Case Report

MODESTUM

The pathological and immunohistochemical profile of tumor angiogenesis in perforated sigmoid carcinoma–Case report and short literature review

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ARTICLE INFO	ABSTRACT
	Background: Little is known about the physiopathological factors or mechanisms that underlie tumor invasion of the serosa and lead to perforation in the peritoneal cavity. The aim of the work was to analyze the pathological and immunohistochemical factors of tumor neoangiogenesis which could influence tumor perforation in colorectal cancer. **Results and discussions:** 451 cases of complicated colorectal carcinomas were statistically analyzed, of which 19 cases were perforated sigmoid tumors. The immunohistochemical detection of mutant p53 proteins was the first molecular parameter examined in the context of the search for markers predicting the natural evolution mode in colorectal carcinomas. **Conclusions**: Both loss of p53 and overexpression of bcl-2 proteins confer immortalization on cancer cells by inhibiting the processes leading to apoptosis. The paper proposes a review of the specialized literature, but also the presentation of a clinical case. **Keywords:** sigmoid cancer, perforation, p53, immunohistochemistry

INTRODUCTION

According to GLOBOCAN 2018 data, the colon cancer is the third most common form of cancer worldwide (10.2% of all new cases) and it has one of the highest incidence rates in various regions in Europe [1]. In Romania, it is the second type of cancer, after lung tumors [2]. It is the 3rd most common form of cancer in men and the 2nd in women in our country [3]. The prevalence of colorectal cancer patients hospitalized in Romania was 116.83/100,000 [4]. 3-10% of all colon cancer cases present as an emergency with perforations [5]. As far as we know, there are no published studies on the epidemiology of perforated colon tumors in Romania. In the guidelines of the European Society of Medical Oncology and according to the national cancer network, perforated colon tumors are considered a negative prognostic factor [6, 7].

The characteristics of perforation of a colon tumor depend on the site of the perforation, which can present as a tumor perforation or as a diastatic perforation, distant from the tumor site [8]. The perforation is a rare complication of colon tumors that calls for emergency surgical intervention. The surgical treatment of perforated colon tumors has changed over time, but long-term prognosis does not seem to be influenced by perforation itself [8]. In [9], perforations were most frequently located in the sigmoid colon (47.3%), followed by perforations in the cecum (24.8%). Several studies have shown that perforations proximal to the tumor had a worse prognosis than those located at the tumor level [10, 11]. According to literature data, most perforated tumors are in stage IV pTNM [12] and are usually accompanied by vascular invasion [13-16]. Perforations were statistically associated with rectal location [17].

The role of angiogenesis in perforated colon tumors has been analyzed in several studies, which showed that perforated tumors express less angiogenesis by immunohistochemical analysis of TP53, VEGF, and CD31 [18-20]. Colonic perforation complications have a dismal prognosis, the mortality rate in perforated colon tumors is 30-40% [21]. Research on the physiopathological factors or

Table 1. Repartition of cases according to gender

		Frequency	Percent	Valid percent	Cumulative percent
Valid	F	9	47.4	47.4	47.4
	M	10	52.6	52.6	100
	Total	19	100	100	

Table 2. Repartition of cases according to age

	N	Minimum	Maximum	Mean	Standard deviation
Age	19	27	92	67.74	18.740
Valid N (listwise)	19				

Table 3. Repatition of cases according to the symptomatology at admission

Symptoms				
		Frequency	Percent	Valid percent
Valid	Abdominal pain	19	100	100
	Flatulence	15	78.94	78.94
	Vomiting	13	68.42	68.42
	Absence of intestinal transit	14	73.68	73.68

Table 4. Repartition of cases according to the grading

	Frequency	Percent	Valid percent	Cumulative percent
Valid	G1	2	10.5	10.5	10.5
	G2	12	63.2	63.2
	G3	5	26.3	26.3
	Total	19	100	100

Table 4. Repartition of cases according to the grading

		Frequency	Percent	Valid percent	Cumulative percent
Valid	G1	2	10.5	10.5	10.5
	G2	12	63.2	63.2	63.2
	G3	5	26.3	26.3	26.3
	Total	19	100	100	

Table 5. Repartition of cases according to the postoperative mortality

	Frequency	Frequency	Percent	Valid percent	Cumulative percent
Valid	>48 postoperative hours	2	10.5	10.5	10.5
	>96 postoperative hours	3	15.8	15.8	26.3
	<48 postoperative hours	2	10.5	10.5	36.8
	No	12	63.2	63.2	100
	Total	19	100	100	

mechanisms that underlie tumor invasion of the serosa and lead to perforation in the peritoneal cavity is limited.

The aim of this work was to analyze the pathological and immunohistochemical factors of tumor neoangiogenesis that, at least from a theoretical point of view, could influence tumor perforation in colorectal cancers.

MATERIAL AND METHODS

A retrospective study of 451 cases of complicated colorectal carcinomas, between 2009-2018, hospitalized in the I and II surgery clinics of the "Sf. Ap. Andrei" County Emergency Clinical Hospital, of which 4.21% (19 cases) were sigmoid tumors with perforation. The observation sheets, histopathological and immunohistochemical examinations were analyzed. The database was created in Excel sheet, the information from the database was entered in the form of numerical (quantitative) and nominal (attributive) variables. The software package used was IBM SPSS statistics version 23.

RESULTS

A total of 58 perforations were identified in the studied group, of which 6 were diastatic perforations and 52 were tumor perforations. Thus, there were 8 perforations at the cecum-ascendant level, 9 at the transverse level, 12 at the descending level, 19 at the sigmoid and 10 at the level of the rectosigmoid junction.

