (III vs. II: 60,46 ± 9,01 vs. 27,17 ± 2,62; p=0.009; III vs. I: 60,46 ± 9,01 vs 24.44 ± 0.89; p=0.032), as well as fecal Gal-1/IFN-γ ratio (III vs. II: 13,64 ± 0,78 vs. 9,76 ± 1,39; p=0.001; III vs. I: 13,64 ± 0,78 vs.10.03 ± 2.96; p=0.048).

Further, we classified CRC patients in two groups, according to histological differentiation rate: well and moderate + poor. In patients with poor tumor tissue differentiation, we detected increased fecal Gal-1 (moderate and poor vs. well: 19353,69 ± 2224,35 vs. 12757,56 ± 1207,58 pg/ml; p=0.026) and systemic Gal-1/TNF-α ratio (moderate and poor vs. well: 503,57 ± 100,01 vs. 69,73 ± 11,61; p=0.042; Figure 1B).

Serum and fecal Gal-1/TNF-α ratios are associated with TNM system and lymph and blood vessels invasion

Patients with CRC were divided in two categories on the basis of TNM stage of disease: I+II (localized tumor) and III+IV (metastatic tumor). There were no patients with TNM stage II. Patients with TNM stages III+IV revealed significantly higher Gal-1/TNF-α ratio in serum (115,03 ± 20,10 vs. 60,51 ± 7,95; p=0.046) and feces (16,84 ± 0,92 vs. 10,36 ± 1,36; p=0.024; Figure 2A).

Patients with CRC were divided in two groups, based on the presence of lymphatic/blood vessel invasion, respectively (+ and -). Increased Gal-1/TNF-α ratio in serum was detected in patients with detectable lymphatic (146,95 ± 28,91 vs. 58,53 ± 24,87; p=0.049) and blood vessel invasion (38,62 ± 4,01 vs. 22,82 ± 3,25; p=0.040; Figure 2B).
Liver, lung and peritoneal metastasis are associated with higher Gal-1/TNF-α ratio

Further, we divided patients in two categories based on presence of lung/liver metastasis or peritoneal carcinomatosis, respectively. Higher Gal-1/TNF-α ratio was found in serum of patients with detectable liver metastasis (48.53 ± 6.95 vs. 28.12 ± 2.87; p=0.005), lung metastasis (70.61 ± 10.09 vs. 28.87 ± 2.51; p=0.001), or peritoneal carcinomatosis (53.79 ± 11.42 vs. 29.71 ± 2.72; p=0.012), in comparison to patients without metastasis/carcinomatosis (Figure 3). In addition, we also found higher Gal-1/IFN-γ ratio in serum of patients with detectable liver metastasis (72.68 ± 12.51 vs. 46.01 ± 3.26; p=0.043), lung metastasis (100.34 ± 25.82 vs. 55.02 ± 5.25; p=0.033), or peritoneal carcinomatosis (89.57 ± 19.57 vs. 54.65 ± 5.46; p=0.033), as illustrated in figure 3. Increased Gal-1/IL-1 ratio was detected in serum of patients with detectable lung metastasis (1001.91 ± 82.09 vs. 791.65 ± 31.63; p=0.027; Figure 3).

Faecal Gal-1 concentration significantly correlates with histological differentiation stage of colon cancer and systemic values of tumor markers AFP, CEA and CA 19-9

Spearman correlation analysis of Gal-1 concentration in stool uncovered positive correlation between Gal-1 value and histological differentiation stage of tumor (r=0.357; P= 0.025). Further analyses also found that fecal Gal-1 significantly correlated with AFP levels (r=0.317; P= 0.028), CA 19-9 levels (r=0.296; P= 0.049), but no significant correlation was found with CEA levels (Figure 4). Serum Gal-1 did not correlate with same parameters and markers of colon cancer (data not shown). Analysis also showed that fecal Gal-1 can be a valuable marker for distinguishing poor and moderate differentiation of tumor tissue (Figure 4). The optimal cut-off value estimated for Gal-1 that allows discrimination between poor and moderate differentiation was 13708.29 pg/g. For this cut-off, we determined sensitivity to be 73.6% and specificity 60.0%.
Biological role of Gal-1 in tumor cell proliferation, invasion, apoptosis, metastasis, immuno-suppression and tumor angiogenesis is well known\textsuperscript{20-27}. It is involved in poor prognosis and the metastatic phenotype\textsuperscript{23,24}. Galectin-1 may act intracellularly as well as extracellularly, after secretion\textsuperscript{28}. Secreted Gal-1 can interact with cell-surface proteins such as fibronectin, integrins, laminin and VEGFR2 and subsequently determines proliferation, adhesion, migration and angiogenesis\textsuperscript{29,30}. These findings highlight the importance of extracellular Gal-1 in tumor biology. In the present study, we analyzed systemic and fecal level of Gal-1 and its ratio with several pro-inflammatory cytokines, in different stages of CRC. We found increased concentration of Gal-1 in stool of CRC patients with higher nuclear grade (III vs. II and III vs. I) and poor tumor tissue differentiation (Figure 1). Previous studies established Gal-1 as a protein commonly elevated in serum of patients with tumors\textsuperscript{8-10}. Also, serum Gal-1 values were significantly increased in patients with metastatic disease compared with patients with localized tumors\textsuperscript{11}. We didn’t find that serum Gal-1 mean values ranged significantly different regard to pathohistological characteristics of tumor, while fecal Gal-1 showed significant alteration according to pathohistological characteristics (Figure 1).

