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Janko Žujović<sup>1,\*</sup>, Milena Vuletić<sup>2</sup>, Miroslav Stojanović<sup>3</sup>, Ranko Lazović<sup>4</sup>,  
Nebojša Đorđević<sup>3</sup>, Miodrag Radunović<sup>4</sup>, Snežana Jančić<sup>2</sup>, Velimir Milošević<sup>5</sup>

**Clinical Significance of Proliferation Index and E-Cadherin Expression in  
Colorectal Adenocarcinoma**

Клинички значај индекса пролиферације и експресије Е-кадхерина у  
колоректалном аденокарциному

<sup>1</sup>Clinical Centre of Montenegro, Centre for Abdominal Surgery, Podgorica, Montenegro

<sup>2</sup>University of Kragujevac, Faculty of Medical Sciences, Department of Pathology, Kragujevac, Serbia

<sup>3</sup>University of Niš, Faculty of Medicine, Niš, Serbia

<sup>4</sup>University of Montenegro, Faculty of Medicine, Podgorica, Montenegro

<sup>5</sup>Codra Hospital, Podgorica, Montenegro

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**\*Correspondence to:**

Janko ŽUJOVIĆ

Clinical Centre of Montenegro, Centre for Abdominal Surgery, Podgorica, Montenegro, Ljubljanska bb,

Podgorica 81101, Montenegro

E-mail: [janko.zujovic@kccg.me](mailto:janko.zujovic@kccg.me)

## Clinical Significance of Proliferation Index and E-Cadherin Expression in Colorectal Adenocarcinoma

Клинички значај индекса пролиферације и експресије Е-кадхерина у колоректалном аденокарциному

### SUMMARY

**Introduction/Objective** The aim of this study is to examine the association of E-cadherin expression and high proliferation index (proIDX) with clinical and pathological indicators of colorectal cancer progression.

**Methods:** The biopsy of 72 patients, obtained by resection of colorectal cancer, was routinely processed at the Institute of Pathology of the Clinical Centre of Montenegro, embedded in paraffin and archived. Based on the archived pathohistological reports, two study groups were formed: the first group ( $n = 72$ ) consisted of operative biopsies of colorectal cancer, and the control group ( $n = 72$ ) consisted of biopsies of adjacent non-tumor tissue. Routine hematoxylin-eosin and immunohistochemical ABC method with anti-Ki67 and anti-E-cadherin antibodies was applied on. After quantification of the results for statistical tests, the software package SPSS for Windows (19.0) was used.

**Results:** In colorectal carcinoma, we observed a significant association of proIDX with pT stage, lymph and blood vessel invasion, perineural invasion, lymph node metastases and distant metastases, and Astler-Coller stage tumor disease. We also observed that the absence of E-cadherin was significantly associated with pT stage, lymph and blood vessel invasion, perineural invasion with lymph node metastases, distant metastases, with C2 and D Astler-Coller tumor stage. E-cadherin expression is associated with proIDX with a significantly high, negative correlation coefficient.

**Conclusion:** Our results indicate that it is possible to differentiate patients into groups with a higher or lower risk of developing metastatic disease, based on the expression of Ki67 and E-cadherin.

**Keywords:** colorectal cancer; E-cadherin expression; proliferative index; clinical significance

### САЖЕТАК

**Увод/Циљ** Циљ студије је испитивање повезаности експресије Е-кадхерина и високог индекса пролиферације (проИДХ) са клиничко-патолошким показатељима прогресије колоректалног карцинома.

**Метод:** Биопсијски материјал 72 пацијента, добијен ресекцијом колоректалног карцинома је у Институту за Патологију КЦЦГ рутински обрађиван, калуљен у парафин и архивиран. На основу архивираних патохистолошких извештаја формиране су две студијске групе: прву групу ( $n = 72$ ) су чиниле оперативне биопсије колоректалног карцинома, а контролну групу ( $n = 72$ ) су чиниле биопсије суседног не-туморског ткива. Примењене су рутински хематоксилин-еозин бојење и имунохистохемијска АВС метода са *anti-Ki67* и анти-Е-кадхерин антителима. Након квантификације резултата за статистичка тестирања је коришћен програмски пакет *SPSS* за *Windows* (19.0).

**Резултати:** У ткиву колоректалног карцинома је запажена сигнификантна повезаност проИДХ са *pT* стадијумом, инвазијом лимфних и крвних судова, перинеуралном инвазијом, метастазама у лимфним чворовима и удаљеним метастазама и Астлер-Колер стадијумом туморске болести. Такође је запажено да је одсуство експресије Е-кадхерина значајно повезано са *pT* стадијумом, инвазијом лимфних и крвних судова, перинеуралном инвазијом, са метастазама у лимфним чворовима, удаљеним метастазама, и са *C2* и *D* Астлер-Колер стадијумом тумора. Експресија Е-кадхерина је значајним, високим али негативним коефицијентом корелације повезана са проИДХ.

**Закључак:** Наши резултати указују да је на основу експресије *Ki67* и Е-кадхерина могуће издиференцирати пацијенте у групе већег, односно мањег ризика од појаве метастатске болести.

**Кључне речи:** колоректални карцином; експресија Е-кадхерина; пролиферативни индекс; клинички значај

## INTRODUCTION

Colorectal cancer is a complex disease caused by the interaction of genetic, epigenetic

and environmental factors. Colorectal carcinogenesis is a consequence of the "interplay" between environmental factors on the one side and different oncogenes, suppressor genes and their products on the other, where cell proliferation is one of the most significant biological events in that process [1].