Statistically analyzing the 19 cases of perforated sigmoid tumors, we found that the male gender predominated, in a proportion of 52.6% of the cases (**Table 1**). The patients came in most cases from rural areas (57.9%), with an average age of 68 (**Table 2**). In all cases, the patients had abdominal pain upon admission, followed by abdominal flatulence in 78.94% of cases and the absence of intestinal transit in 73.68% of cases (**Table 3**). As an associated pathology, cardiac pathology was frequently found in 36.84% of cases, followed by respiratory diseases and type II diabetes. In all analyzed cases, Hartmann's colectomy was performed. The histopathological examination
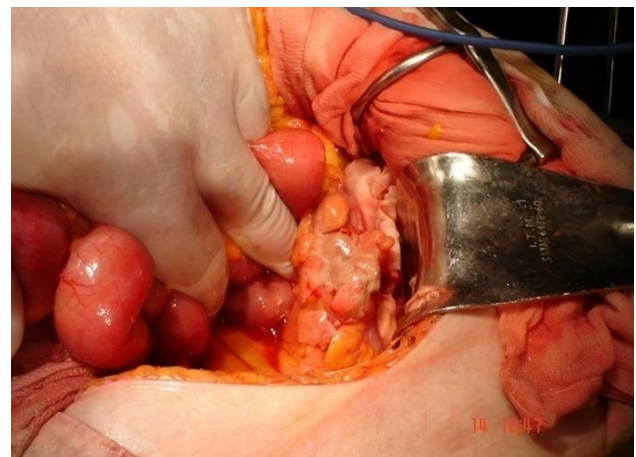

Figure 1. Perforated sigmoid tumor (surgery II clinic) (Source: Personal archive from Emergency Clinical Hospital Galati, reprinted with permission of the patient)

of the operative parts highlighted the frequency of medium differentiated G2 adenocarcinoma in 63.2% of cases (**Table 4**). The death rate was 36.84% of the cases, most at more than 96 hours postoperatively (**Table 5**).

CASE REPORT

We present the case of a 69 year old patient, who was admitted to the surgery II clinic of the "St. Ap. Andrei" Emergency Hospital Galati, for diffuse abdominal pain, abdominal flatulence, absence of the intestinal transit, vomiting, symptoms which started 4 days before admission. From paraclinical laboratory explorations, WBC 15,450/mmc, HB 11.5 g/dl, serum potassium 2.9 mmol/L were highlighted; on empty abdominal X-ray, paraumbilical hydroaerial levels were described; on ultrasound, distended intestinal loops with accentuated peristalsis and liquid content.

Emergency surgery was performed for the diagnosis of intestinal occlusion. Intraoperatively, intestinal occlusion due to perforated sigmoid tumor was found (**Figure 1**), with localized peritonitis and retractile mesenteritis. We performed a Hartmann type colectomy (**Figure 2**), with favorable postoperative evolution.
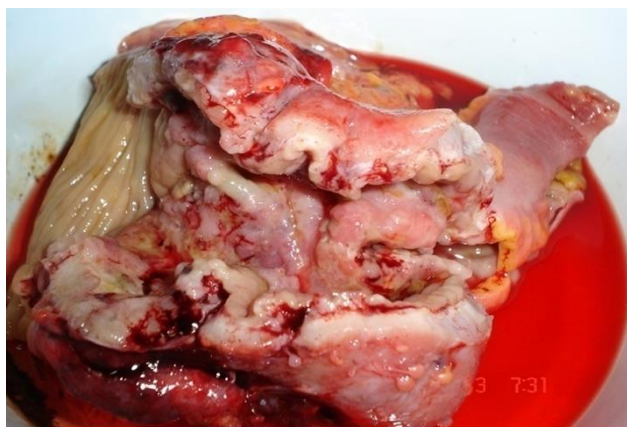
Figure 2. Stenotic perforated sigmoid tumor–Rezection piece longitudinally sectioned (surgery II clinic) (Source: Personal archive from Emergency Clinical Hospital Galati, reprinted with permission of the patient)

Table 6. The antibodies used for immunohistochemical tests

PAU	Kit	Clone	Producer	Platform
CK7	CONFIRM anti-cytokeratin 7 rabbit monoclonal primary antibody	SP52	Ventana	Automatised
CK20	CONFIRM anti-cytokeratin 7 rabbit monoclonal primary antibody	SP33	Ventana	Automatised
CDX2	CDX-2	EPR2764Y	Cell marque	Automatised
Bcl-2	CONFIRM anti-bcl-2 mouse monoclonal primary antibody	124	Ventana	Automatised
P53	Anti-p53 primary antibody	Bp53-11	Ventana	Automatised
mCEA	CEA mouse monoclonal antibody	CEA31	Cell marque	Automatised
ß-catenin	Beta-catenin	14	Cell marque	Automatised

Note. PAU: Primary antibodies used

Tissue biopsy was sent to the histopathology laboratory; 6 paraffin blocks were processed. 10 slides were prepared which stained with hematoxylin and eosin stain. 7 immunohistochemical markers were performed on the selected block.

An automatic staining device was used for the immunohistochemical tests (**Table 6**).

The descriptive histopathological report of the operative piece was of a fragment of the colonic wall with minimal marginally intercepted mucosa showing at the level of the muscularis propria and subserosa malignant tumor infiltration made up of large neoplastic cells, with abundant eosinophilic cytoplasm, with moderate nuclear pleomorphism, some hyperchromatic nuclei, with condensed chromatin, others vesicular, with proeminent eosinophilic nucleoli, with intense mitotic activity (28 typical and atypical mitoses/10 microscopic fields of objective 40/0.55); the tumor cells are arranged in the form of glandular structures, with complex architecture and zonal formation of cribriform areas, some with necrotic material and cellular detritus in lumen–appearance of colonic adenocarcinoma, moderately differentiated histologically (G2); marked stromal desmoplastic reaction; zonal tumor necrosis with hematic overexpression; moderately associated polymorphous inflammatory infiltrate (lymphocytes, plasma cells, polymorphonuclear neutrophils and eosinophils) arranged diffusely interstitially; reduced intratumoral lymphocyte response (1 lymphocyte/microscopic objective field 40/0.55); present perivascular invasion; present lymphovascular invasion (LV1), present perineural invasion (Pn1).