Indeed, in recent studies feces has been used as a sample for testing different biomarkers\textsuperscript{5,6}. For instance, fecal calprotectin (FC), a biomarker of intestinal inflammation that has been in clinical use for years\textsuperscript{5-7}, has been also shown to be elevated in CRC and suggested for screening high risk groups for CRC\textsuperscript{31}. Today, researchers are testing diagnostic accuracy of different fecal markers in the detection of cancerous lesions of the colorectum in order to find the most accurate for CRC screening. According to available literature, this is the first study testing fecal Gal-1 for detection of severe and progressive forms of CRC. It had been suggested that ratio of counterregulatory cytokines is a reliable marker of the disease progression\textsuperscript{32}. Therefore, we considered ratios of Gal-1 and pro-inflammatory cytokines and showed predomination of Gal-1 over pro-inflammatory cytokines TNF-\(\alpha\), IL-1\(\beta\) and IFN-\(\gamma\) in patients with CRC with progressive disease. Gal-1/TNF-\(\alpha\) ratio in serum and feces had a higher trend in patients with advanced TNM stage (III+IV) as well as detectable lymphatic and blood vessel invasion (Figure 2). In line with this finding,
enhanced Gal-1/TNF-α and Gal-1/IFN-γ ratios were detected in serum of patients with presence of lung/liver metastasis or peritoneal carcinomatosis, respectively, while enhanced Gal-1/IL-1 ratio was detected only in serum of patients with lung metastasis (Figure 3). Based on these findings, we believe that Gal-1/TNF-α ratio could be a predictor for the advanced stages of colorectal cancer.

The role of Gal-1 in the onset, progression and resolution of inflammation is well established. Previous studies revealed that Gal-1 inhibit cell growth and induce the apoptosis of activated immune cells. Gal-1 has been shown to skew the balance toward type-2 immune response, simultaneously inhibiting IFNγ, TNFα, IL-2, and IL-12 production and facilitating IL-5 secretion, in vitro and in vivo. Some studies suggest that Gal-1 might inhibit T-cell effector functions or induce the death of tumor infiltrating leukocytes and subsequently suppress strong immune response derived by proinflammatory cytokines. We are first to describe prevailing of Gal-1 over TNF-α, IL-1β and IFN-γ in stool of patients with severe and progressive forms of CRC (Figure 2 and 3). In line with our finding Camby I et al. conclude that tumor cells may impair T-cell effector functions through the secretion of Gal-1, that favors genesis of an immunosuppressive environment at a tumor site.

Further in this study, we envisage the possible role of fecal galectin-1 as a biomarker in preceding disease severity. We found positive correlation between Gal-1 value in feces and histological differentiation stage of tumor and biomarkers AFP and CA 19-9, respectively (Figure 4). Interestingly, we didn’t find correlation of serum Gal-1 with the same parameters and markers of disease severity. Also, values of Gal-1 in feces are about two to three times higher than in serum, what makes measurement in feces a more sensitive method. Analysis of Receiver Operating Characteristic (ROC) curves of Gal-1 and disease parameters and markers for CRC revealed that Gal-1 could predict poor differentiated type of tumor, at good sensitivity and specificity. According to our findings, fecal Gal-1 could be a valuable marker for CRC severity.
CONCLUSION

In summary, increased local values of Gal-1, reflected through higher fecal concentration, in CRC patients with higher nuclear grade and poor tumor tissue differentiation may be considered as a sign of the tumor’s malignant progression and, consequently, of a poor prognosis for patients. Predominance of Gal-1 over pro-inflammatory cytokines TNF-α, IL-1β and IFN-γ in patients with advanced and progressive disease may implicate an immunomodulatory role of Gal-1 in limiting ongoing proinflammatory processes and preventing potent antitumor immune response. Furthermore, the fecal values of Gal-1 can be used as a valuable marker for CRC severity. These observations point on possible role of fecal Gal-1 as state marker of CRC and its potential use as therapeutic target.