A nuclear antigen is isolated from the proliferating cells, which was used to produce a monoclonal Ki67 antibody of IgG1 class. The antigen detected by the Ki67 antibody is present in the cell nuclei during the G<sub>1</sub>, S, G<sub>2</sub> and M phases on the cell cycle. It cannot be detected in the G<sub>0</sub> phase [2].

Cadherin adhesion molecules also play an important role in colorectal carcinogenesis [3]. Cadherins are a family of glycoproteins that perform calcium (Ca<sub>2+</sub>) dependent intercellular adhesion at intercellular junctions [4]. Cadherins participate in the processes of embryogenesis and they are involved in the malignant transformation of cells, where a significant reduction of their expression occurs. It has been proved that cadherins inhibit invasion and it is hypothesized that they activate the process of malignant cell dissemination [5, 6]. E-cadherin plays a key role in establishing epithelial architecture, in differentiation and in maintaining cell polarity [4]. Numerous studies have shown that E-cadherin is a suppressor of tumor invasion and metastasis [6, 7].

Considering the fact that the loss of E-cadherin molecules results in a disruption of cytoskeletal structure, which allows cell separation from tumor and increased cell migration [6], this study aims is to examine the correlation between E-cadherin expression and proliferation index (proIDX) on the one side, and clinical and pathological indicators of colorectal cancer progression on the other.

## **METHODS**

### **Patients and samples**

The biopsies and the operative material of 72 patients were obtained by resection of

colorectal tumor at the Centre for Abdominal Surgery of the Clinical Centre of Montenegro (CCM) between January 2010 and December 2012. At the Institute of Pathology CCM, 5–15 tissue samples were obtained from each surgery, according to the established protocol, depending on the size of the tumor, including 2–3 biopsies of the adjacent, non-tumorous colorectal tissue. After fixation the bioptic material was routinely processed, embedded in paraffin and archived. Based on the standard pathological reports from that period, an experimental group was formed that consisted of operative biopsies of colorectal adenocarcinoma ( $n = 72$ ). The control group ( $n = 72$ ) consisted of operative biopsies of the adjacent non-tumorous colorectal tissue (epithelial cells), from the same patients in the experimental group. The study protocol was approved by the local Ethics Committee.

### **Histopathology and Immunohistochemistry**

On paraffin blocks, where samples of tumor tissue and regional lymph nodes were embedded, 3–5  $\mu\text{m}$  thick cuts were made. For histopathological verification of lesions, the routine hematoxylin-eosin (HE) method was applied.

Representative samples of tumor and adjacent non-tumor tissue were used for immunohistochemical analysis. Immunostaining was performed using the avidin-biotin-peroxidase complex (ABC) method (Vectastain ABC Elite-kit, Vector Laboratories, Burlingame, CA), with mouse monoclonal anti-E-cadherin antibody (DAKO, Denmark, clone NCH-38, 1:50) and rabbit monoclonal anti-Ki67 antibody (Abcam, Burlingame, CA, USA, clone SP6, 1:100).

Expression of E-cadherin in carcinoma cells was measured in 10 fields of microscopic magnification  $400\times$  (mean value represents the final result for the case) and classified in the following manner: Intramembranous immunohistochemical reaction with  $< 10\%$  of immunoreactive cells was considered a negative immunoreaction (–), and the presence of

intramembrane expression in  $\geq 10\%$  was evaluated as a positive reaction; an immunohistochemical reaction present in  $< 50\%$  of cells was classified as moderate expression (1+); a positive immunohistochemical reaction present in  $>50\%$  of cells was classified as pronounced expression (2+) of E-cadherin.

To evaluate the expression of Ki67+ cells per  $\text{mm}^2$  by area, test system M42 according to Weibel was used. Objective micrometer calibrated the test system on a Nikon Eclipse Ni MBP 99 400 microscope at a magnification of 400, with a measurement field of  $0.016 \text{ mm}^2$ . To test the density of Ki67+ cells per  $\text{mm}^2$ , 10 "hot-spots" were counted successively. The absolute value of the density of positive cells in the "hot-spot" was determined stereometrically [8]. The means of the obtained values of the "hot-spots" represents the final number of Ki67+ cells in  $\text{mm}^2$  per case. The median was subsequently determined and the absolute values of the density of positive cells were divided into two groups: those with the low level of expression (the value  $\leq$  the median value) and those with the high level of expression (values  $>$  the median value). From absolute determined values of Ki67 regarding deviation from median Ki67 index (proIDX) was obtained.

### **Statistical Analysis**

The statistical software package SPSS for Windows (19.0 IBM Corp. Armonk, NY; USA) was used. To analyze the statistical significance of parametric and nonparametric features, between and within the groups,  $\chi^2$ -test (chi-square test), the Kruskal–Wallis test, Fisher exact test and Student's t-test were used. Afterwards, the Kolmogorov-Smirnov normality test, correlation analysis (Spearman's rank correlation coefficient).

P values less than 0.05 were considered statistically significant.

## RESULTS

This study included 72 patients with colorectal adenocarcinoma (42 men, mean age  $66.1 \pm 10.6$  years, range 34–83, and 30 women, mean age  $61.4 \pm 14.2$  years, range 27–83). The main clinical and pathological characteristics of colorectal adenocarcinoma are shown in Table 1.

### **Immunohistochemical expression of Ki67 and E-cadherin in colorectal adenocarcinoma and adjacent non-tumor tissue**

Immunohistochemical analysis of the Ki67 expression, gave absolute values of the number of immunopositive cells in  $\text{mm}^2$  of tissue. Ki67 expression reported by the number of immunopositive cells in  $\text{mm}^2$  (median = 3098.9708; min = 1475.3, max = 4799.2) in colorectal carcinoma tissue, was significantly higher compared to the control group-adjacent, non-tumor tissue (median = 547.227; min = 431.5, max = 760.2;  $p < 0.001$ ) (Figure 1).