Tumor proliferation extends intramurally to the level of pericolic adipose tissue and at certain levels submillimetre distance from the mesothelium; one of the fragments shows the appearance of neoplastic proliferation with adipocytic entourage and extension to the level of the serosa with its excess (pT4a). Mesothelium-lined tissue fragments with focally prominent underlying hematic extravasates and mixed inflammatory overcolonization of diffuse disposition with zonal perivascular and perineural aggregation (perivegetoneuritis appearance); areas of necrosis with hematic extravasation and massive neutrophilic granulocytic colonization (appearance of acute abscessed epiloitis), with marginal areas of liponecrosis and histocytic rearrangement. Undetectable microorganisms in the necrotic areas (**Figure 3**, **Figure 4**, and **Figure 5**).
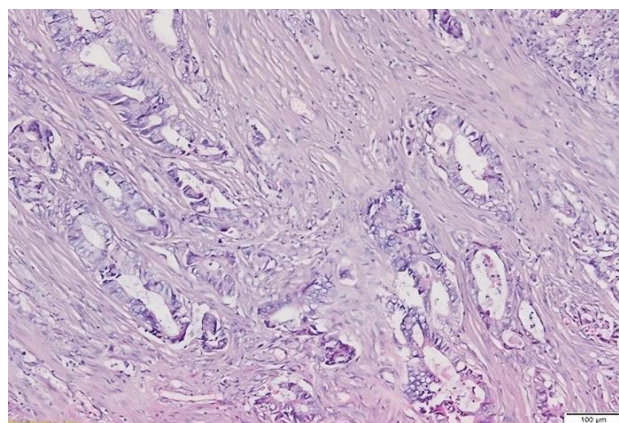
Figure 3. Deep muscularis propria with appearance of adenocarcinomatous remodeling: irregular glandular inserts, with intracytoplasmic mucin vacuoles and hemato-leukocyte accumulation in sporadic lumens (10x) (Source: Personal archive from Emergency Clinical Hospital Galati, reprinted with permission of the patient)

Figure 4. Neoplastic extension in the pericolonic adipose tissue, with a large area of extensive tumor necrosis, with mixed hematic and inflammatory overcolonization, with frequent neutrophilic granulocytes and eosinophils (10x) (Source: Personal archive from Emergency Clinical Hospital Galati, reprinted with permission of the patient)

Figure 5. Perivascular neoplastic extension and small intravenous neoplastic embolus (10x) (Source: Personal archive from Emergency Clinical Hospital Galati, reprinted with permission of the patient)

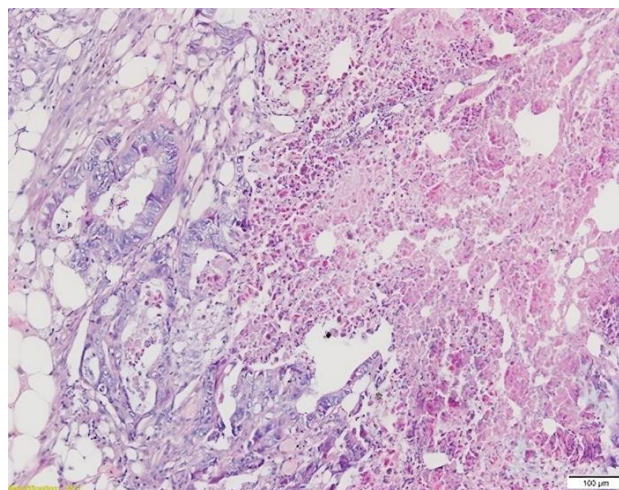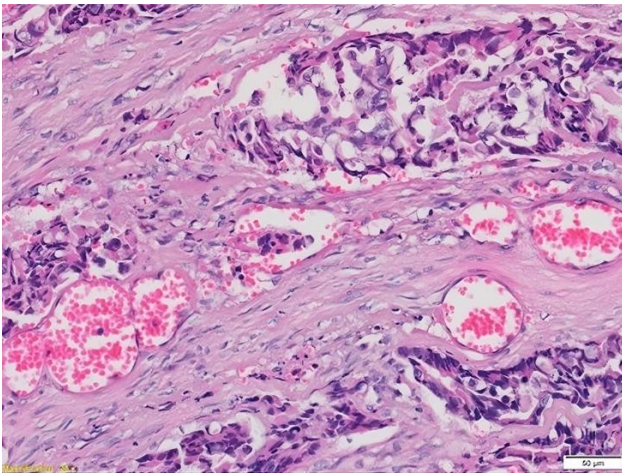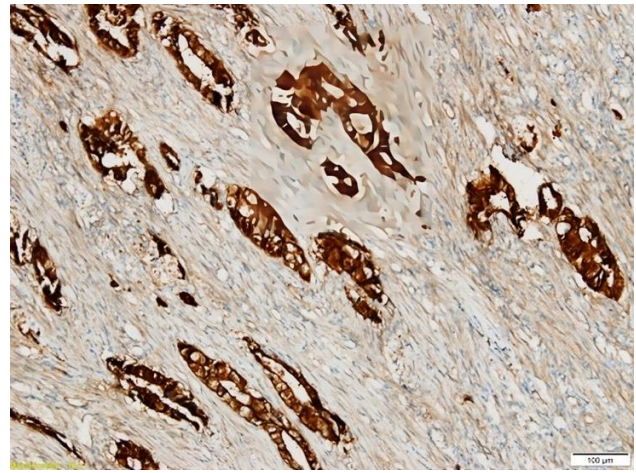**Figure 7.** Immunohistochemical reactivity for beta catenin: submembrane cytoplasmic reaction and nuclear accumulation, high intensity, diffuse in tumor cells (10x) (Source: Personal archive from Emergency Clinical Hospital Galati, reprinted with permission of the patient)
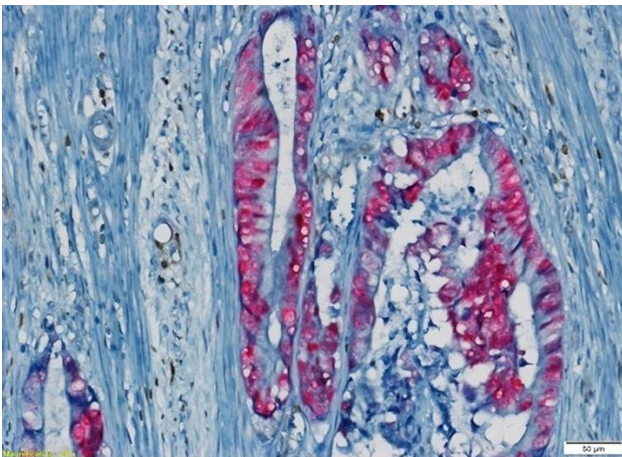
β-catenin: Predominantly cytoplasmic and submembrane, zonally nuclear, high-intensity, diffuse positive reaction in tumor cells–nuclear accumulation suggests abnormal functioning of the Wnt-beta catenin pathway (**Figure 7**).

