ORIGINAL ARTICLE

UDC: 616.348/.351-006-07
https://doi.org/10.2298/VSP171201007J

Fecal galectin-1 as a potential marker for colorectal cancer and disease severity

Fecesni galektin-1 – potencijalni marker kolorektalnog karcinoma i težine bolesti

Milan Jovanović*†, Nevena Gajović‡, Nataša Zdravković§, Marina Jovanović§, Milena Jurišević‖, Danilo Vojvodić†¶, Darko Mirković*†, Boško Milev*†, Veljko Marić, Nebojša Arsenijević‡**

Military Medical Academy, *Clinic for General Surgery, ¶Institute for Medical Research, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Kragujevac, Faculty of Medical Sciences, ‡Center for Molecular Medicine and Stem Cell Research, §Department of Internal Medicine, ‖Department of Pharmacy, Kragujevac, Serbia; University of East Sarajevo, Faculty of Medicine, **Department of Surgery, Foča, Bosnia and Herzegovina

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 the 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: tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interferon gamma (IFN-γ), in the patients with CRC and the relationship with clinicopathological aspects of the disease. **Methods.** The blood samples and feces liquid fraction of 58 patients with CRC were analyzed. The serum and fecal levels of TNF-α, IL-1β and IFN-γ and gal-1 were measured using sensitive enzyme-linked immunosorbent assay (ELISA) kits. **Results.** The fecal level of gal-1 was increased in the CRC patients with higher nuclear grade and poor tumor tissue differentiation. The gal-1/TNF-α ratio in the serum and feces had a higher trend in the patients with the advanced tumor-node-metastasis (TNM) stage as well as the detectable lymphatic and blood vessel invasion. The gal-1/TNF-α and gal-1/IFN-γ ratios were increased in the serum of patients with presence of lung/liver metastasis or peritoneal carcinomatosis, while the enhanced gal-1/IL-1 ratio was detected only in the serum of patients with lung metastasis. A positive correlation between the gal-1 value in feces and histological differentiation of tumor and biomarkers alpha-fetoprotein (AFP) and cancer antigen-19-9 (CA 19-9), respectively, was also observed. The fecal values of gal-1 higher than 13,708.29 pg/g presented a highly sensitive and specific marker for histological differentiation of tumor tissue. **Conclusion.** We believe that the predomination of gal-1 over pro-inflammatory cytokines TNF-α, IL-1β and IFN-γ in the patients with advanced and progressive CRC may implicate on an immunomodulatory role of gal-1 in the limiting ongoing proinflammatory processes. The fecal values of gal-1 can be used as a valuable marker for the severity of CRC.

Key words:
colorectal neoplasms; carcinoma; feces; galectin-1; disease progression.

Apstrakt

Uvod/Cilj. Kolorektalni karcinom (*colorecral carcinoma* – CRC) je jedan od najučestalijih karcinoma na svetu. CRC se često dijagnostikuje u uznapredovalim 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: faktoru nekroze tumora alfa (TNF-α), interleukina-1 beta (IL-1β) i interferona-gama (IFN-γ) kod bolesnika sa CRC i odnosa sa kliničko-patološkim aspektima bolesti. **Metode.** Analizirani su uzorci krvi i tečne frakcije fecesa 58 bolesnika sa CRC. Serumski i fekalni nivoi TNF-α, IL-1β, IFN-γ i gal-1 su mereni korišćenjem senzitivnog *enzyme-linked immunosorbent assay* (ELISA) testa. **Rezultati.** Fekalni nivo gal-1 povećan je kod bolesnika sa CRC sa velikim nuklearnim gradusom i slabo diferentovanim tumorskim tkivom. Odnos gal-1/TNF-α u serumu i fecesu značajno je veći kod bolesnika sa uznapredovalim tumor-nodus-metastaza (TNM) stadijumom, kao i detektabilnom invazijom limfnih i krvnih sudova. Odnosi gal-1/TNF-α i gal-1/IFN-γ su povećani u serumima bole-

Correspondence to: Nevena Gajović, University of Kragujevac, Faculty of Medical Sciences, Center for Molecular Medicine and Stem Cell Research, Svetozara Markovica 69, 34 000 Kragujevac, Serbia. E-mail: gajovicnevena@yahoo.com

snika sa metastazama u plućima/jetri ili peritonealnom karcinomatozom, dok je povećan odnos gal-1/IL-1 detektovan samo u serumu bolesnika sa metastazama u plućima. Takođe, primećena je pozitivna korelacija između vrednosti gal-1 u fecesu i histološkog tipa tumora i biomarkera alfa-fetoproteina (AFP) i *cancer antigen* 19-9 (CA 19-9). Vrednosti gal-1 u fecesu veće od 13,708.29 pg/g predstavljaju visoko osetljiv i specifičan marker za histološku diferencijaciju tumorskog tkiva. **Zaključak.** Naši rezultati ukazuju na to da predominacija gal-1 nad proinflamacijskim citokinima, TNF-α, IL-1β, IFN-γ, kod bolesnika sa uznapredovalom 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:
kolorektalne neoplazme; karcinom; stolica; galektin-1; bolest, progresija.

