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Ki-67, p53 and bcl-2 analysis in colonic *versus* rectal adenocarcinoma

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Abstract

This paper develops a comparative study between similar subtypes of colonic and rectal adenocarcinoma, based on their immunohistochemical profiles for Ki-67, p53 and bcl-2 markers, in order to evaluate the prediction value for the investigated markers, according to the histologic subtype and location. Thirty cases of adenocarcinoma were investigated, 15 with colonic and 15 with rectal location. For both locations, the cases included five well-differentiated, five moderately differentiated and five low differentiated subtypes. The immunohistochemical investigation was performed using Ki-67, p53 and bcl-2 antibodies (DAKO) and streptavidin–biotin method (LSAB Kit, DAKO). The semiquantitative analysis of the immunohistochemical reactions was based on the Ki-67 and p53 index, respectively, counted as number of positive cells from 100 positive and negative cells. For bcl-2, the reaction was considered positive, respectively negative, for a percentage of positive cells higher, respectively smaller, than 5%. All 30 cases (100%) were positive for Ki-67. The mean value of Ki-67 index for colonic, respectively rectal adenocarcinoma was 55.8%, respectively 59.6%. No statistical correlation was found between the proliferative activity and location ($p = 0.502$). Ten cases (66%) of colonic adenocarcinoma were positive for p53, with a mean value of 42.5% for p53 index. Eight cases (53%) of rectal adenocarcinoma were positive for p53, with a mean value of 21.1% for p53 index. There was a statistic correlation between the apoptotic activity and location ($p = 0.005$). The positive reaction for bcl-2 was present in seven (46.6%) and nine (60%) from the 15 cases of colonic and, respectively, rectal adenocarcinoma. The statistic analysis revealed that bcl-2 cannot be significantly associated with any of the two locations ($p = 0.48144$, 95% CI). The use of the Wald tests permitted the assessment of the predictive power for the investigated markers according to the pathologic subtype and location. Thus, p53 is on the first position ($W = 16.56$, $p = 0.00004$, 95% CI), followed by Ki-67 ($W = 4.49$, $p = 0.034$, 95% CI), whereas bcl-2 cannot be considered a predictive factor ($W = 2.5$, $p = 0.107$, 95% CI). The immunohistochemical evaluation of Ki-67, p53 and bcl-2 yields refined information on colorectal tumor biology. Our study confirms, from the statistic point of view, the role of p53, followed by Ki-67, as predictive factors.

***Keywords*:** colorectal carcinoma, Ki-67, p53, bcl-2.

Introduction

In the developed countries, colorectal carcinoma (CRC) is the second cause of death by cancer [1]. Annually, half of a million people die because of this disease [2].

As a current rate of incidence, approximately 6% of the population will develop colorectal cancer during their course of life. With respect to other types of cancers, CRC occupies the third place both in men (after broncho-pulmonary and gastric cancer) as well as in women (after mammary gland and cervix cancer) [2].

The study of CRC morphology becomes thus important for the refinement of the diagnosis, the correct staging, the choice of an efficient individualized therapy, the modification of the prognosis and, consequently, of the survival rate.

The evaluation of colon cancer prognosis is performed according to four types of factor established by *College of American Pathologists Consensus Statement 1999* [3].

The first category includes the local extent of tumor assessed pathologically, regional lymph node metastases, blood or lymphatic vessel invasion, residual tumor classification, preoperative CEA elevation.

The second category (A and B) comprises histologic grade, radial margin, tumor classification after neoadjuvant therapy, histologic type, histologic features associated with MSI–H, loss of heterozygosity at 18q and allelic loss of deleted in colon cancer gene, tumor border configuration.

In the third category, there are present the DNA contents, other molecular markers, perineural invasion, microvessel density, cell proteins and carbohydrates, peritumoral fibrosis, purulent peritumoral inflammatory reaction, foci of neuroendocrine differentiation within any histologic type, nucleolar organizing regions,

proliferation indices. The fourth category is represented by tumor size, gross tumor configuration.

With respect to the histologic degree of tumor differentiation – factor from the IIA category, multiple variable studies consider it is an independent prognosis factor. The lesser differentiated the carcinoma, the more invasive it can be, with an increased potential for metastases in the lymph nodes and/or at further distance. The utility of this parameter is limited by the inter-observational variability [4].