In conclusion, the case analyzed from the histopathological and immunohistochemical point of view is a conventional colonic Adenocarcinoma with low histological grade G2 (moderately differentiated), invasive in the serous (pT4a); lymphovascular invasion present (LV1); perineural invasion present (Pn1), indeterminate nodal status in the absence of information (pNX), indeterminate resection (RX), epiploic tissue with hematic extravasation, mixed inflammatory infiltration, areas of abscessation and hematoleukocyte serositis; immunophenotype: CK7-, CK20+, CDX2+, mCEA+, nuclear beta catenin; p53+ (diffuse), bcl2-.

Figure 6. Dual immunohistochemical staining for bcl2 and p53: Cytoplasmic brown reaction (blc2) in peritumoral lymphocytes; red nuclear reaction (p53), of predominantly high intensity, in very common tumor cells (Source: Personal archive from Emergency Clinical Hospital Galati, reprinted with permission of the patient)

The results of the immunohistochemical tests were the following (**Table 6**):

CK7: Absent (negative) reaction in tumor cells. Positive cytoplasmic reaction, in rare mesothelial cells.

CK20: Cytoplasmic positive reaction, high intensity, diffuse in tumor cells.

CDX2: Diffuse, high-intensity nuclear positivity in tumor cells. High-intensity nuclear positivity in epithelial cells of the remaining colonic mucosa.

Bcl2-p53. Red nuclear reaction (p53), of high intensity, in frequent invasive tumor cells (70%)–the appearance of the color reaction suggests the existence of a mutation in the TP53 gene. Brown cytoplasmic reaction (blc2) of variable intensity, in inflammatory cells and absent (negative) in tumor cells (**Figure 6**).

CEA: Cytoplasmic positivity of variable intensity, predominantly moderate and high, diffuse in tumor cells.

DISCUSSION

In the presented case, we identified a perforated sigmoid tumor of adenocarcinoma type NOS G2, pT4aNx LV1, Pn1, CK7, CK20+, CDX2+, p53+. Bcl2-, CEA+, βcatenin+. If the immunohistochemical markers characteristic of colorectal cancer are also identified in this patient (CK7-, CK20+, CDX2+, CEA+), the immunohistochemical profile with p53+. Bcl2-, and βcatenin+ can explain the local aggressive character of this tumor with free perforation and lymphovascular and perineural invasions present.

Some studies show that patients with tumor perforation have a poor prognosis even after curative resection of colon cancer, if it is in the Dukes B stage [22-25].

Colorectal carcinoma presents several genetic alterations, of which p53 deletions and point mutations are present most frequently [26, 27]. Sporadic cancer is characterized by TP53 mutations [28].

The tumor suppressor protein p53 (encoded by TP53) is involved in DNA damage repair, cell cycle regulation, apoptosis, aging and cellular senescence and it is mutated in ~50% of cancers [29-31]. Immunohistochemical detection of mutant p53 proteins was the first molecular parameter examined in the context of the search for new prognostic

markers in colorectal carcinomas. Both loss of p53 protein expression and overexpression of bcl-2 proteins lead to cancer cell immortality by inhibiting apoptotic cell death processes [32].

A major inhibitor of angiogenesis is the suppressor gene p53. This gene arrests cell cycle progression under non-viable conditions by inducing hypoxia-mediated apoptosis [33]. The function of P53 is mediated by the proteins it produces, which arrest cells in the G1 phase of the cell cycle or induce apoptosis upon detection of DNA damage. The role of nuclear accumulation of p53 protein as a prognostic factor in human cancer has been extensively studied lately. However, there is no conclusive evidence regarding its usefulness in assessing the prognosis of patients [34]. Over the years, several studies have been carried out that had controversial results regarding the histochemical role of p 53 expression [35-40].

Although the study in [41] conducted in 2001 could not demonstrate a significant association between p53 expression levels and clinical outcomes in colon cancer patients, in 2004, it was shown that p53 can be a potential biological diagnostic marker [42], and it was shown that p53 status is associated with the prognosis of colon cancer [43].

The immunohistochemical expression of the p53 protein was identified as an independent prognostic factor in CRC patients; p53 overexpression being associated with a reduced survival rate [44-47].

Another study conducted in 2004 showed that right-sided colon tumors can develop in a p53-independent manner and therefore p53 status in cancer cells has prognostic value only for left-sided colorectal tumors [48].