Introduction

Colorectal cancer (CRC) is one of the most common cancers and the fourth cause of cancer-related deaths [1]. Despite the constant achievements in the understanding of cancer biology, the morbidity and mortality rates of CRC continue to increase [1]. In most cases, the CRC is diagnosed at the 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 [2–4]. 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 [4]. Novel studies point to the significance of fecal markers measurement in the detection and prediction of disease severity [5–7].

A large body of evidence indicates that galectins participate in a variety of normal cellular functions, and are disregulated in CRC [8–11]. Among all known galectins, galectin-1 (gal-1) is well characterized. gal-1 is a multifunctional β-galactoside-binding lectin produced by a variety of vascular, interstitial, epithelial, immune cells as well as neoplastic cells [12, 13]. It can be located either inside the cells in nucleus and cytosol, or in the extracellular space [12, 13]. 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 [14, 15]. An elevated expression of gal-1 was observed in tissues of various solid malignant tumors, whereas low, or no expression was found in the normal tissues [8–11]. The immunomodulatory role of gal-1 is also known, and its strong influence on inflammation is well-established [16].

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

Methods

Ethical approvals

The study was conducted at the Clinical Center in Kragujevac, Serbia, and the Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia, after the study protocol had been approved by relevant Ethics Committees of the Clinical Center in 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. A diagnosis was based on the endoscopic and histological criteria. The exclusion criteria included no well-defined pathology, no adequate clinical documents available and previous treatment with radiation and chemotherapy. The 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 a clinical stage by TNM were recorded and analyzed in the study. The blood and stool samples were taken before the surgery and stored at -80°C until enzyme-linked immunosorbent assay (ELISA**).**

Feces liquid fraction preparation

The stool samples (1–10 g) were collected in the morning in the 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 [17, 18]. The supernatant fluid was collected and stored at -80°C until ELISA.

Evaluation of tumor markers in serum

The serum levels of alpha-fetoprotein (AFP), carcinoembryonic 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 in Kragujevac.

Determination of galectin-1, TNF-α, IL-1β and IFN-γ in serum and feces

The serum and fecal concentrations of gal-1 and cytokines were measured, as described [19], by using the sensitive ELISA kits (R&D Systems, Minneapolis, MN, for gal-1, enzyme-linked immunosorbent assay tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interferon-gamma (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. The serum/fecal samples, or the 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 4 mol/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. The values of measured cytokines were presented as pg/mL of serum and pg/g of feces, respectively.

Statistical analysis

The statistical analyses were performed by using the SPSS 20.0 software. The results were reported as the mean and standard error (SE). A statistically significant difference between the means of two groups was determined using the Student's *t*-test for the independent samples if the data had normal distribution, or Mann-Whitney *U*-test for the data without normal distribution. The Pearson's or Spearman's correlation, where appropriate, evaluated the possible relationship between the cytokines and disease severity and progression in the patients with CRC. The numerical values were assigned to different histological differentiation stages (well = 1; moderate + poor = 2). A 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 a statistically significant.

Results

Fifty-eight patients with CRC were enrolled in the study. There was no significant difference in the gender distribution (34 males and 24 females). The patients were similar in age (mean age 66 ± 1 years). The clinical and pathological characteristics of these patients are presented in Table 1.

Table 1

Baseline characteristics of patients

Characteristics	Values
Gender (male/female), n	34/24
Age (years), mean (range)	66 (50–82)
Site (P/D/R), n	14/34/10
Nuclear grade (I/II/III), n	7/37/14
Stage (TNM: I/II/III/IV), n	32/0/14/12
Necrosis (well/moderate/absent), n	15/43/0

P – proximal colon; D – distal colon; R – rectum; TNM – tumor-node-metastasis; n – number.

The serum and fecal concentration of gal-1 and a ratio between gal-1 and pro-inflammatory mediators, with regard to histopathologic characteristics of CRC

The patients with CRC were categorized into 3 groups according to the nuclear grade of tumor tissue: I, II and III, and analyzed for the systemic and fecal values of ratio between the gal-1 and pro-inflammatory mediators (TNF-α, IL-1β and IFN-γ). As shown in Figure 1A, the CRC patients with a higher nuclear grade appeared to have the higher fecal level of gal-1 (III *vs* II: 21,936.14 ± 3,601.19 *vs* 13,286.97 ± 782,97 pg/mL; $p = 0.020$; III *vs* I: 21,936.14 ± 3,601.19 *vs* 1,5724.30 ± 1,903.49 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 the 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 the CRC patients into two groups, according to the histological differentiation rate: well and moderate + poor. In the patients with the poor tumor tissue differentiation, we detected the increased fecal gal-1 (moderate and poor *vs* well: 19,353.69 ± 2,224.35 *vs* 12,757.56 ± 1,207.58 pg/mL; $p = 0.026$) and the 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).