The factors named generically "other molecular markers" (the third category) include tumor suppressor genes (LOH 1p/p53, LOH 8p, LOH 1p, LOH 5q), oncogenes (K-ras, c-myc), apoptosis and cell suicide-related genes (bcl-2, BAX), DNA synthesis-related genes, TGF and EGF-R genes, cyclin-dependent kinase inhibitor genes, angiogenesis-related genes, adhesion molecule and glycoprotein genes, matrix metalloproteases and inhibitors, metastasis suppressor genes. In correlation with these molecular markers, a critical analysis of the literature [3] reveals the following faulty points: large number of single studies on single factors (i.e. p53 [5–8] or bcl-2 [9–11]), small number of papers on a large number of individual molecular factors [12–17], conflicting results from various studies of same factor, almost no statistically robust studies on most factors, almost no multivariate analysis on most factors.

Also, in the third category can be included the proliferation indices (Ki-67, PCNA), established by immunohistochemistry or by flow cytometric method, the achieved studies providing different results [18–20]. The inconsistencies can be explained by the technique differences and by the subjective component in the assessment of the immunostaining (strong *versus* weak staining) and in overall interpretation (average or region of most intense activity only).

Starting from the multitude of information existent on the prognosis factors, we aimed to perform a complex morphologic investigation on a series of adenocarcinomas with colonic and rectal location. The novelty of our approach results from the researches oriented toward the following two objectives: (i) the comparison of immunohistochemical profiles for Ki-67, p53 and bcl-2 markers between two similar pathologic subtypes of colonic and rectal carcinoma; (ii) the evaluation of the prediction value for the investigated markers according to the histologic subtype and location.

Material and methods

The study group consisted of 30 cases of adenocarcinoma, 15 with colonic and 15 with rectal location, diagnosed at the Pathology Laboratory, Emergency University Hospital Bucharest. For both locations, the 15 cases included five well-differentiated, five moderately differentiated and five low differentiated subtypes. The immunocytochemical investigation was performed using Ki-67, p53 and bcl-2 antibodies (DAKO) and streptavidin–biotin method (LSAB Kit, DAKO).

Semiquantitative analysis of immunocytochemical reactions

For the assessment of the proliferative activity, the positivity index Ki-67 (Ki-67 index) was calculated as number of positive cells from 100 positive and negative cells in microscopic fields investigated with ×40 magnification [21].

Similarly, the expression of p53 was quantified with respect to the percentage of positive nuclei, yielding a p53 index. The reaction was interpreted as having a low, moderate or marked expression when the percentage of the colored nuclei represented less than 20%, between 20% and 50% or more than 50%, respectively [22].

The bcl-2 expression was evaluated relative to the percentage of the tumoral cells with cytoplasmic reaction, the reaction being considered positive, respectively negative, for a percentage of positive cells higher, respectively smaller, than 5%.

For the statistic processing of data we used the STATISTICA software, specially designed for medical research. For the comparison of the mean values for a parameter corresponding to more data series, we applied the Kruskal–Wallis and ANOVA tests.

Results

Evaluation of Ki-67 marker – colonic *vs.* rectal adenocarcinoma

All 30 cases of adenocarcinoma presented positive immunohistochemical reaction for Ki-67 (100% positivity), with a Ki-67 index varying on an extremely wide range, from 5 to 95% (Figures 1 and 2).

In the individual evaluation of each case, we noted the fact that for all three pathologic subtypes with colonic location the proliferation index has very high values, over 90%, as opposed to the rectal location where only in the low differentiated form the Ki-67 index is over 90%, the other two forms having maximum values between 80 and 90%. If, for the colonic location, in the well and moderately differentiated subtypes there was one case with a very small Ki-67 index value – 5%, the minimum values in the rectal location are very close for the well differentiated form (8%) and much higher in the other two forms – for the low differentiated form the smallest value is 72%.