However, it was noted that loss of p53 expression in colon cancer may be a predictor of a more aggressive tumor phenotype and that other clinicopathological features may also require consideration along with p53 status to design an effective strategy follow-up to improve patient survival [28].

However, the association between immunohistochemical expression of p53 and clinicopathological features in CRC has not been fully elucidated. p53 immunoreactivity has been considered to be closely associated with clinicopathological variables, including pathologic type, lymphocytic infiltration, degree of tumor differentiation and tumor location in CRC [45]. The study in [49] highlights that p53 overexpression is associated with well or moderately differentiated tumors, located in the rectum and with better overall survival.

Beta-catenin has been identified as an integral membrane protein that, in association with e-cadherin, facilitates cell-cell interactions [50]. Beta-catenin function is under the control of the intracellular Wnt signaling pathway. Mutations in APC genes, beta-catenin genes and genes of various important components of the Wnt signaling pathway cause intracellular overaccumulation of beta-catenin protein, followed by its nuclear translocation. The intranuclear presence of beta-catenin stimulates the activity of various other transcription factors leading to cell proliferation and tumorigenesis [51, 52].

The most common genetic abnormality found in colorectal tumorigenesis is the mutation of the APC gene, which is an important component of the Wnt signaling pathway. Dysfunction in the Wnt pathway causes abnormal localization and nuclear accumulation of beta-catenin [51]. Because colorectal tumorigenesis that occurs as a result of mutation in the APC gene (85% of cases) follows a gradual sequence in several steps, the expression of beta-catenin by immunohistochemistry (nuclear positivity) can be used to determine the malignant potential of colorectal polyps and adenomas [53, 54]. The identification of the presence of beta-catenin increases the possibility of prophylactic interventions and sustained follow-up of benign tumors, thus preventing the development of a malignancy [55].

A study conducted in 2019 performed a descriptive evaluation of beta-catenin expression in various colorectal neoplasms. The result showed the gradual intracellular translocation of beta-catenin from membranous to nuclear expression in a stepwise manner following the sequence polyp---adenoma---carcinoma, which confirmed the findings in [52, 55]. Also in this study, a statistically significant positive correlation was obtained between the subcellular localization of beta-catenin together with the membranous, cytoplasmic and nuclear scores corresponding to the AJCC-TNM stage (r = 0.512; p < 0.001) of colorectal adenocarcinoma, in accordance with the research in [52, 56].

Angiogenesis is an essential process for the growth and progression of solid tumors in general. In in vitro experiments, the expression of an anti-apoptotic protein in endothelial cells (Bcl-2) is stimulated.

Bcl-2 expression has been shown to inhibit apoptosis of tumor cells [57, 58]. However, in most clinical trials, cytoplasmic bcl-2 expression has been correlated with better prognosis [59-62]. In patients with colorectal carcinoma, it was observed a direct association of bcl-2 expression with a favorable prognosis [35]. Similar results were also reported in [38]. In contrast, the correlation of bcl-2 with a low survival rate was reported by Bhatavdekar et al. on a group of 48 patients. The combined prognostic role of p53 and bcl-2 expression has also been investigated in recent studies. It was reported that a group of colorectal adenocarcinoma patients with the most clinically indolent phenotype had tumors with the presence of bcl-2 expression and the absence of nuclear p53 expression [38].

Microsatellite instability (MSI) is another important biomarker in colorectal cancer, with a crucial diagnostic, prognostic and predictive role [59]. The role of mismatch repair deficiency (MMR-D) in colorectal cancer has been explored since the nineties. Knowing the molecular mechanisms of MMR-D in colorectal cancers has led to the characterization of a subset of colorectal cancer with distinctive molecular and clinicopathologic features [61]. MSI can now be detected with 2 different approaches: immunohistochemistry (IHC) and polymerase chain reaction (PCR). IHC searches for MLH1, MSH2, MSH6, and PMS2 staining on tumor samples to identify the loss of protein expression that characterizes MMR-D as a surrogate for MSI [61].

Circulating tumor DNA (ctDNA) is another promising prognostic and predictive biomarker in the personalized management of patients with [62], but there aren't any studies yet that include only perforated colon tumors. Prospective studies such as CIRCULATE, COBRA, Dynamic II/III, or ACT3 use ctDNA to guide management of patients with CRC.

In the literature, we did not find any published studies on the immunohistochemical profile of perforated colon tumors. Only one very recent study, from 2022 [63], also includes perforated or obstructive tumors in the analysis. The authors show that 66.4% of the cases were CDX2-positive tumors, as is evident from our study, and the loss of immunohistochemical expression of this marker would be associated with rectal

localization, poor differentiation, advanced disease stage and lymphovascular invasion.

CONCLUSIONS

We conclude that p53 mutations, bcl-2 expression and tumor angiogenesis are events related to the process of metastasis and local invasion in colorectal carcinoma. The interplay between bcl-2, p53 and angiogenesis, as well as their combined prognostic role in colorectal carcinoma, deserves further investigation.

REFERENCES

1. Xie Y, Shi L, He X, Luo Y. Gastrointestinal cancers in China, the USA and Europe. Gastroenterol Rep (Oxf). 2021;9(2):91-104. https://doi.org/10.1093/gastro/goab010 PMid: 34026216 PMCid:PMC8128023

2. Gheju A, Jurescu A, Tăban S, Al-Jobory D, Lazăr F, Dema A. Different disease characteristics in young patients with colorectal cancer: A large retrospective study in a city in Romania. J Int Med Res. 2021;49:5. https://doi.org/10.1177/03000605211016630 PMid:34034541 PMCid:PMC8161876

3. Valean S, Chira R, Dumitrascu D. Epidemiological trends in digestive cancers in Romania, 1955-2012, compared to alcohol consumption. Correlation or coincidence? Clujul Med. 2018;91(4):376-86. https://doi.org/10.15386/cjmed-1067 PMid:30564012 PMCid:PMC6296719