Immunohistochemical analysis of E-cadherin expression showed that there is a statistically significant difference in the expression of this marker between colorectal carcinoma tissue and adjacent non-tumor tissue. Namely, in colorectal adenocarcinoma, the absence of E-cadherin expression was found in a significant 52.8% of cases ( $p < 0.001$ ). It is also observed that the expression of E-cadherin is significantly increased (38.9%,  $p < 0.001$ ) in the adjacent non-tumor tissue compared to the colorectal carcinoma, where only 4.2% of cases expressed E-cadherin (Figure 2).

### **Association and correlation analysis between Ki67 expression/proliferative index (proIDX) and clinical and pathological characteristics of colorectal cancer (Table 2)**

There was a significant correlation of proliferation index (proIDX) with the pT stage of the tumor because in 100% of cases there was the correlation between low pro IDX and pT1

and in 91.7% of cases between low proIDX and the pT2 stage of the tumor. This correlation is defined by a significant, positive correlation coefficient ( $r = 0.352$ ,  $p = 0.002$ ).

High proIDX was associated with lymphatic invasion in 82.1% of cases, while in 87.9% of cases low proIDX was associated with tumors in which lymphatic vessel invasion was not identified. Pro IDX was associated with lymph vessel invasion with a significant, positive and high correlation coefficient ( $r = 0.697$ ,  $p = 0.000$ ).

High proIDX was significantly associated with vascular invasion in 90.9% of cases, while in 92.3% of cases with neural invasion, there were lower but significant and positive correlation coefficients ( $r = 0.347$  and  $r = 0.397$ ,  $p = 0.003$  and  $p = 0.001$ ).

There was a significant correlation between high proIDX and lymph node metastases. High proIDX was significantly associated with metastases in 1–3 lymph nodes in 84.2%, with metastases of 4–6 lymph nodes in 100% and with metastases of seven and more lymph nodes in 100% of cases. It is also noteworthy that low proIDX was associated with the absence of lymph node metastases in 94.3% of cases. Lymph node metastases were highly, positively, and significantly correlated with proIDX ( $r = 0.766$ ,  $p = 0.000$ ).

High proliferation index (Figure 3) was in 100% of cases associated with distant metastases with a significant and positive correlation coefficient ( $r = 0.354$ ,  $p = 0.002$ ).

ProIDX was significantly associated with Astler-Coller stage of tumor disease. Low proIDX was significantly associated with stage B1 in 90.9% and stage B2 in 100% of cases. At the same time, high proIDX was significantly associated with stage C2 in 90% and with stage D in 100% of cases. This correlation was defined by a highly significant and high correlation coefficient ( $r = 0.818$ ,  $p = 0.000$ ).

In this study, no significant correlation was found between the proIDX/Ki67 expression and subjects' gender, age, localization of the tumor in the colorectum and the histological grade of the tumor ( $p > 0.05$ ).

## **Association and correlation analysis between E-cadherin expression and clinical and pathological characteristics of colorectal cancer (Table 2)**

A statistically significant correlation between E-cadherin expression and pT stage of the tumor was observed. In 63% of cases, there was a correlation between absent E-cadherin expression and pT3 stage of the tumor ( $p = 0.039$ ). There was also a significant correlation between moderate E-cadherin expression and pT1 and pT2 stages ( $p = 0.021$ ). This correlation was defined by a significant, inverse, moderate correlation coefficient ( $r = -0.515$ ).

There was a statistically significant correlation between E-cadherin expression and lymphatic vessel invasion in colorectal cancer. Namely, the absence of E-cadherin expression was associated with lymphatic vessel invasion in 87.2% of cases. In cases where lymph vessel invasion was not identified, there was a moderate expression of E-cadherin in 78.8% of cases. E-cadherin expression was also associated with lymph vessel invasion with a high negative and significant correlation coefficient ( $r = -0.726$ ,  $p = 0.000$ ).

The expression of E-cadherin was associated with the invasion of blood vessels with a significant, negative and weak correlation coefficient ( $r = -0.311$ ,  $p = 0.008$ ), so that in 90.9% of cases, the absence of E-cadherin expression was associated with vascular invasion.

The absence of E-cadherin expression was significantly associated with nerve invasion in 92.3% of cases. Neural invasion and E-cadherin expression were associated with weak, significant negative correlation coefficient ( $r = -0.293$ ,  $p = 0.013$ ).

In a significant number of cases, the absence of E-cadherin expression was associated with lymph node metastases. In 94.7% of cases, there was an absence of E-cadherin expression associated with metastases in 1–3 lymph nodes, in 100% of cases with metastases in 4–6 lymph nodes, as well as in 100% of cases with metastases in seven or more lymph nodes. In lymph nodes, where no metastatic deposits were identified, moderate expression of



E-cadherin was present in a significant number of cases (85.7%,  $p < 0.001$ ). Lymph node metastases and E-cadherin expression were associated with a significant, high but negative correlation coefficient ( $r = -0.729$ ,  $p = 0.000$ ).

In 100% of cases, there was a significant correlation between the absence of E-cadherin expression and distant metastases.

There was a statistically significant correlation between E-cadherin expression and the Astler-Coller stage of tumor disease. Moderate expression of E-cadherin was associated with B1 and B2 Astler-Coller stage in a significantly higher number of cases (81.2% and 90.5%,  $p = 0.021$ ). Absence of E-cadherin expression was significantly associated with C2 and D Astler-Coller stage tumors in 100% of cases ( $p < 0.001$ ). E-cadherin expression was associated with the Astler-Coller stage of tumor disease with a very high, negative correlation coefficient ( $r = -0.875$ ,  $p = 0.000$ ).