Calculating the mean value of the Ki-67 index for each pathologic subtype, we noted an increase in the proliferative activity in both locations, as follows:

- For the colonic adnocarcinoma (Table 1): from the well differentiated form (48% mean Ki-67 index) to the low differentiated form (60.8% mean Ki-67 index); the moderately differentiated form had an intermediate mean Ki-67 index – 58.6%. Nevertheless, the ANOVA test applied for the comparison of mean Ki-67 values showed there are no statistically significant differences with respect to the pathologic subtypes ($p = 0.674$, $F = 0.39$, 95% CI).
- For the rectal adenocarcinoma (Table 2): from the well differentiated form (35.2% mean Ki-67 index) to the low differentiated form (83.4% mean Ki-67 index);

the moderately differentiated form had an intermediate mean Ki-67 index – 60.2%. The ANOVA test applied for the comparison of the comparison of mean Ki-67 values with respect to the pathologic subtypes of rectal adenocarcinoma indicated the existence of statistically significant differences (p = 0.018, F = 5.61, 95% CI).

Table 1 – *Statistic indicators of Ki-67 according to the degree of differentiation of colonic adenocarcinoma*

Groups	Mean Ki-67	Mean		Standard deviation	Standard error	Min.	Max.
		-95%	*+95%*				
Well differentiated ADK	48.0%	31.6%	64.4%	35.0%	7.8%	5.0%	94.0%
Moderately differentiated ADK	58.6%	43.0%	74.2%	33.4%	7.5%	5.0%	98.0%
Low differentiated ADK	60.8%	46.1%	75.5%	31.4%	7.0%	15.0%	95.0%
Total	**55.8%**	**47.2%**	**64.4%**	**33.2%**	**4.3%**	**5.0%**	**98.0%**

Table 2 – *Statistic indicators of Ki-67 according to the degree of differentiation of rectal adenocarcinoma*

Groups	Mean Ki-67	Mean		Standard deviation	Standard error	Min.	Max.
		-95%	*+95%*				
Well differentiated ADK	35.2%	21.1%	49.3%	32.8%	14.6%	8.0%	88.0%
Moderately differentiated ADK	60.2%	52.1%	68.3%	18.9%	8.5%	37.0%	80.0%
Low differentiated ADK	83.4%	78.7%	88.1%	11.0%	4.9%	72.0%	99.0%
Total	**59.6%**	**52.2%**	**67.0%**	**29.3%**	**7.6%**	**8.0%**	**99.0%**

Based on our results, we performed a comparison between the mean values of Ki-67 index for colonic (55.8%) and rectal adenocarcinoma (59.6%), in order to establish the existence of a correlation between the proliferative activity and location. The Kruskal–Wallis and ANOVA tests indicated the fact that there are no significant differences between the mean values of Ki-67 in colonic adenocarcinoma, with respect to those computed in rectal adenocarcinoma (p = 0.502, 95% CI).

Evaluation of p53 marker – colonic *vs.* rectal adenocarcinoma

From the 15 cases of colonic adenocarcinoma, 10 presented positive reaction for p53 (66% positivity), with a p53 index varying on an extremely large range, between 0 and 95% (Figure 3). From the 15 cases of rectal adenocarcinoma, eight presented positive reaction for p53 (53% positivity), with a p53 index varying on an extremely large range, between 0 and 100% (Figure 4).

In the individual evaluation of each case, we noted that:

▪ For the well and low differentiated subtypes with colonic location, there are very high values of p53 index, between 90 and 100%, as opposed to the rectal location where only in the well differentiated form the p53 index reached 90%, the other two forms having maximum values of 20% and 62%, respectively.

▪ For the colonic location, only in the moderately and low differentiated subtypes there existed negative cases.

▪ For the rectal location, in all three pathologic subtypes there existed negative cases.

Calculating the mean value of p53 index for each pathologic subtype, we observed a decrease in the apoptotic activity in both locations as follows:

▪ For colonic adenocarcinoma (Tables 3 and 4): from the well-differentiated form (62.6% p53 index) to the low differentiated form (36% mean p53 index), the moderately differentiated form having the lowest mean value – 29%. Because the study group consisted from a small number of cases, the estimation standard error obtained for p53 was very high (9.7% – Table 3), a normalization of the data (value series) being necessary (Table 4). The ANOVA test applied for the comparison of p53 mean values after the normalization of the value series showed the existence of a statistically significant difference between the p53 mean values and pathologic subtypes (p = 0.007, F = 5.413, 95% CI).