Declaration of interest
The authors declare that they have no competing interests.

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REFERENCES


34. He, J. and Baum, L.G. Presentation of galectin-1 by extracellular matrix triggers T cell death. J. Biol. Chem. 2004; 279: 4705-4712


**Table 1. Baseline characteristics of patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>34/24</td>
</tr>
<tr>
<td>Age (mean [range])</td>
<td>66 [50–82] years</td>
</tr>
<tr>
<td>Site (P/D/R)</td>
<td>14/34/10</td>
</tr>
<tr>
<td>Nuclear grade (I/II/III)</td>
<td>7/37/14</td>
</tr>
<tr>
<td>Stage (TNM: I/II/III/IV)</td>
<td>32/0/14/12</td>
</tr>
<tr>
<td>Necrosis (well/moderate/absent)</td>
<td>15/43/0</td>
</tr>
</tbody>
</table>

Note: P: proximal colon; D: distal colon; R: rectum.
Figure 1. Serum and fecal values of Gal-1 and mediators of inflammation and their ratio in patients with CRC, based on histo-pathological characteristics of tumor.

A. Increased concentration of Gal-1 and Gal-1/IFN-γ ratio in feces and Gal-1/TNF-α ratio in serum, in patients with higher nuclear grade of CRC. Patients with CRC were divided in three groups, based on nuclear grade (I, II and III). Serum and fecal levels of all mentioned biomarkers were determined by ELISA. Gal-1/IFN-γ and Gal-1/TNF-α ratios were evaluated for each patient, separately.

B. Increased concentration of Gal-1 in feces and Gal-1/TNF-α ratio in serum of patients with poor histological differentiation of CRC. Patients with CRC were divided in two groups, according to histological differentiation rate (well and moderate + poor). Statistical significance was tested by Mann–Whitney Rank Sum test or independent samples t-test, where appropriate.

Figure 2. Concentrations of Gal-1 and Gal-1/TNF-α ratio in serum and feces of patients with CRC, based on clinico-pathological characteristics of tumor.

A. Increased concentration of Gal-1/TNF-α ratio in serum and feces in patients with higher TNM stage of CRC. Patients with CRC were divided in two groups, based on TNM stage (I+II and III+IV). Serum and fecal levels of all mentioned biomarkers were determined by ELISA. Gal-1/TNF-α ratio was evaluated for each patient, separately.

B. Increased Gal-1/TNF-α ratio in serum of patients with detectable lymphatic and blood vessel invasion of CRC. Patients with CRC were divided in two groups, based on the presence of lymphatic/blood vessel invasion (+ and -). Serum levels of all mentioned biomarkers were determined by ELISA. Gal-1/TNF-α ratio was evaluated for each patient, separately. Statistical significance was tested by Mann–Whitney Rank Sum test or independent samples t-test, where appropriate.
Figure 3. Sistemic values of Gal-1/TNF-α, Gal-1/IL-1 and Gal-1/IFN-γ ratios in patients with CRC, based on tumor progression.

A. *Increased Gal-1/TNF-α and Gal-1/IFN-γ ratios in patients with detectable liver metastasis.* Patients with CRC were divided in two groups, based on presence of liver metastasis (+ and -).

B. *Increased Gal-1/TNF-α, Gal-1/IL-1 and Gal-1/IFN-γ ratios in patients with detectable lung metastasis.* Patients with CRC were divided in two groups, based on presence of lung metastasis (+ and -).

C. *Increased Gal-1/TNF-α and Gal-1/IFN-γ ratios in patients with detectable peritoneal carcinomatosis.* Patients with CRC were divided in two groups, based on presence of carcinomatosis in peritoneum (+ and -).

Serum levels of all mentioned biomarkers were determined by ELISA. Gal-1/TNF-α, Gal-1/IL-1 and Gal-1/IFN-γ ratios were evaluated for each patient, separately. Statistical significance was tested by Mann–Whitney Rank Sum test or independent samples t-test, where appropriate.

Figure 4. Fecal concentration of Gal-1 was positively associated with poorly differentiated tumor and systemic values of tumor markers AFP, CEA and CA 19-9, in patients with CRC. Relationships between values of Gal-1 in feces and histological differentiation stage of tumor tissue and concentrations of AFP, CEA and CA 19-9 in serum were examined by Spearman correlation test. ROC curve illustrates the specificity and sensitivity of fecal Gal-1 in attempt to differentiate histological differentiation stage of tumor tissue: well/moderate vs. poor differentiated.