In this study, there was also a highly significant correlation between E-cadherin expression and proIDX defined by a high, negative correlation coefficient ( $r = -0.794$ ,  $p = 0.000$ ).

No statistical significance was observed between E-cadherin expression and subjects' gender, age, tumor localization and histological grade of colorectal cancer ( $p > 0.05$ ).

## DISCUSSION

Numerous studies have observed the association of cell proliferation with the aggressive biological behavior of tumors of different localization [9–12], while the expression of Ki67 protein has been identified as a good predictor of recurrence and as an independent prognostic factor of survival rate [12, 13].

The expression of Ki67 protein during the cell cycle is strictly regulated by the balance between synthesis and degradation, which means that its half-life is short and is only 60–90

minutes long. During the cell cycle Ki67 becomes detectable in the mid-late G<sub>1</sub> phase when levels are low, and then its expression increases through the S and G<sub>2</sub> phases, and peaks in the early M phase. In the late stages of mitosis, the expression of Ki67 falls sharply and then disappears [14].

In this study, we observed that low proIDX has a good correlation coefficient significantly associated with pT<sub>1</sub> and pT<sub>2</sub> stages, while high proIDX is associated with significant and high correlation coefficients to lymph vessel invasion, blood vessel invasion, neural invasion, lymph node metastases and distant metastases. In a recent study by Tong et al 2020, a sample of 1,090 subjects with colorectal cancer observed a significant association of Ki67 expression with pT stage, histological grade, AJCC stage, and lymph node metastases [15].

The proliferation index calculated in our study indicates the existence of a very strong relationship between Ki67 expression and Astler Collier stage, supported by the high correlation coefficient that defined this relationship (Spearman rho = 0.818). High proIDX is significantly associated with C2 and D stages, which is consistent with the suggestion of Li et al. 2016, that a high proliferative index may be a useful biomarker to aid in the assessment of disease outcome in patients with stage III and IV colorectal cancer [16].

In this study, no significant association was found between Ki67/proIDX expression with gender, age of the subjects, localization of the tumor in the colon and with the histological grade of colorectal cancer. However, there is an observation in the literature about a significant correlation the high expression of Ki67 with an older age of patients [17].

Invasion and metastasis of malignant tumors, in addition to the kinetics and proliferative capacity of tumor cells, include the interaction between tumor cells themselves and between tumor cells and their microenvironment in which changes in cell adhesion play an important role [3, 6].

The expression of E-cadherin on epithelial cell membranes maintains cell connections and suppresses cell invasion. Mutation of the CDH1 gene which encodes E-cadherin results in the formation of a mutated, dysfunctional protein, which further results in decreased cell adhesion and uncontrolled growth of malignant cells [18]. Many studies have shown an association between reduced /absent E cadherin expression and progression of tumors [3, 6].

We identified a loss of E-cadherin expression in 52.8% of colorectal cancers, which is in complete agreement with the results of Kim et al. 2016, who examined 689 colorectal cancers in 52% of cases to verify the loss of E-cadherin expression [6]. Also, in agreement with other reports [3, 19], a significant difference in E-cadherin expression between tumor and adjacent non-tumor tissue was observed in our study.

As expected, the data on the expression of E-cadherin in relation to the clinical-pathological characteristics of colorectal cancer are heterogeneous and contradictory [5, 6]. In this study, we identified a significant association between reduced and/or absent E-cadherin expression with pT tumor stage, lymph and blood vessel invasion, perineural invasion, lymph node metastases, distant metastases, and Astler-Coller stage tumor disease. Other authors have observed that loss of E-cadherin expression is associated with lymph node metastases, but not with other parameters of colorectal cancer progression [6]. Kim et al, 2016 found significantly lower E-cadherin expression in cases with infiltrative tumor growth, lymph node metastasis and distant metastasis [6].

Our results indicate the existence of a highly significant relationship between the loss of E-cadherin expression and proIDX/Ki67 expression defined by a high, negative correlation coefficient. Also, a significant inverse correlation between E-cadherin expression and proIDX was observed in other tumors as such esophageal squamous cell carcinoma [20].

In this study, we did not observe a significant association of E-cadherin expression with gender, age of the subjects, tumor localization in the colorectum, the mode of tumor growth

or with the histological grade of the tumor. Kim et al. 2016, reported a significant association between the loss of E-cadherin expression with infiltrative tumor growth [6], while Palaghia et al. 2016, reported an association of negative expression of this marker with the age of subjects, with a higher prevalence in younger patients [3].

It has been observed that loss of E-cadherin expression stimulates Wnt , Rho GTPs and PI3K/AKT signaling ,which are thought to play an active role in the epithelial-mesenchymal transition (EMT) process [21, 22]. E- cadherin dysregulation promotes dysfunctions of these signaling pathways and affects the polarity, survival, invasion, and migration of cancer cells [4, 5, 21]. E cadherin dysregulation occurs mainly due to somatic alterations of the CDH1 gene, which is often an early stage in colorectal carcinogenesis. In addition to colorectal cancer, somatic mutations in the CDH1 gene have also been identified in sporadic diffuse gastric cancers, lobular breast cancer, ovarian cancer, and prostate cancer [23].

## CONCLUSION

High proliferative index/high levels of Ki67 expression and loss/reduced expression of E-cadherin are both mutually and strongly correlated with indicators of colorectal cancer progression. The proliferation index and expression of E-cadherin do not depend on gender, age, histological grade, localization and growth pattern of colorectal cancer.

Finally, this study supports the view that it is possible to differentiate patients into groups with a higher or lower risk of developing metastatic disease, based on the expression of these two biomarkers.