The serum and fecal gal-1/TNF-α ratios are associated with the TNM system and lymph and blood vessels invasion

The patients with CRC were divided into 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. The patients with the TNM stages III+IV revealed a significantly higher gal-1/TNF-α ratio in the 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).

The patients with CRC were divided into two groups, based on the presence of lymphatic/blood vessel invasion, respectively (+ and -). The increased gal-1/TNF-α ratio in the serum was detected in the 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).

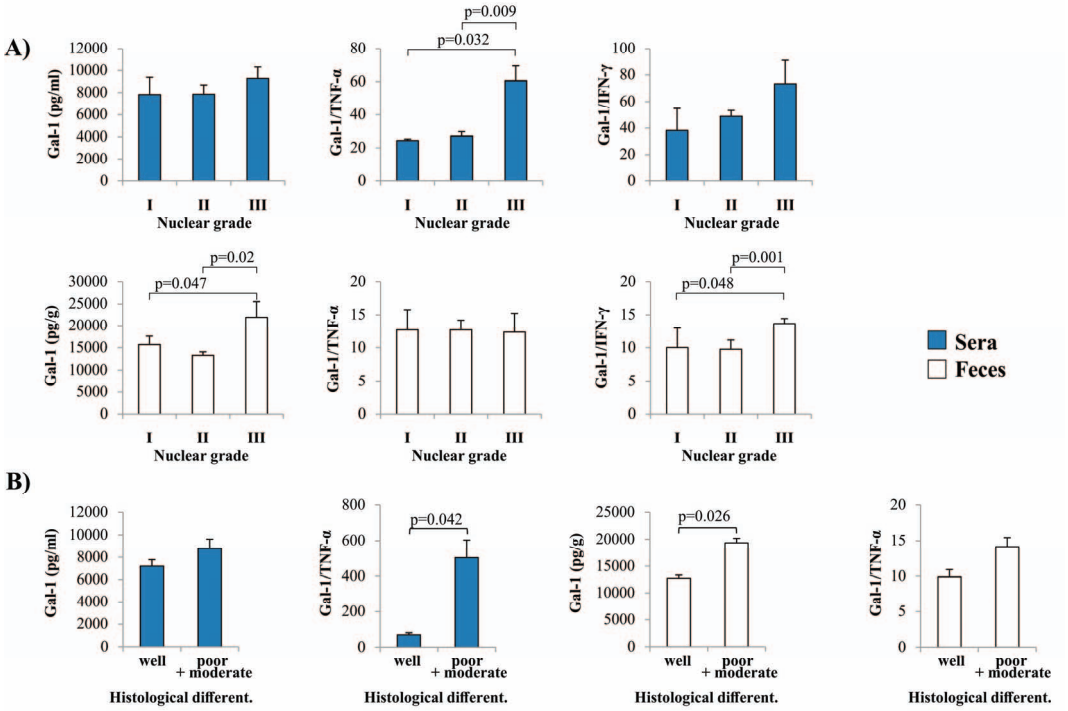

Fig. 1 – The serum and fecal values of galectin-1 (gal-1) and mediators of inflammation and their ratio in patients with colorectal cancer (CRC), based on histopathological characteristics of tumor.
A) The increased concentration of gal-1 and the gal-1/IFN-γ ratio in feces and gal-1/tumor necrosis factor (TNF)-α ratio in the serum of patients with higher nuclear grade of CRC. The patients with CRC were divided into three groups, based on a nuclear grade (I, II and III). The serum and fecal levels of all mentioned biomarkers were determined by enzyme linked immunosorbent assay (ELISA). The gal-1/interferon-gamma (IFN-γ) and the gal-1/tumor necrosis factor alpha (TNF-α) ratios were evaluated for each patient, separately.
B) The increased concentration of gal-1 in feces and the gal-1/TNF-α ratio in the serum of patients with poor histological differentiation of CRC. The patients with CRC were divided into two groups, according to a histological differentiation rate (well and moderate + poor). A statistical significance was tested by the Mann–Whitney Rank Sum test, or the independent samples *t*-test, where appropriate.