4. Ionescu EM, Tieranu CG, Maftei D. Colorectal cancer trends of 2018 in Romania–An important geographical variation between northern and southern lands and high mortality versus European averages. J Gastrointest Canc. 2021; 52:222-8. https://doi.org/10.1007/s12029-020-00382-3 PMid:32152824

5. Chen TM, Huang YT, Wang GC. Outcome of colon cancer initially presenting as colon perforation and obstruction. World J Surg Onc. 2017;15:164. https://doi.org/10.1186/s12957-017-1228-y PMid:28841901 PMCid:PMC5574146

6. Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K. ESMO consensus guidelines for management of patients with colon and rectal cancer: A personalized approach to clinical decision making. Ann Oncol Off J Eur Soc Med Oncol. 2012;23:2479-516. https://doi.org/10.1093/annonc/mds236 PMid:23012255

7. Benson AB, Venook AP, Bekaii-Saab T, Chan E, Chen YJ, Cooper HS. Colon cancer, version 3.2014. J Natl Compr Cancer Netw. 2014;12:1028-59. https://doi.org/10.6004/jnccn.2014.0099 PMid:24994923

8. Otani K, Kawai K, Hata K. Colon cancer with perforation. Surg Today. 2019;49:15-20. https://doi.org/10.1007/s00595-018-1661-8 PMid:29691659

9. Tan KK, Hong CC, Zhang J, Liu JZ, Sim R. Predictors of outcome following surgery in colonic perforation: An institution's experience over 6 years. J Gastrointest Surg. 2011; 15(2):277-84. https://doi.org/10.1007/s11605-010-1330-8 PMid:20824374

10. Tan KK, Hong CC, Zhang J, Liu JZ, Sim R. Surgery for perforated colorectal malignancy in an Asian population: An institution's experience over 5 years. Int J Colorectal Dis. 2010; 25(8):989-95. https://doi.org/10.1007/s00384-010-0945-2 PMid:20390285

11. Khan S, Pawlak SE, Eggenberger JC, Lee CS, Szilagy EJ, Margolin DA. Acute colonic perforation associated with colorectal cancer. Am Surg. 2001;67(3):261-4. https://doi.org/10.1177/000313480106700312 PMid: 11270886

12. Gök MA, Kafadar MT, Yeğen SF. Perforated colorectal cancers: Clinical outcomes of 18 patients who underwent emergency surgery. Prz Gastroenterol. 2021;16(2):161-5. https://doi.org/10.5114/pg.2021.106667 PMid:34276844 PMCid:PMC8275966

13. Constantin GB, Firescu D, Voicu D, et al. The influence of the type of surgery on the immediate postoperative results in patients with colorectal cancer operated in emergency. Chirurgia. 2020;115(2):227-35. https://doi.org/10.21614/chirurgia.115.2.227 PMid:32369727

14. Medina-Arana V, Martínez-Riera A, Delgado-Plasencia L, et al. Clinicopathological analysis of factors related to colorectal tumor perforation: Influence of angiogenesis. Med (Baltimore). 2015;94(15):e703. https://doi.org/10.1097/MD.0000000000000703 PMid:25881846 PMCid: PMC4602503

15. Mihailov R, Firescu D, Constantin GB, et al. Nomogram for prediction of postoperative morbidity in patients with colon cancer requiring emergency therapy. Med Sci Monit. 2022;28:e936303. https://doi.org/10.12659/MSM.936303 PMid:35768977 PMCid:PMC9254725

16. Buzia OD, Madalina C, Tatu AL, et al. Stability and microbiological analysis of aloe barbadensis and chamomilla matricaria pharmaceutical forms. Rom J Oral Rehabil. 2022;14(1):216-28.

17. Rodríguez-González D, Martínez-Riera A, Delgado-Plasencia L, et al. Lymphs nodes and lymph node ratio as predictive factors of survival in perforated and non-perforated T4 colorectal tumors. J Surg Oncol. 2013;108:176-81. https://doi.org/10.1002/jso.23373 PMid: 23832524

18. Barbera-Guillem E, Nelson B, Barr B, et al. B lymphocyte pathology in human colorectal cancer. Experimental and clinical therapeutic effects of partial B cell depletion. Cancer Immunol Immunother. 2000;48:541-9. https://doi.org/10.1007/PL00006672 PMid:10630306 PMCid:PMC11037163

19. Minhajat R, Mori D, Yamasaki F, et al. Endoglin (CD105) expression in angiogenesis of colon cancer: Analysis using tissue microarrays and comparison with other endothelial markers. Virchows Arch. 2006;448:127-34. https://doi.org/10.1007/s00428-005-0062-8 PMid:16177881

20. Markowitz SD, Bertagnolli MM. Molecular basis of colorectal cancer. N Engl J Med. 2009;361:2449-60. https://doi.org/10.1056/NEJMra0804588 PMid:20018966 PMCid:PMC2843693

21. Gök MA, Kafadar MT, Yeğen SF. Perforated colorectal cancers: Clinical outcomes of 18 patients who underwent emergency surgery. Prz Gastroenterol. 2021;16(2):161-5. https://doi.org/10.5114/pg.2021.106667 PMid:34276844 PMCid:PMC8275966

22. Saha AK, Smith KJ, Sue-Ling H, Sagar PM, Burke D, Finan PJ. Prognostic factors for survival after curative resection of Dukes' B colonic cancer. Colorectal Dis. 2011;13:1390-4. https://doi.org/10.1111/j.1463-1318.2010.02507.x PMid:21073647

23. Constantin GB, Firescu D, Voicu D, et al. The importance of systemic inflammation markers in the survival of patients with complicated colorectal cancer, operated in emergency. Chirurgia. 2020;115(1):39-49. https://doi.org/10.21614/chirurgia.115.1.39 PMid:32155398