**Conflict of interest:** None declared.

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**Table 1.** Clinicopathological features of colorectal carcinoma studied

<b>Clinicopathological factors</b>	<b>Number of cases (%)</b>	<b>Age (min/max/<math>\bar{X}</math>)</b>
Males	42 (58.33)	34/83/66.1 ± 10.6
Females	30 (52.77)	27/83/61.4 ± 14.2
<b>Localization</b>	<b>Number of cases (%)</b>	
Cecum(+ileocecum)/Ascendant colon/Transverse colon/Sigma (+descendant colon)/Rectum (+anorectum + rectosigma)	3(4.2)/11(15.3)/1(1.4)/9(12.5)/48(66.7)	
<b>Macroscopic appearance of the tumor</b>	<b>Number of cases (%)</b>	
Ulceroinfiltrative/Infiltrative/Vegetatively-infiltrative/Vegetative/Ulcerative	27(37.5)/25(34.7)/16(22.2)/3(4.2)/1(1.4)	
<b>Histological grade</b>	<b>Number of cases (%)</b>	
Grade I/Grade II/Grade III	4(5.6)/63(87.5)/5(6.9)	
<b>Invasion of lymphatic vessels</b>	<b>Number of cases (%)</b>	
Not identified/Present	33(45.8)/39(54.2)	
<b>Invasion of blood vessels</b>	<b>Number of cases (%)</b>	
Not identified/Present	61(84.7)/11(15.3)	
<b>Nerve invasion</b>	<b>Number of cases (%)</b>	
Not identified/Present	59(81.9)/13(18.1)	
<b>Pathological stage of the tumor(pT)</b>	<b>Number of cases (%)</b>	
pT <sub>1</sub> / pT <sub>3</sub> / pT <sub>4</sub>	2(2.8)/54(75.0)/4(5.6)	
<b>Lymph node metastases</b>	<b>Number of cases (%)</b>	
No deposits/Deposits in 1–3 lymph nodes/Deposits in 4–6 lymph nodes/Deposits in seven and more lymph nodes	35(48.6)/19(26.4)/8(11.1)/10(13.9)	
<b>Distant metastases</b>	<b>Number of cases (%)</b>	
Not identified/Present	64(88.9)/8(11.1)	
<b>Astler Coller stage of tumor</b>	<b>Number of cases (%)</b>	
B1/B2/C1/C2/D	11(15.3)/21(29.2)/3(4.2)/30(41.7)/7(9.7)	

**Table 2.** Association of Ki67 and E-cadherin expression with clinico-pathological parameters

Type of tumor		Colorectal carcinoma				
Markers		Ki67		E-cadherin		
Tested parameters		low	high	-	+	++
		n (%)	n (%)	n (%)	n (%)	n (%)
Gender	Male	21 (50.0)	21 (50.0)	22 (52.4)	20 (47.6)	0 (0.0)
	Female	15 (50.0)	15 (50.0)	16 (53.3)	11 (36.7)	3 (10.0)
Significance	p	/		<b>0.096</b>		
Age of the subjects	≤ 65	14 (42.4)	19 (57.6)	18 (54.5)	14 (42.4)	1 (3.0)
	> 65	22 (56.4)	17 (43.6)	20 (51.3)	14 (43.6)	2 (5.1)
Significance	p	<b>0.237</b>		<b>0.891</b>		
Localization	Rectum (anorectum + rectosigma)	23 (47.9)	25 (52.1)	26 (54.2)	19 (39.6)	3 (6.2)
	Syigma (+sin. colon)	7 (77.8)	2 (22.2)	4 (44.4)	5 (55.6)	0 (0.0)
	Colon ascendens	5 (45.5)	6 (54.5)	5 (45.5)	6 (54.5)	0 (0.0)
	Transverse colon	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)
	Cecum(ileocecum)	1 (33.3)	2 (66.6)	2 (66.7)	1 (33.3)	0 (0.0)
Significance	p	<b>0.369</b>		<b>0.893</b>		
Tumor growth pattern	Ulceroinfiltrative	12 (44.4)	15 (55.6)	16 (59.3)	10 (37.0)	1 (3.7)
	Infiltrative	13 (52.0)	12 (48.0)	11 (44.0)	13 (52.0)	1 (4.0)
	Vegetative	3 (100)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)
	Vegetatively-infiltrative	7 (43.8)	9 (56.2)	11 (68.8)	5 (31.2)	0 (0.0)
	Ulcerovegetative	1 (100)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
Significance	p	<b>0.328</b>		<b>0.124</b>		
Histological grade of the tumor	Grade I	3 (75.0)	1 (25.0)	2 (50.0)	2 (50.0)	0 (0.0)
	Grade II	32 (50.8)	31 (49.2)	31 (49.2)	29 (46.0)	3 (4.8)
	Grade III	1 (20.0)	4 (80.0)	5 (100.0)	0 (0.0)	0 (0.0)
Significance	p	<b>0.245</b>		<b>0.285</b>		
Lymphatic invasion	Not identified	<b>29 (87.9)</b>	4 (12.1)	4 (12.1)	<b>26 (78.8)</b>	3 (9.1)
	Lymphatic invasion present	7 (17.9)	<b>32 (82.1)</b>	<b>34 (87.2)</b>	5 (12.8)	0 (0.0)
Significance	p	< <b>0.001*</b>		< <b>0.001*</b>		
Blood vessels invasion	Not identified	35 (57.4)	26 (42.6)	28 (45.9)	30 (49.2)	3 (4.9)
	Blood vessels invasion present	1 (9.1)	<b>10 (90.9)</b>	<b>10 (90.9)</b>	1 (9.1)	0 (0.0)
Significance	p	<b>0.003*</b>		<b>0.022*</b>		
Nerve invasion	Not identified	35 (59.3)	24 (40.7)	26 (44.1)	31 (52.5)	2 (3.4)
	Nerve invasion present	1 (7.7)	<b>12 (92.3)</b>	<b>12 (92.3)</b>	0 (0.0)	1 (7.7)
Significance	p	< <b>0.001</b>		<b>0.020*</b>		