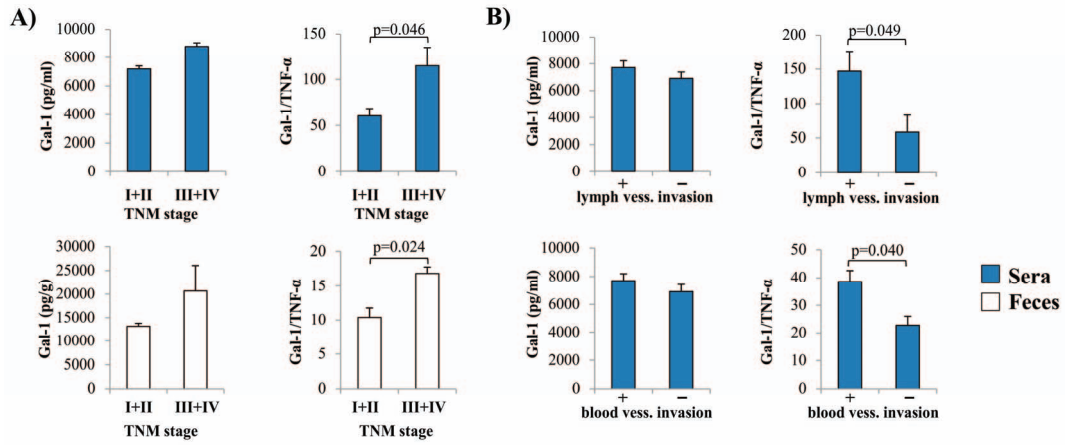

Fig. 2 – The concentrations of galectin-1 (gal-1) and gal-1/tumor necrosis factor (TNF) ratio in the serum and feces of patients with colorectal cancer (CRC), based on clinicapathological characteristics of tumor.
A) The increased concentration of gal-1/TNF-α ratio in the serum and feces in the patients with a higher tumor-node-metastasis (TNM) stage of CRC. The patients with CRC were divided into two groups, based on a TNM stage (I+II and III+IV). The serum and fecal levels of all mentioned biomarkers were determined by enzyme linked immunosorbent assay (ELISA). The gal-1/TNF-α ratio was evaluated for each patient, separately.
B) The increased gal-1/TNF-α ratio in the serum of patients with detectable lymphatic and blood vessel invasion of CRC. The patients with CRC were divided into two groups, based on the presence of lymphatic/blood vessel invasion (+ and -). The serum levels of all mentioned biomarkers were determined by ELISA. The gal-1/TNF-α ratio was evaluated for each patient, separately. A statistical significance was tested by the Mann–Whitney Rank Sum test, other independent samples *t*-test, where appropriate.

Liver, lung and peritoneal metastasis are associated with a higher gal-1/TNF-α ratio

Further, we divided patients into two categories based on presence of lung/liver metastasis, or peritoneal carcinomatosis, respectively. A higher gal-1/TNF-α ratio was found in the 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 the patients without metastasis/carcinomatosis (Figure 3). In addition, we also found a higher gal-1/IFN-γ ratio in the 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. An increased gal-1/IL-1 ratio was detected in the serum of patients with detectable lung metastasis (1,001.91 ± 82.09 vs. 791.65 ± 31.63; $p = 0.027$; Figure 3).

The Spearman's correlation analysis of gal-1 concentration in stool uncovered a positive correlation between the 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 the AFP levels (r = 0.317; $p = 0.028$), the CA 19-9 levels (r = 0.296; $p = 0.049$), but there was no significant correlation found with the CEA levels (Figure 4). The serum gal-1 did not correlate with same parameters and markers of colon cancer (data not shown). The analysis also showed that the 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 13,708.29 pg/g. For this cut-off, we determined sensitivity to be 73.6% and specificity 60.0%.