▪ For rectal adenocarcinoma (Tables 5 and 6): from the well-differentiated form (45.2% mean p53 index) to the low differentiated form (6% mean p53 index); the moderately differentiated form had an intermediate mean p53 index – 24%. Here too, the small number of cases resulted in a very high estimation standard error (8.3% – Table 5), a normalization of the data being necessary (Table 6). Following this normalization the ANOVA test showed the existence of a statistically significant difference between the mean values of p53 in accordance with the pathologic subtypes (p = 0.0001, F = 10.402, 95% CI).

Table 3 – *Statistic indicators of p53 according to the degree of differentiation of colonic adenocarcinoma (study group of 15 cases)*

Groups	Mean p53	Mean		Standard deviation	Standard error	Min.	Max.
		-95%	*+95%*				
Well differentiated ADK	62.6%	28.1%	97.1%	27.8%	12.4%	22.0%	95.0%
Moderately differentiated ADK	29.0%	-7.9%	65.9%	29.7%	13.3%	0.0%	70.0%
Low differentiated ADK	36.0%	-25.8%	97.8%	49.8%	22.3%	0.0%	100.0%
Total	42.5%	21.8%	63.3%	37.5%	9.7%	0.0%	100.0%

Table 4 – *Statistic indicators of p53 according to the degree of differentiation of colonic adenocarcinoma (normalization of value series)*

Groups	Mean p53	Mean		Standard deviation	Standard error	Min.	Max.
		-95%	*+95%*				
Well differentiated ADK	62.6%	50.7%	74.5%	25.5%	5.7%	22.0%	95.0%
Moderately differentiated ADK	29.0%	16.2%	41.8%	27.3%	6.1%	0.0%	70.0%
Low differentiated ADK	36.0%	14.6%	57.4%	45.7%	10.2%	0.0%	100.0%
Total	42.5%	33.1%	52.0%	36.5%	4.7%	0.0%	100.0%

Table 5 – *Statistic indicators of p53 according to the degree of differentiation of colonic adenocarcinoma (study group of 15 cases)*

Groups	Mean p53	Mean		Standard deviation	Standard error	Min.	Max.
		-95%	*+95%*				
Well differentiated ADK	45.2%	26.7%	63.7%	43.1%	19.3%	0.0%	90.0%
Moderately differentiated ADK	24.0%	12.6%	35.4%	26.5%	11.8%	0.0%	62.0%
Low differentiated ADK	6.0%	2.2%	9.8%	8.9%	4.0%	0.0%	20.0%
Total	25.1%	17.0%	33.1%	32.1%	8.3%	0.0%	90.0%

Table 6 – *Statistic indicators of p53 according to the degree of differentiation of colonic adenocarcinoma (normalization of value series)*

Groups	Mean p53	Mean		Standard deviation	Standard error	Min.	Max.
		-95%	*+95%*				
Well differentiated ADK	45.2%	26.7%	63.7%	39.5%	8.8%	0.0%	90.0%
Moderately differentiated ADK	24.0%	12.6%	35.4%	24.3%	5.4%	0.0%	62.0%
Low differentiated ADK	6.0%	2.2%	9.8%	8.2%	1.8%	0.0%	20.0%
Total	25.1%	17.0%	33.1%	31.2%	4.0%	0.0%	90.0%

Based on our results, we performed a comparison between the mean values of p53 index for colonic (42.5%) and rectal adenocarcinoma (21.1%), in order to establish the existence of a correlation between the apoptotic activity and location. The Kruskal–Wallis and ANOVA tests indicated significant differences between the mean values of p53 in colonic adenocarcinoma, with respect to those computed in rectal adenocarcinoma ($p = 0.005$, $F = 7.921$, 95% CI).

Evaluation of Bcl-2 marker – colonic *vs.* rectal adenocarcinoma

The positive immunocytochemical reaction for bcl-2 (Figure 5) was present in seven (46.6%) and nine (60%) from the 15 cases of colonic and rectal adenocarcinoma, respectively (Table 7). The global appreciation of the pathologic subtypes in accordance with the locations yielded similar immunoreactivity in well-differentiated adenocarcinoma and slightly increased in moderately and low differentiated adenocarcinoma. The number of positive bcl-2 cases was higher in the rectal as opposed to the colonic location, for moderately (three *vs.* two cases) and low differentiated subtypes (four *vs.* three cases).