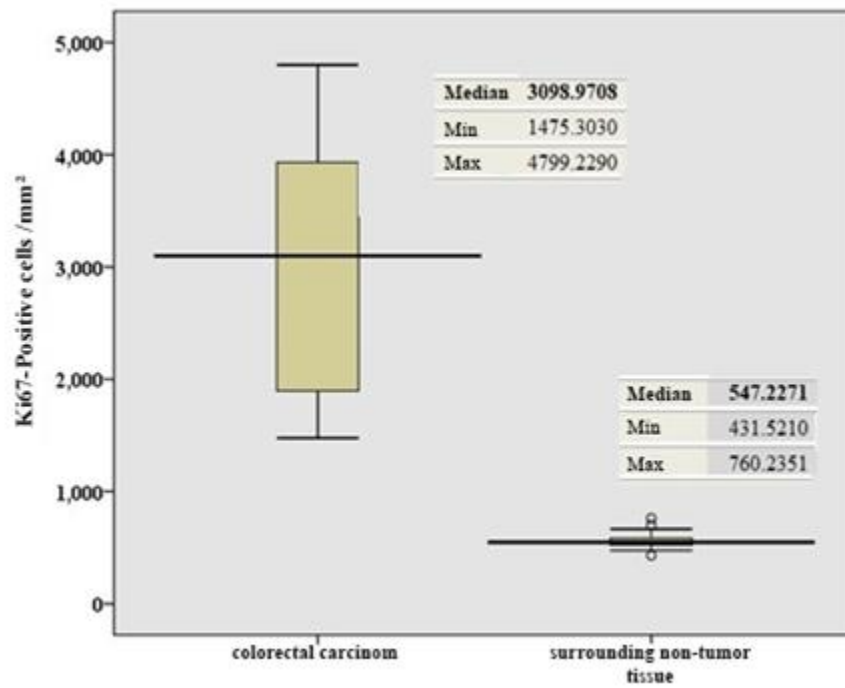


<b>Lymph node metastases</b>	No deposit	<b>33 (94.3)</b>	2 (5.7)	2 (5.7)	<b>30 (85.7)</b>	3 (8.6)
	Deposits in 1–3 LN	3 (15.8)	<b>16 (84.2)</b>	<b>18 (94.7)</b>	1 (5.3)	0 (0.0)
	Deposits in 4–6 LN	0 (0.0)	<b>8 (100.0)</b>	<b>8 (100.0)</b>	0 (0.0)	0 (0.0)
	Deposits in > 7 LN	0 (0.0)	<b>10 (100.0)</b>	<b>10 (100.0)</b>	0 (0.0)	0 (0.0)
<b>Significance</b>	p	<b>&lt; 0.001*</b>		<b>&lt; 0.001*</b>		
<b>Distant metastases</b>	Not identified	36 (56.2)	28 (43.8)	30 (46.9)	31 (48.4)	3 (4.7)
	Metastases present	0 (0.0)	<b>8 (100.0)</b>	<b>8 (100.0)</b>	0 (0.0)	0 (0.0)
<b>Significance</b>	p	<b>0.003*</b>		<b>0.018*</b>		
<b>Pathological stage of the tumor</b>	pT <sub>1</sub>	<b>2 (100.0)</b>	0 (0.0)	0 (0.0)	<b>2 (100.0)</b>	0 (0.0)
	pT <sub>2</sub>	<b>11 (91.7)</b>	1 (8.3)	0 (0.0)	<b>10 (83.3)</b>	2 (16.7)
	pT <sub>3</sub>	21 (38.9)	33 (61.1)	<b>34 (63.0)</b>	19 (35.2)	1 (1.9)
	pT <sub>4</sub>	2 (50.0)	2 (50.0)	4 (100.0)	0 (0.0)	0 (0.0)
<b>Significance</b>	p	<b>0.005*</b>		<b>&lt; 0.001*</b>		
<b>Astler Coller stage of tumor</b>	B1	<b>10 (90.9)</b>	1 (9.10)	<b>0 (0.0)</b>	<b>9 (81.8)</b>	2 (18.2)
	B2	<b>21 (100.0)</b>	0 (0.0)	1 (4.8)	<b>19 (90.5)</b>	1 (4.8)
	C1	2 (66.7)	1 (33.3)	0 (0.0)	3 (100)	0 (0.0)
	C2	3 (10.0)	<b>27 (90.0)</b>	<b>30 (100.0)</b>	0 (0.0)	0 (0.0)
	D	0 (0.0)	<b>7 (100.0)</b>	7 (100.0)	0 (0.0)	0 (0.0)
<b>Significance</b>	p	<b>&lt; 0.001*</b>		<b>&lt; 0.001*</b>		

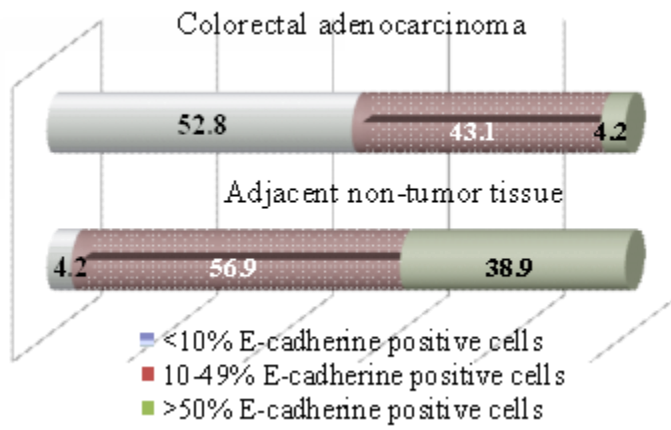
**Table 3.** Correlation matrix of clinical-pathological parameters and expression of colorectal adenocarcinoma markers