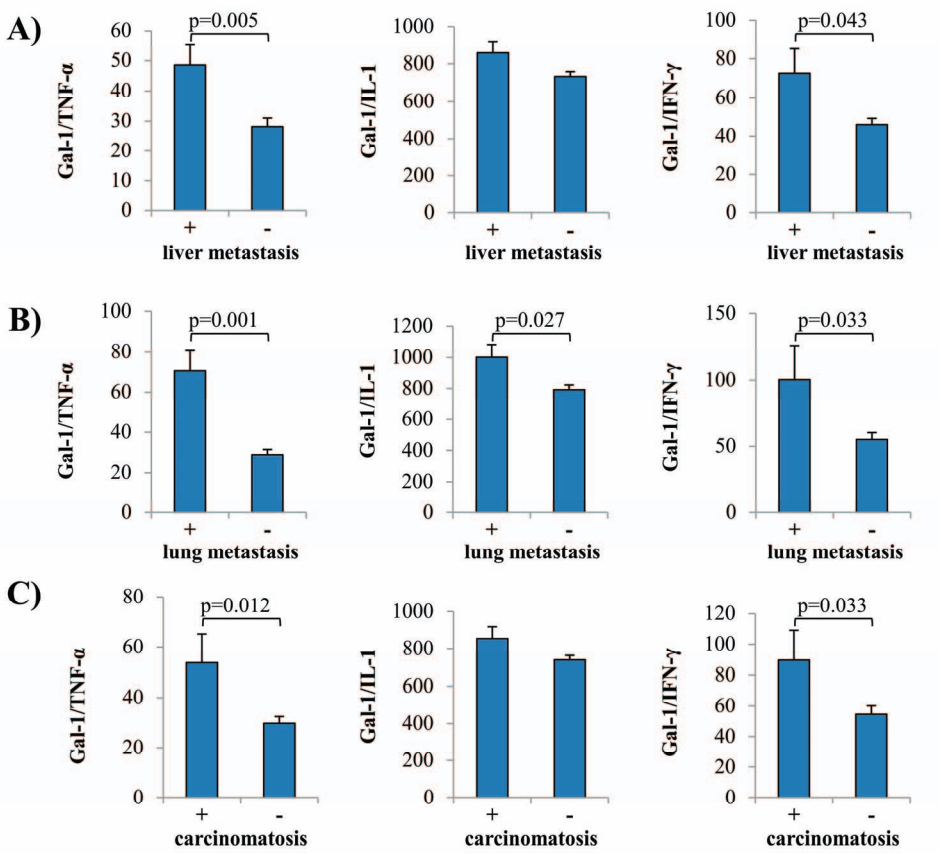

Fig. 3 – The systemic values of galectin-1 (gal-1)/tumor necrosis factor (TNF) α, gal-1/interleukin-1 (IL-1) and gal-1/interferon-gamma (IFN-γ) ratios in the patients with colorectal cancer (CRC), based on tumor progression.
A) The increased gal-1/TNF-α and gal-1/IFN-γ ratios in the patients with detectable liver metastasis. The patients with CRC were divided into two groups, based on the presence of liver metastasis (+ and -).
B) The increased Gal-1/TNF-α, Gal-1/IL-1 and Gal-1/IFN-γ ratios in the patients with detectable lung metastasis. The patients with CRC were divided into two groups, based on the presence of lung metastasis (+ and -).
C) The increased gal-1/TNF-α and gal-1/IFN-γ ratios in the patients with detectable peritoneal carcinomatosis. The patients with CRC were divided into two groups, based on the presence of carcinomatosis in peritoneum (+ and -).
The serum levels of all mentioned biomarkers were determined by enzyme linked immunosorbent assay (ELISA). The gal-1/TNF-α, gal-1/IL-1 and gal-1/IFN-γ ratios were evaluated for each patient, separately. A statistical significance was tested by the Mann–Whitney Rank Sum test, or the independent samples *t*-test, where appropriate.

Variables	Fecal Gal-1	
	Spearman`s rho	*p* value
Histological type	0,357	0.025
AFP	0,317	0.028
CEA	0,230	0.115
CA 19-9	0,296	0.049

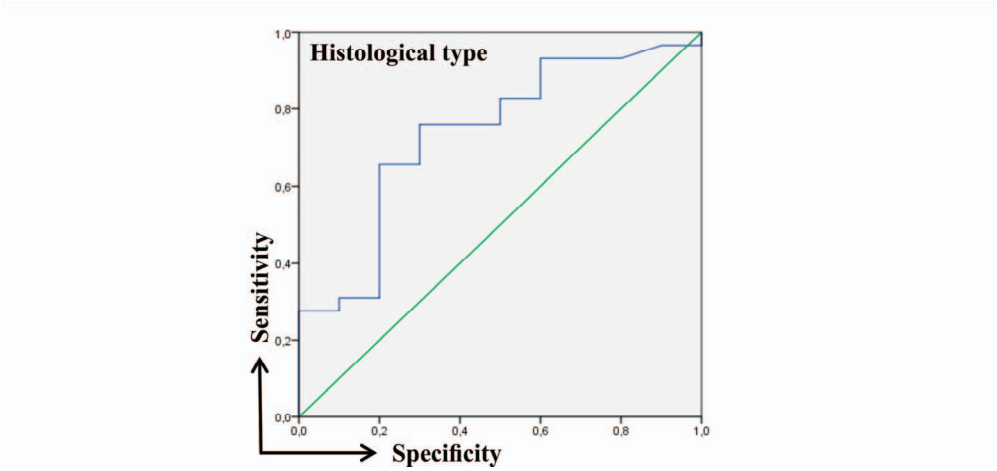

Fig. 4 – Fecal concentration of galectin-1 (gal-1) was positively associated with the poorly differentiated tumor and systemic values of tumor markers alpha-fetoprotein (AFP), carcino-embryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9), in the patients with colorectal cancer (CRC). The relationships between the values of gal-1 in feces and a histological differentiation stage of tumor tissue and concentrations of AFP, CEA and CA 19-9 in the serum were examined by the Spearman's correlation test. The receiver operations characteristic (ROC) curve illustrates the specificity and sensitivity of fecal gal-1 in an attempt to differentiate a histological differentiation stage of tumor tissue: well/moderate vs. poor differentiated.