Table 7 – *Bcl-2 marker in colonic/rectal adenocarcinoma*

	bcl-2 – colonic adenocarcinoma		bcl-2 – rectal adenocarcinoma	
	No. of cases	*%*	*No. of cases*	*%*
Positive	7	46.67%	9	60%
Negative	8	53.33%	6	40%
Total	15		15	

Based on our results, we assessed the presence or the absence of the bcl-2 marker in order to establish a correlation between the antiapoptotic activity and the colonic or rectal location, respectively. The developed statistic analysis (Pearson χ^2 test, correlation coefficient) revealed the fact that bcl-2 cannot be significantly associated with any of the two locations ($p = 0.48144$, 95% CI).

Correlation study of Ki-67, p53 and bcl-2 markers

The next stage consisted in a multiple correlation study, associated mainly with the location of the adenocarcinoma (Tables 8 and 9).

Table 8 – *Multiple correlation between the investigated markers and adenocarcinoma location*

Test	Value
Correlation coefficient r	0.367538
r^2	0.135084
F	6.039047
p – significance level 95% CI	0.000739
Standard error of estimate	0.472954

The results of the multiple correlation test indicate a moderate correlation between all three investigated markers and adenocarcinoma location ($r = 0.36$, $p = 0.00073$, 95% CI).

Table 9 – *Partial coefficients of multiple correlation between investigated markers and adenocarcinoma location*

Partial correlation	Correlation coefficient	Standard error	t	p *95% confidence range*
Intercept		0.236232	433.1488	0.000000
Ki-67	-0.413711	0.157404	-2.6283	0.009742
p53	-0.724400	0.186036	-3.8939	0.000165
bcl-2	-0.157609	0.165337	-0.9533	0.342442

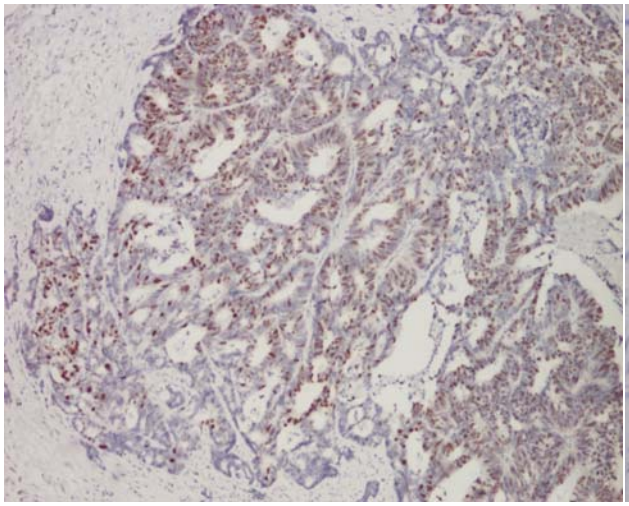

Figure 1 – ***Well-differentiated adenocarcinoma: positive reaction for Ki-67; Ki-67 index 70% (IHC, anti-Ki-67, ob. ×10)***

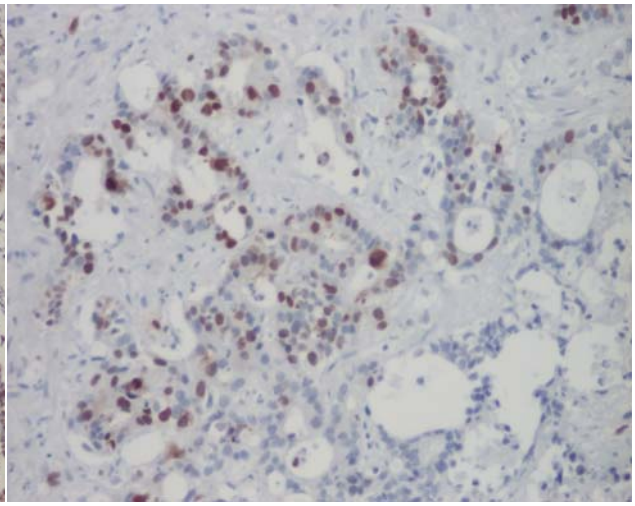

Figure 2 – ***Moderately differentiated adenocarcinoma: positive reaction for Ki-67; Ki-67 index 30% (IHC, anti-Ki-67, ob. ×20)***