24. Mihailov R, Firescu D, Voicu D, et al. Challenges and solutions in choosing the surgical treatment in patients with complicated colon cancer operated in an emergency– A retrospective study. Chirurgia. 2021;116(3):312-0. https://doi.org/10.21614/chirurgia.116.3.312 PMid:34191712

25. Mihailov R, Firescu D, Constantin GB, et al. Mortality risk stratification in emergency surgery for obstructive colon cancer-external validation of international scores, american college of surgeons national surgical quality improvement program surgical risk calculator (SRC), and the dedicated score of french surgical association (AFC/OCC score). Int J Environ Res Public Health. 2022;19(20):13513. https://doi.org/10.3390/ijerph192013513 PMid:36294094 PMCid:PMC9603747

26. Constantin GB, Firescu D, Mihailov R, et al. A novel clinical nomogram for predicting overall survival in patients with emergency surgery for colorectal cancer. J Pers Med. 2023;13(575):1-17. https://doi.org/10.3390/jpm13040575 PMid:37108961 PMCid:PMC10145637

27. Eguchi K, Yao T, Konomoto T, et al. Discordance of P53 mutations of synchronous colorectal carcinomas. Mod Pathol. 2000;13:131-9. https://doi.org/10.1038/modpathol.3880024 PMid:10697269

28. Conlin A, Smith G, Carey FA, Wolf CR, Steele RJC. The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. BMJ. 2005;54:1283-6. https://doi.org/10.1136/gut.2005.066514 PMid:15843421 PMCid:PMC1774675

29. Da-Zhong C, Xi-Long O, Ting Y. The association of p53 expression levels with clinicopathological features and prognosis of patients with colon cancer following surgery Oncol Lett. 2017;13(5):3538-46. https://doi.org/10.3892/ol.2017.5929 PMid:28521456 PMCid:PMC5431169

30. Allegra CJ, Paik S, Colangelo LH, et al. Prognostic value of thymidylate synthase, Ki-67, and p53 in patients with Dukes' B and C colon cancer: A national cancer institute-national surgical adjuvant breast and bowel project collaborative study. J Clin Oncol. 2003;21:241-50. https://doi.org/10.1200/JCO.2003.05.044 PMid:12525515

31. Luțenco V, Rebegea L, Beznea A, et al. Innovative surgical approaches that improve individual outcomes in advanced breast cancer. Int J Womens Health. 2024;16:555-60. https://doi.org/10.2147/IJWH.S447837 PMid:38577150 PMCid:PMC10992669

32. Feroz W, Sheikh AMA. Exploring the multiple roles of guardian of the genome: P53. Egypt J Med Hum Genet. 2020;21:49. https://doi.org/10.1186/s43042-020-00089-x

33. Hassin O, Oren M. Drugging p53 in cancer: One protein, many targets. Nat Rev Drug Discov. 2023;22:127-44. https://doi.org/10.1038/s41573-022-00571-8 PMid:36216888 PMCid:PMC9549847

34. Hu X, Xue Z, He K, et al. Strategies to optimize treatment for locally advanced rectal cancer. Cancers. 2023;15:219. https://doi.org/10.3390/cancers15010219 PMid:36612213 PMCid:PMC9818694

35. Liebl MC, Hofmann TG. The role of p53 signaling in colorectal cancer. Cancers. 2021;13:2125. https://doi.org/10.3390/cancers13092125 PMid:33924934 PMCid:PMC8125348

36. Kruschewski M, Mueller K, Lipka S, et al. The prognostic impact of p53 expression on sporadic colorectal cancer is dependent on p21 status. Cancers. 2011;3:1274-84. https://doi.org/10.3390/cancers3011274 PMid:24212661 PMCid:PMC3756413

37. Wang H. MicroRNAs and apoptosis in colorectal cancer. Int J Mol Sci. 2020;21:5353. https://doi.org/10.3390/ijms21155353 PMid:32731413 PMCid:PMC7432330

38. Taha A, Taha-Mehlitz S, Petzold S, et al. Prognostic value of immunohistochemical markers for locally advanced rectal cancer. Molecules. 2022;27:596. https://doi.org/10.3390/molecules27030596 PMid:35163861 PMCid:PMC8839263

39. Rahman N, Khan H, Zia A, et al. Bcl-2 modulation in p53 signaling pathway by flavonoids: A potential strategy towards the treatment of cancer. Int J Mol Sci. 2021;22:11315. https://doi.org/10.3390/ijms222111315 PMid:34768743 PMCid:PMC8582810

40. Michel M, Kaps L, Maderer A, Galle PR, Moehler M. The role of p53 dysfunction in colorectal cancer and its implication for therapy. Cancers. 2021;13:2296. https://doi.org/10.3390/cancers13102296 PMid:34064974 PMCid:PMC8150459

41. Kasurinen JH, Hagström J, Kaprio T, et al. Prognostic values of tissue and serum angiogenic growth factors depend on the phenotypic subtypes of colorectal cancer. Cancers. 2023;15:3871. https://doi.org/10.3390/cancers15153871 PMid:37568687 PMCid:PMC10417397

42. Watanabe T, Wu TT, Catalano PJ, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med. 2001;344:1196-206. https://doi.org/10.1056/NEJM200104193441603 PMid:11309634 PMCid:PMC3584633

43. Resnick MB, Routhier J, Konkin T, Sabo E, Pricolo VE. Epidermal growth factor receptor, c-MET, beta-catenin, and p53 expression as prognostic indicators in stage II colon cancer: A tissue microarray study. Clin Cancer Res. 2004;10:3069-75. https://doi.org/10.1158/1078-0432.CCR-03-0462 PMid:15131045

44. Nasif WA, El-Emshaty HM, Tabll A, Elmasry SA, Attallah AM, Elhak Gad NA. Immunoreactivity evaluation of mutant p53 gene product with DNA ploidy pattern in colorectal carcinoma. Hepatogastroenterology. 2004;51:1001-6.