Discussion

Biological role of gal-1 in the tumor cell proliferation, invasion, apoptosis, metastasis, immuno-suppression and tumor angiogenesis is well-known [20–27]. It is involved in a poor prognosis and the metastatic phenotype [23, 24]. Gal-1 may act intracellularly as well as extracellularly, after secretion [28]. Secreted gal-1 can interact with the cell-surface proteins such as fibronectin, integrins, laminin and vascular endothelial growth factor receptor 2 (VEGFR2) and subsequently determines the proliferation, adhesion, migration and angiogenesis [29, 30]. These findings highlight the importance of extracellular gal-1 in tumor biology. In the present study, we analyzed the systemic and fecal level of gal-1 and it`s ratio with several pro-inflammatory cytokines, in different stages of CRC. We found the increased concentration of gal-1 in the stool of CRC patients with a 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 the serum of patients with tumors [8–10]. Also, the serum gal-1 values were significantly increased in the patients with metastatic disease compared with the patients with localized tumors [11]. We did not find that the serum gal-1 mean values ranged significantly differently histopathological characteristics of tumor, while the fecal gal-1 showed a significant alteration according to the histopathological characteristics (Figure 1).

Indeed, in the recent studies, feces was used as a sample for testing different biomarkers [5, 6]. For instance, fecal calprotectin (FC), a biomarker of intestinal inflammation that has been in clinical use for years [5–7], also proved to be elevated in CRC and suggested for screening the high risk groups for CRC [31]. Today, the researchers test diagnostic accuracy of different fecal markers in the detection of cancerous lesions of the colorectum in order to find the most accurate one for CRC screening. According to the available literature, this is the first study testing fecal gal-1 for the detection of severe and progressive forms of CRC.

It was suggested that ratio of counterregulatory cytokines was a reliable marker of the disease progression [32]. Therefore, we considered the ratios of gal-1 and pro-inflammatory cytokines and showed the predomination of gal-1 over pro-inflammatory cytokines TNF-α, IL-1β and IFN-γ in the patients with CRC with progressive disease. The gal-1/TNF-α ratio in the serum and feces had a higher trend in the patients with the advanced TNM stage (III+IV) as well as detectable lymphatic and blood vessel invasion (Figure 2). In line with this finding, the enhanced gal-1/TNF-α and gal-1/IFN-γ ratios were detected in the serum of patients with presence of lung/liver metastasis or peritoneal carcinomatosis, while the enhanced gal-1/IL-1 ratio was detected only in the serum of patients with lung metastasis (Figure 3). Based on these findings, we believe that the gal-1/TNF-α ratio could be a predictor of the advanced stages of colorectal cancer.

The role of gal-1 in the onset, progression and resolution of inflammation is well-established [16]. Previous studies revealed that gal-1 inhibit cell growth and induce the apoptosis of activated immune cells [33, 34]. Gal-1 was shown to

skew the balance toward the type-2 immune response, simultaneously inhibiting IFNγ, TNFα, IL-2 and IL-12 production and facilitating IL-5 secretion, *in vitro* and *in vivo* [35–37]. Some studies suggest that gal-1 might inhibit T-cell effector functions, or induce the death of tumor infiltrating leukocytes and subsequently suppress a strong immune response derived by proinflammatory cytokines [14, 36, 38, 39]. We are first to describe prevailing of gal-1 over TNF-α, IL-1β and IFN-γ in the stool of patients with the severe and progressive forms of CRC (Figure 2 and 3). In line with our finding, Camby et al. [14] concluded that the tumor cells may impair the 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 gal-1 as a biomarker in preceding disease severity. We found a positive correlation between the gal-1 value in feces and histological differentiation tumor of stage and biomarkers AFP and CA 19-9, respectively (Figure 4). Interestingly, we did not find a correlation of serum gal-1 with the same parameters and markers of disease severity. Also, the values of gal-1 in feces are about two to three times higher than in the serum, what makes measurement in feces a more sensitive method. The analysis of receiver operating characteristic (ROC) curves of gal-1 and the disease parameters and markers for CRC revealed that gal-1 could predict a poor differentiated type of tumor, at good sensitivity and specificity. According to our findings, fecal gal-1 could be a valuable marker for the CRC severity.

Conclusion

In summary, the increased local values of gal-1, reflected through a higher fecal concentration, in the CRC patients with a 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 the patients. The predominance of gal-1 over proinflammatory cytokines TNF-α, IL-1β and IFN-γ in the patients with advanced and progressive disease may implicate immunomodulatory role of gal-1 in limiting the ongoing proinflammatory processes and preventing a potent antitumor immune response. Furthermore, the fecal values of gal-1 can be used as a valuable marker for the CRC severity. These observations point to a possible role of fecal gal-1 as a state marker of CRC and its potential use as a therapeutic target.

Declaration of interest

The authors declare that they have no competing interests.

Acknowledgements

This work was supported by grants from the Serbian Ministry of Education, Science and Technological Development (175071, 175069 and 175103), and from the Faculty of Medical Sciences, Kragujevac, Serbia (project JP 04/15). The authors thank Milan Milojević and Aleksandar Ilić for excellent technical assistance.