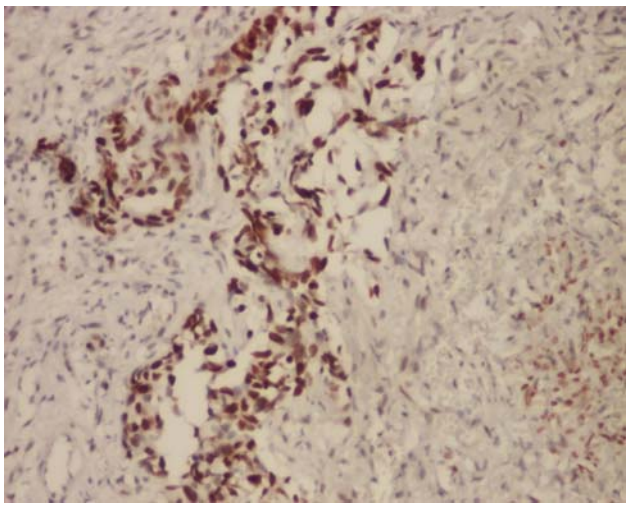

Figure 3 – ***Low differentiated adenocarcinoma: positive reaction for p53; p53 index 100% (IHC, anti-p53, ob. ×20)***

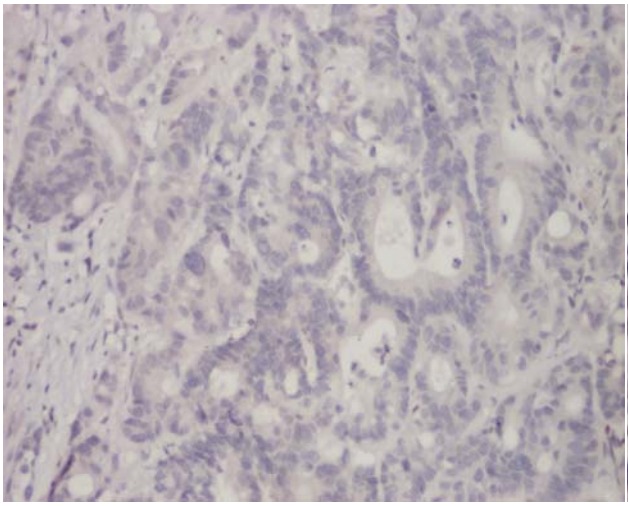

Figure 4 – ***Moderately differentiated adenocarcinoma: negative reaction for p53 (IHC, anti-p53, ob. ×20)***

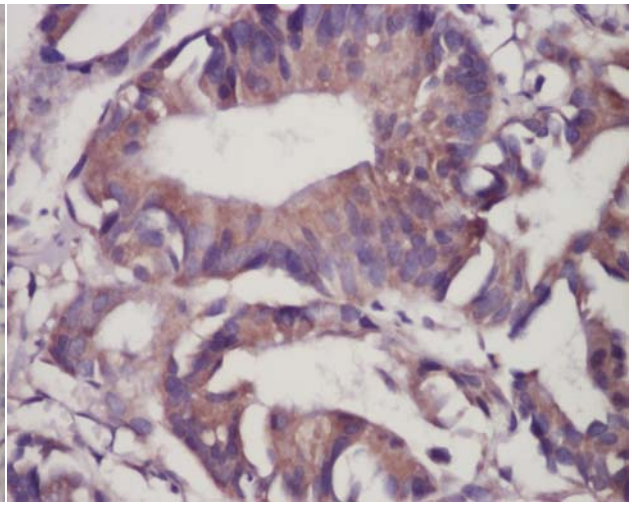

Figure 5 – ***Well-differentiated adenocarcinoma: positive reaction for bcl-2 (IHC, anti-bcl-2, ob. ×40)***

The analysis of partial correlation coefficients calculated within the multiple correlations denotes the association degree of each marker with adenocarcinoma location. Thus, we noted the existence of a strong reverse correlation between p53 and adenocarcinoma location (r = -0.72, p = 0.00016, 95% CI), as well as a moderate reverse correlation between Ki-67 and adenocarcinoma location (r = -0.41, p = 0.009, 95% CI), i.e. high values of p53 and Ki-67 respectively are associated with the colonic location of adenocarcinoma.