	Gender	Age	Localiza tion.	pT stage	Histol. Grade	AC Stage	Growth pattern	Lymph vessel invas.	Blood vessel invas.	Neural invasion	Lymph node metast.	Distant metastas.	Ki67	E- cadherin
<b>Gender</b>	r p 1.000 /	0.188 0.113	-0.058 0.630	0.102 0.394	-0.113 0.345	0.034 0.776	-0.204 0.086	-0.071 0.555	-0.202 0.088	0.043 0.722	0.024 0.840	-0.030 0.803	0.000 1.000	0.077 0.519
<b>Age</b>	r p 0.188 0.113	1.000	0.099 0.408	-0.141 0.238	0.012 0.917	-0.025 0.833	-0.057 0.635	-0.029 0.807	0.068 0.569	-0.046 0.700	0.021 0.860	0.072 0.546	-0.031 0.798	0.015 0.904
<b>Localiza tion</b>	r p 0.058 0.630	0.099 0.408	1.000	0.158 0.184	0.088 0.464	0.104 0.384	-0.155 0.193	0.160 0.179	-0.037 0.754	-0.079 0.511	0.141 0.238	0.037 0.757	0.053 0.661	-0.060 0.617
<b>pT stage</b>	r p 0.102 0.394	0.141 0.238	0.158 0.184	1.000	0.154 0.196	<b>0.638</b> <b>0.000</b>	-0.131 0.274	<b>0.429</b> <b>0.000</b>	0.128 0.284	0.142 0.236	<b>0.372</b> <b>0.001</b>	0.187 0.117	<b>0.352</b> <b>0.002</b>	<b>-0.515</b> <b>0.000</b>
<b>Histol. Grade</b>	r p 0.113 0.345	0.012 0.917	0.088 0.464	0.154 0.196	1.000	0.120 0.316	-0.104 0.382	0.115 0.336	-0.017 0.889	0.084 0.484	0.224 0.058	-0.014 0.908	0.197 0.098	-0.171 0.150
<b>AC stage</b>	r p 0.034 0.776	0.025 0.833	0.104 0.384	<b>0.638</b> <b>0.000</b>	0.120 0.316	1.000	-0.082 0.494	<b>0.756</b> <b>0.000</b>	<b>0.468</b> <b>0.000</b>	<b>0.437</b> <b>0.000</b>	<b>0.726</b> <b>0.000</b>	<b>0.503</b> <b>0.000</b>	<b>0.818</b> <b>0.000</b>	<b>-0.875</b> <b>0.000</b>
<b>Growth pattern</b>	r p 0.204 0.086	0.057 0.635	-0.155 0.193	-0.131 0.274	-0.104 0.382	-0.082 0.494	1.000	0.071 0.551	-0.120 0.316	0.061 0.609	-0.153 0.201	-0.083 0.489	0.059 0.625	-0.013 0.912
<b>Lymph vessel invasion</b>	r p 0.071 0.555	0.029 0.807	0.160 0.179	<b>0.429</b> <b>0.000</b>	0.115 0.336	<b>0.756</b> <b>0.000</b>	0.071 0.551	1.000	<b>0.391</b> <b>0.001</b>	<b>0.359</b> <b>0.002</b>	<b>0.698</b> <b>0.000</b>	<b>0.325</b> <b>0.005</b>	<b>0.697</b> <b>0.000</b>	<b>-0.726</b> <b>0.000</b>
<b>Blood vessel invasion</b>	r p 0.202 0.088	0.068 0.569	-0.037 0.754	0.128 0.284	-0.017 0.889	<b>0.468</b> <b>0.000</b>	-0.120 0.316	<b>0.391</b> <b>0.001</b>	1.000	<b>0.403</b> <b>0.000</b>	<b>0.255</b> <b>0.030</b>	<b>0.710</b> <b>0.000</b>	<b>0.347</b> <b>0.003</b>	<b>-0.311</b> <b>0.008</b>
<b>Neural invasion</b>	r p 0.043 0.722	0.046 0.700	-0.079 0.511	0.142 0.236	0.084 0.484	<b>0.437</b> <b>0.000</b>	0.061 0.609	<b>0.359</b> <b>0.002</b>	<b>0.403</b> <b>0.000</b>	1.000	<b>0.279</b> <b>0.018</b>	<b>0.638</b> <b>0.000</b>	<b>0.397</b> <b>0.001</b>	<b>-0.293</b> <b>0.013</b>
<b>Lymph node metastase s</b>	r p 0.024 0.840	0.021 0.860	0.141 0.238	<b>0.372</b> <b>0.001</b>	0.224 0.058	<b>0.726</b> <b>0.000</b>	-0.153 0.201	<b>0.698</b> <b>0.000</b>	<b>0.255</b> <b>0.030</b>	<b>0.279</b> <b>0.018</b>	1.000	0.156 0.190	<b>0.766</b> <b>0.000</b>	<b>-0.729</b> <b>0.000</b>
<b>Distant metastase s</b>	r p 0.030 0.803	0.072 0.546	0.037 0.757	0.187 0.117	-0.014 0.908	<b>0.503</b> <b>0.000</b>	-0.083 0.489	<b>0.325</b> <b>0.005</b>	<b>0.710</b> <b>0.000</b>	<b>0.638</b> <b>0.000</b>	0.156 0.190	1.000	<b>0.354</b> <b>0.002</b>	<b>-0.315</b> <b>0.007</b>
<b>Ki67</b>	r p 0.000 1.000	0.031 0.798	0.053 0.661	<b>0.352</b> <b>0.002</b>	0.197 0.098	<b>0.818</b> <b>0.000</b>	-0.059 0.625	<b>0.697</b> <b>0.000</b>	<b>0.347</b> <b>0.003</b>	<b>0.397</b> <b>0.001</b>	<b>0.766</b> <b>0.000</b>	<b>0.354</b> <b>0.002</b>	1.000	<b>-0.794</b> <b>0.000</b>
<b>E- cadherin</b>	r p 0.077 0.519	0.015 0.904	-0.060 0.617	<b>-0.515</b> <b>0.000</b>	-0.171 0.150	<b>-0.875</b> <b>0.000</b>	-0.013 0.912	<b>-0.726</b> <b>0.000</b>	<b>-0.311</b> <b>0.008</b>	<b>-0.293</b> <b>0.013</b>	<b>-0.729</b> <b>0.000</b>	<b>-0.315</b> <b>0.007</b>	<b>-0.794</b> <b>0.000</b>	1.000