R E F E R E N C E S

1. Global Burden of Disease Cancer Collaboration. Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, et al. The Global Burden of Cancer 2013. JAMA Oncol 2015; 1(4): 505–27.
2. *Zdravkovic N, Pavlovic M, Radosavljevic G, Jovanovic M, Arsenijevic A, Zdravkovic N*, et al. Serum levels of immunosuppressive cytokines and tumor markers in metastatic colorectal carcinoma. JBUON 2017; 22(5): 1–8.
3. *Zdravkovic ND, Jovanovic IP, Radosavljevic GD, Arsenijevic AN, Zdravkovic ND, Mitrovic SLJ,* et al. Potential Dual Immunomodulatory Role of VEGF in Ulcerative Colitis and Colorectal Carcinoma. Int J Med Sci 2014; 11(9): 936–47
4. *Wu KL, Chen HH, Pen CT*, *Yeh WL, Huang EY, Hsiao CC*, et al. Circulating Galectin-1 and 90K/Mac-2BP Correlated with the Tumor Stages of Patients with Colorectal Cancer. Biomed Res Int 2015; 2015: 306964.
5. *Wagner M, Peterson CG, Ridefelt P, Sangfelt P, Carlson M.* Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. World J Gastroenterol 2008; 14(36): 5584–9; discussion 5588.
6. *Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I.* Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease Gastroenterology 2000; 119(1): 15–22.
7. *Tibble J, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S,* et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000; 47(4): 506–13.
8. *Kim HJ, Jeon HK, Cho YJ, Park YA, Choi JJ, Do IG*, et al. High galectin-1 expression correlates with poor prognosis and is involved in epithelial ovarian cancer proliferation and invasion. Eur J Cancer. 2012; 48(12): 1914–21.
9. *Zhang P, Zhang P, Shi B, Zhou M, Jiang H, Zhang H*, et al. Galectin-1 overexpression promotes progression and chemoresistance to cisplatin in epithelial ovarian cancer. Cell Death Dis 2014; 5: e991.
10. *Ouyang J, Plütschow A, Pogge von Strandmann E, Reiners KS, Ponader S, Rabinovich GA*, et al. Galectin-1 serum levels reflect tumor burden and adverse clinical features in classical Hodgkin lymphoma. Blood 2013; 121(17): 3431–3
11. *Chen L, Yao Y, Sun L, Zhou J, Liu J, Wang J*, et al. Clinical implication of the serum galectin-1 expression in epithelial ovarian cancer patients. J Ovarian Res 2015; 8: 78.
12. *Al-Salam S, Hashmi S.* Galectin-1 in early acute myocardial infarction. PLoS One 2014; 9(1): e86994.
13. *Nakahara S, Raz A.* Biological modulation by lectins and their ligands in tumor progression and metastasis. Anticancer Agents Med Chem 2008; 8(1): 22–36.
14. *Camby I, Le Mercier M, Lefranc F, Kiss R.* Galectin-1: a small protein with major functions. Glycobiology 2006; 16(11): 137R–57R.
15. *Spano D, Russo R, Di Maso V, Rosso N, Terracciano LM, Roncalli M*, et al. Galectin-1 and its involvement in hepatocellular carcinoma aggressiveness. Mol Med. 2010; 16(3–4): 102–15.
16. *Almkvist J, Karlsson A.* Galectins as inflammatory mediators. Glycoconj J 2004; 19(7–9): 575–81.
17. *Heilmann RM, Cranford SM, Ambrus A, Grützner N, Schellenberg S, Ruaux CG*, et al. Validation of an enzyme-linked immunosorbent assay (ELISA) for the measurement of canine S100A12. Vet Clin Pathol 2016; 45(1): 135–47.