Finally, the statistic analysis focused on the correlations between the investigated markers as predictive factors – prognosis, differentiation degree of tumor (pathologic subtypes of adenocarcinoma) and colonic/rectal location.

The use of the Wald test – based on a χ^2 distribution (Table 10) permits the assessment of the predictive power for the investigated markers according to the pathologic subtype and location. We placed the values obtained in a hierarchy from the point of view of the predictive power. Thus, p53 has the highest predictive power (W = 16.56, p = 0.00004, 95% CI), followed by Ki-67 (W = 4.49, p = 0.034, 95% CI). Bcl-2 cannot be considered as a predictive factor (W = 2.5, p = 0.107, 95% CI).

Table 10 – *Wald test results*

Test	Estimates	Standard error	Wald statistics	p
Intercept	0.961333	0.152512	**39.7322**	0.000000
Ki-67	-0.343447	0.162060	**4.4913**	0.034068
p53	-0.789623	0.193996	**16.5673**	0.000047
bcl-2	-0.184767	0.114921	2.5849	0.107885

Discussions

Ki-67 is a nuclear protein that is present in all cell cycle phases except for the G0 and early G1 phase making it a good marker for cycling cells [23]. Ki-67 proliferative index has a prognostic and/or predictive value in different tumor types [24], however in colorectal cancer the results seem to be conflicting [25–30]. According to the literature Ki-67 cannot be correlated with the clinic and pathologic parameters that define colorectal carcinoma (location, age, sex, five years survival rate, degree of differentiation), although there can be found a strong statistically significant correlation with metastases development [31]. For this reason, Ki-67 has a limited usage as discriminatory prognosis factor [26].

Recent studies [20] established the fact that an increased expression of Ki-67 indicates a better survival in rectal and recto-sigmoid cancer. In rectal cancer, increased proliferative activity tumors have a better response to radiotherapy [20, 32]: the irradiation destroys preferentially the quick dividing cells, while the cell population with a low proliferation activity presents an increase in the radioresistance [29].

In some case series of colorectal cancer, the assessment of Ki-67 index by immunohistochemical assay showed a wide range in the percentage of Ki-67 reacting cells (mean values): 25% [33, 34], 38% [18], 45.4% [35], 48.8% [36], 50% [37], 66.3% [38].

The results obtained on our study group were characterized by 100% positivity for Ki-67 in both locations. Mean values of Ki-67 index (55.8% and 59.6%, respectively) were situated towards the upper portion of the range reported in the mainstream. Statistically significant correlations between proliferation activity and pathologic subtype were absent for colonic carcinoma, but present for rectal adenocarcinoma. Interesting were the differences between colonic and rectal locations for mean Ki-67 index in the well differentiated (48% *vs.* 35.2%) and low differentiated subtype (60.8% *vs.* 83.4%). Nevertheless, the proliferation index was not statistically significant correlated with the location (p = 0.502, 95% CI). This assertion is in consensus with the literature data, underlining the concept of colorectal cancer as a whole.

P53 is a tumor suppressor gene. Approximately half of colorectal cancers present mutations in p53 gene, with a higher frequency in the distal colon tumors (including rectal) and a low frequency in proximal tumors [39]. It is not yet well established how p53 gene mutations and/or protein expression are causally related with prognostic variables and prognosis or with survival [30]. The results obtained following the interpretations of the statistic analysis showed that p53 could not be correlated with clinic and pathologic variables [5, 8, 40, 41] or with the proliferation index [5, 42]. According to the literature, the deletion of p53 with the overexpression of p53 protein is correlated with a low rate of survival, thus being an independent prognosis factor [7, 9]; moreover, p53 negative tumors respond favorably to chemotherapy and radiotherapy [43, 44].