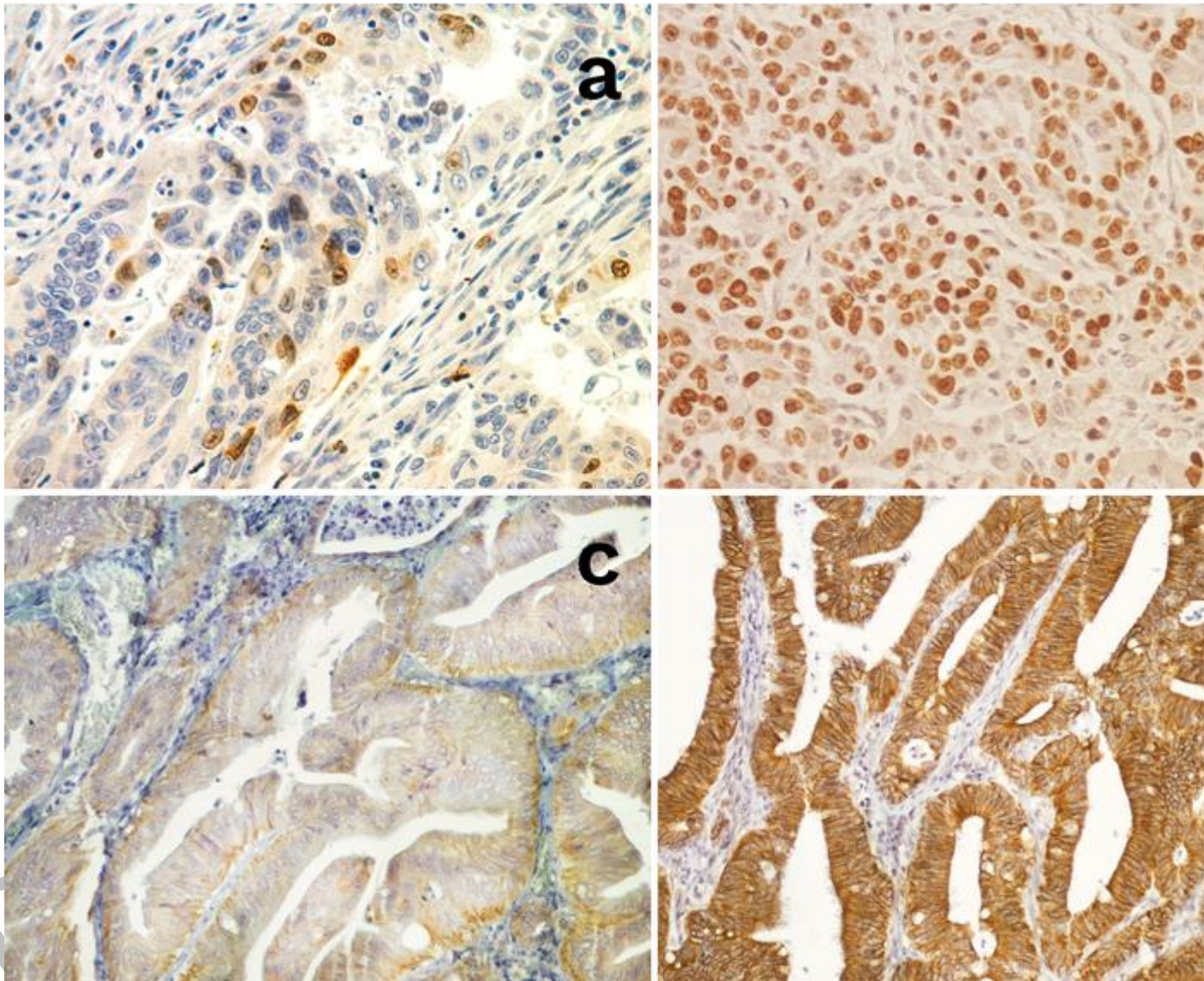
\*statistically significant difference  $p < 0,05$ ; Spearman correlation coefficient



**Figure 1.** Distribution of Ki67-immunopositive cells in mm<sup>2</sup> of examined tissue



**Figure 2.** E-cadherin expression in colorectal cancer and adjacent non-tumor tissue



**Figure 3.** Immunohistochemical expression of Ki67 and E-cadherin in colorectal adenocarcinoma; a – low Ki67 expression index; b – high Ki67 expression index; c – lack of expression and expression of trace E-cadherin; d – pronounced E-cadherin expression (ABC × 400)