18. *Prakash N, Stumbles P, Mansfield C.* Initial Validation of Cytokine Measurement by ELISA in Canine Feces. Open J Vet Med 2013; 3: 282–8.
19. *Jovanovic M, Zdravkovic N, Jovanovic I, Radosavljevic G, Gajovic N, Zdravkovic N*, et al. TGF-β as a marker of ulcerative colitis and disease severity. Ser J Exp Clin Res 2017; 1–1. DOI: 10.1515/SJECR-2017-0019.
20. *Wu MH, Hong TM, Cheng HW, Pan SH, Liang YR, Hong HC*, et al. Galectin-1-mediated tumor invasion and metastasis, upregulated matrix metalloproteinase expression, and reorganized actin cytoskeletons. Mol Cancer Res 2009; 7(3): 311–8.
21. *Thijssen VL, Postel R, Brandwijk RJ, Dings RP, Nesmelova I, Satijn S*, et al. Galectin-1 is essential in tumor angiogenesis and is a target for antiangiogenesis therapy. Proc Natl Acad Sci U S A 2006; 103(43): 15975–80.
22. *Tang D, Gao J, Wang S, Yuan Z, Ye N, Chong Y*, et al. Apoptosis and anergy of T cell induced by pancreatic stellate cells-derived galectin-1 in pancreatic cancer. Tumour Biol 2015; 36(7): 5617–26.
23. *Grosset AA, Labrie M, Vladoiu MC, Yousef EM, Gaboury L, St-Pierre Y.* Galectin signatures contribute to the heterogeneity of breast cancer and provide new prognostic information and therapeutic targets. Oncotarget 2016; 7(14): 18183–203.
24. *Astorgues-Xerri L, Riveiro ME, Tijeras-Raballand A, Serova M, Rabinovich GA, Bieche I,* et al. OTX008, a selective small-molecule inhibitor of galectin-1, downregulates cancer cell proliferation, invasion and tumour angiogenesis. Eur J Cancer 2014; 50(14): 2463–77.
25. *Jung EJ, Moon HG, Cho BI, Jeong CY, Joo YT, Lee YJ*, et al. Galectin-1 expression in cancer-associated stromal cells correlates tumor invasiveness and tumor progression in breast cancer. Int J Cancer 2007; 120(11): 2331–8.
26. *Croci DO, Salatino M, Rubinstein N, Cerliani JP, Cavallin LE, Leung HJ*, et al. Disrupting galectin-1 interactions with N-glycans suppresses hypoxia-driven angiogenesis and tumorigenesis in Kaposi's sarcoma. J Exp Med 2012; 209(11): 1985–2000.
27. *Brandt B, Abou-Eladab EF, Tiedge M and Walzel H.* Role of the JNK/c-Jun/AP-1 signaling pathway in galectin-1-induced T-cell death. Cell Death Dis 2010; 1: e23
28. *Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T*, et al. Galectins: a family of animal beta-galactoside-binding lectins. Cell 1994; 76(4): 597–8.
29. *D'Haene N, Sauvage S, Maris C, Adanja I, Le Mercier M, Decaestecker C*, et al. VEGFR1 and VEGFR2 involvement in extracellular galectin-1- and galectin-3-induced angiogenesis. PLoS One 2013; 8(6): e67029.
30. *Suzuki O, Abe M.* Galectin-1-mediated cell adhesion, invasion and cell death in human anaplastic large cell lymphoma: regulatory roles of cell surface glycans. Int J Oncol 2014; 44(5): 1433–42.
31. *Johne B, Kronborg O, Tøn HI, Kristinsson J, Fuglerud P.* A new fecal calprotectin test for colorectal neoplasia. Clinical results and comparison with previous method. Scand J Gastroenterol 2001; 36(3): 291–6.
32. *Borovcanin M, Jovanovic I, Radosavljevic G, Djukic Dejanovic S, Stefanovic V, Arsenijevic N,* et al. Antipsychotics can modulate the cytokine profile in schizophrenia: attenuation of the type-2 inflammatory response. Schizophr Res 2013; 147(1): 103–9.
33. *Blaser C, Kaufmann M, Muller C, Zimmermann C, Wells V, Mallucci L*, et al. Beta-galactoside-binding protein secreted by activated T cells inhibits antigen-induced prolif eration of T cells. Eur J Immunol 1998; 28(8): 2311–9.
34. *He J, Baum LG.* Presentation of galectin-1 by extracellular matrix triggers T cell death. J Biol Chem 2004; 279(6): 4705–12.
35. *Allione A, Wells V, Forni G, Mallucci L, Novelli F.* Betagalactoside-binding protein (beta GBP) alters the cell cycle, upregulates expression of the alpha- and beta-chains of the IFN-gamma receptor, and triggers IFN-gamma-mediated apoptosis of activated human T lymphocytes. J Immunol 1998; 161(5): 2114–9.
36. *Baum L.G, Blackall DP, Arias-Magallano S, Nanigian D, Uh SY, Browne JM*, et al. Amelioration of graft versus host disease by galectin-1. Clin Immunol 2003; 109(3): 295–307.
37. *Santucci L, Fiorucci S Rubinstein N, Mencarell, A, Palazzett, B, Federici B*, et al. Galectin-1 suppresses experimental colitis in mice. Gastroenterology 2003; 124(5): 1381–94.
38. *van der Leij J, van den Berg A, Blokzijl T, Harms G, van Goor H, Zwiers P,* et al. Dimeric galectin-1 induces IL-10 production in T-lymphocytes: an important tool in the regulation of the immune response. J Pathol 2004; 204(5): 511–8.
39. *van den Brule, FA, Waltregny, D, and Castronovo, V.* Increased expression of galectin-1 in carcinoma-associated stroma predicts poor outcome in prostate carcinoma patients. J Pathol 2001; 193(1): 80–7.

Received on December 11, 2017.
Revised on January 10, 2018.
Accepted on January 11, 2018.
Online First January, 2018.