The prevalence if p53 positive colorectal carcinoma is circumscribed to a wide range: 17% [45], 39% [41], 42% [15, 34, 40, 46], 45.6% [17], 46,1% [18], 57% [47], 60% [6, 48], 65% [8], 66% [49], 67,3% [5, 50, 51], 72% [52], 74% [16], 77% [37], 78.9% [35], 80% [53].

In our study, 66% of colonic and 53% of rectal adenocarcinoma cases were p53 positive. There were established statistically significant differences between mean values of p53 and pathologic subtypes for both locations. Furthermore, the statistic analysis indicated significant differences between mean p53 values for colonic adenocarcinoma, as opposed to those calculated for rectal adenocarcinoma (p = 0.005, 95% CI). This result, different from those reported in the literature makes further investigations on p53 marker mandatory, in view of confirmation on a higher number of cases of the correlation obtained in this study. Thus, the perspective of expanding our knowledge on the different evolution of colonic and rectal carcinoma from the point of view of p53 action mechanism becomes extremely interesting.

Bcl-2 is a gene involved in the cell cycle regulation by inhibiting apoptosis (antiapoptotic oncoprotein) in some cell systems under physiological and neoplastic conditions. The role of bcl-2 in colorectal tumorigenesis is believed to be in the early stages of carcinogenesis [9]. A decrease in the levels of bcl-2 can

lead cells to death by apoptosis while its overexpression protects against this programmed cell death, determining carcinogenic transformation [11]. In the '90s, many studies have examined the value of the immunohistochemical expression of bcl-2 in colorectal cancer, but results have been contradictory [9, 10, 12, 13, 34, 54]. A significant association between bcl-2 expression and tumor stage was demonstrated [41].

The prevalence of bcl-2 protein immunocytochemical expression in colorectal cancers varies greatly from one study to another: 16.7% [47], 20% [37], 24% [52], 27% [33], 28.1% [35], 29% [41], 29.5% [11], 31% [15], 43% [16], 45% [49], 46.3% [46], 46.7% [17], 51.9% [14, 19], 60% [48], 67% [34], 70% [32].

In our study, 46.6% of the colonic and 60% of the rectal adenocarcinomas were bcl-2 positive. With respect to their location, bcl-2 could not be significantly correlated with any of the two locations ($p = 0.48144$, 95% CI), the result pleading, as in the case of the proliferation index, for the support of the general mechanism of colorectal carcinogenesis, without differentiations depending to the intestinal segment involved.

On the basis of partial correlation coefficients analysis calculated in the multiple correlation, it was possible to evaluate the degree of association of every marker with the adenocarcinoma location (colonic *vs.* rectal). Consequently, we obtained a strong reverse correlation between p53 and location ($r = -0.72$, $p = 0.00016$, 95% CI) and a moderate reverse correlation between Ki-67 and location ($r = -0.41$, $p = 0.009$, 95% CI), the higher values of p53 and Ki-67 being associated with colonic adenocarcinoma.

The statistic analysis also focused on the correlation between the investigated markers as prediction factors – prognosis, differentiation degree of tumor (pathologic subtypes of adenocarcinoma) and colonic/rectal location.

Our study confirms, from the statistical point of view, the predictive value of p53 marker, followed by proliferation index Ki-67. The results obtained for bcl-2 marker did not permit its validation as prognosis factor.

We must underline the fact that the investigated markers have a relative value in the prognosis evaluation, due to the high value of Wald statistic ($W = 39.73$, $p = 0.000$, 95% CI) computed in the case of "intercept" parameter (potential predictive factor). Consequently, the investigated markers cannot be considered as independent prognosis factors, the integration within the clinic and biologic tumoral framework being requested for further development of our researches.

Conclusions

The immunohistochemical evaluation of Ki-67, p53 and bcl-2 yields refined information on colorectal tumor biology. The absence of statistically significant correlations between Ki-67, bcl-2 and location, respectively (colonic *vs.* rectal) supports the concept of colorectal carcinoma. The presence of statistically significant correlations between p53 and location (colonic *vs.* rectal) requires an intensified and extended further research on a higher number of cases, in order to sustain the data obtained in this study. The status of the molecular markers as prognosis factors is well known without a unanimously accepted value hierarchy.

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