45. Bouzourene H, Gervaz P, Cerottini JP, et al. p53 and Ki-ras as prognostic factors for Dukes' stage B colorectal cancer. Eur J Cancer. 2000;36:1008-15. https://doi.org/10.1016/S0959-8049(00)00036-8 PMid:10885605

46. Perrone G, Vincenzi B, Santini D, et al. Correlation of p53 and bcl-2 expression with vascular endothelial growth factor (VEGF), microvessel density (MVD) and clinico-pathological features in colon cancer. Cancer Lett. 2004; 208:227-34. https://doi.org/10.1016/j.canlet.2003.11.032 PMid:15142682

47. Pu XX, Huang GL, Guo HQ, et al. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. J Gastroenterol Hepatol. 2010;25: 1674-80. https://doi.org/10.1111/j.1440-1746.2010.06417.x PMid:20880178

48. El-Serafi MM, Bahnassy AA, Ali NM, et al. The prognostic value of c-Kit, K-ras codon 12, and p53 codon 72 mutations in Egyptian patients with stage II colorectal cancer. Cancer. 2010;116:4954-64. https://doi.org/10.1002/cncr.25417 PMid:20652953

49. Tocu G, Mihailov R, Serban C, Stefanescu BI, Tutunaru D, Firescu D. The contribution of procalcitonin, C-reactive Protein and Interleukin-6 in the diagnosis and prognosis of surgical sepsis: An observational and statistical study. J Multidiscip Healthc. 2023;16:2351-9. https://doi.org/10. 2147/JMDH.S422359 PMid:37601324 PMCid:PMC10439796

50. Lan YT, Chang SC, Li AF, et al. p53 protein accumulation as a prognostic marker in sporadic colorectal cancer. Int J Colorectal Dis. 2007;22:499-506. https://doi.org/10.1007/ s00384-006-0194-6 PMid:17021749

51. Li Y, Wang D, Ge H, Güngör C, Gong X, Chen Y. Cytoskeletal and cytoskeleton-associated proteins: Key regulators of cancer stem cell properties. Pharmaceuticals. 2022;15:1369. https://doi.org/10.3390/ph15111369 PMid: 36355541 PMCid:PMC9698833

52. Muto S, Enta A, Maruya Y, et al. Wnt/β-catenin signaling and resistance to immune checkpoint inhibitors: From non-small-cell lung cancer to other cancers. Biomedicines. 2023;11:190. https://doi.org/10.3390/biomedicines 11010190 PMid:36672698 PMCid:PMC9855612

53. Wong SC, Lo ES, Lee KC, Chan JK, Hsiao WL. Prognostic and diagnostic significance of β-catenin nuclear immunostaining in colorectal cancer. Clin Cancer Res. 2004;10:1401-8. https://doi.org/10.1158/1078-0432.CCR-0157-03 PMid:14977843

54. Vallée A, Lecarpentier Y, Vallée JN. The key role of the WNT/β-catenin pathway in metabolic reprogramming in cancers under normoxic conditions. Cancers. 2021;13:5557. https://doi.org/10.3390/cancers13215557 PMid:34771718 PMCid:PMC8582658

55. Zhu Y, Li X. Advances of Wnt signalling pathway in colorectal cancer. Cells. 2023;12:447. https://doi.org/10. 3390/cells12030447 PMid:36766788 PMCid:PMC9913588

56. Bhattacharya I, Barman N, Maiti M, Sarkar R. Assessment of beta-catenin expression by immunohistochemistry in colorectal neoplasms and its role as an additional prognostic marker in colorectal adenocarcinoma. Med Pharm Rep. 2019;92(3):246-52. https://doi.org/10.15386/ mpr-1218 PMid:31460505 PMCid:PMC6709962

57. Kobayashi M, Honma T, Matsuda Y, Suzuki Y. Narisawa R, Ajioka Y. Nuclear translocation of beta-catenin in colorectal cancer. Br J Cancer. 2000;82:1689-93.

58. Decuypere JP, Parys JB, Bultynck G. Regulation of the autophagic Bcl-2/beclin 1 interaction. Cells. 2012;1:284-312. https://doi.org/10.3390/cells1030284 PMid:24710477 PMCid:PMC3901098

59. Qiu Y, Li P, Ji C. Cell death conversion under hypoxic condition in tumor development and therapy. Int J Mol Sci. 2015;16:25536-51. https://doi.org/10.3390/ijms161025536 PMid:26512660 PMCid:PMC4632814

60. Ionescu VA, Gheorghe G, Bacalbasa N, Chiotoroiu AL, Diaconu C. Colorectal cancer: From risk factors to oncogenesis. Medicina. 2023;59:1646. https://doi.org/10. 3390/medicina59091646   PMid:37763765   PMCid: PMC10537191

61. Kunac N, Filipović N, Kostić S, Vukojević K. The expression pattern of Bcl-2 and Bax in the tumor and stromal cells in colorectal carcinoma. Medicina. 2022;58:1135. https://doi.org/10.3390/medicina58081135 PMid:36013602 PMCid:PMC9416041

62. Yang D, He L, Ma S, et al. Pharmacological targeting of Bcl-2 induces caspase 3-mediated cleavage of HDAC6 and regulates the autophagy process in colorectal cancer. Int J Mol Sci. 2023;24:6662. https://doi.org/10.3390/ijms 24076662 PMid:37047634 PMCid:PMC10095469

63. Singh J, Rajesh NG, Dubashi B, et al. Pattern of expression of CDX2 in colorectal cancer and its role in prognosis: An ambispective observational study. Indian J Med Paediatr Oncol. 2022;11:1750207. https://doi.org/10.1055/s-0042-1750207