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

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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
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

**Table 1. Repartition of cases according to gender**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid percent</th>
<th>Cumulative percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>9</td>
<td>47.4</td>
<td>47.4</td>
<td>47.4</td>
</tr>
<tr>
<td>M</td>
<td>10</td>
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<td>52.6</td>
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</tr>
<tr>
<td>Total</td>
<td>19</td>
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<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Repartition of cases according to age**

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valid</td>
<td>19</td>
<td>27</td>
<td>92</td>
<td>67.74</td>
<td>18.740</td>
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</tbody>
</table>

**Table 3. Repartition of cases according to the symptomatology at admission**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>19</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Flatulence</td>
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<td>78.94</td>
<td>78.94</td>
</tr>
<tr>
<td>Vomiting</td>
<td>13</td>
<td>68.42</td>
<td>68.42</td>
</tr>
<tr>
<td>Absence of intestinal transit</td>
<td>14</td>
<td>73.68</td>
<td>73.68</td>
</tr>
</tbody>
</table>

**Table 4. Repartition of cases according to the grading**

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid percent</th>
<th>Cumulative percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;48 postoperative hours</td>
<td>2</td>
<td>10.5</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>&gt;96 postoperative hours</td>
<td>3</td>
<td>15.8</td>
<td>15.8</td>
<td>26.3</td>
</tr>
<tr>
<td>&lt;48 postoperative hours</td>
<td>2</td>
<td>10.5</td>
<td>10.5</td>
<td>36.8</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>63.2</td>
<td>63.2</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5. Repartition of cases according to the postoperative mortality**

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid percent</th>
<th>Cumulative percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;48 postoperative hours</td>
<td>2</td>
<td>10.5</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>&gt;96 postoperative hours</td>
<td>3</td>
<td>15.8</td>
<td>15.8</td>
<td>26.3</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>10.5</td>
<td>10.5</td>
<td>36.8</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**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 “Sf. 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/mmcc, 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—Reztection 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

<table>
<thead>
<tr>
<th>PAU</th>
<th>Kit</th>
<th>Clone</th>
<th>Producer</th>
<th>Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK7</td>
<td>CONFIRM anti-cytokeratin 7 rabbit monoclonal primary antibody</td>
<td>SP52</td>
<td>Ventana</td>
<td>Automatised</td>
</tr>
<tr>
<td>CK20</td>
<td>CONFIRM anti-cytokeratin 7 rabbit monoclonal primary antibody</td>
<td>SP33</td>
<td>Ventana</td>
<td>Automatised</td>
</tr>
<tr>
<td>CDX2</td>
<td>CDX-2</td>
<td>EPR2764Y</td>
<td>Cell marque</td>
<td>Automatised</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>CONFIRM anti-bcl-2 mouse monoclonal primary antibody</td>
<td>124</td>
<td>Ventana</td>
<td>Automatised</td>
</tr>
<tr>
<td>PS3</td>
<td>Anti-p53 primary antibody</td>
<td>Bp53-11</td>
<td>Ventana</td>
<td>Automatised</td>
</tr>
<tr>
<td>mCEA</td>
<td>CEA mouse monoclonal antibody</td>
<td>CEA31</td>
<td>Cell marque</td>
<td>Automatised</td>
</tr>
<tr>
<td>β-catenin</td>
<td>Beta-catenin</td>
<td>14</td>
<td>Cell marque</td>
<td>Automatised</td>
</tr>
</tbody>
</table>

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 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 prominent 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 intramura 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 epiloiptis), 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)
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-.

**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 polypl-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
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.

Author contributions: RM, AB, & OMM: Writing -original draft; RM, DV, & OMM: investigation; RM & GT: formal analysis; RM & GS: project administration; RM: conceptualization; AB, AT, & GT: resources; AB, RB, & DEG: supervision; CP, DV, AT, & EN: methodology; IT & VL: data curation & software; LR & GBC: visualization; LR & RB: validation; & GBC & EN: writing -review & editing. All authors have agreed with the results and conclusions.

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Ethical statement: The authors stated that the study was conducted in accordance with the Declaration of Helsinki and the case presentation was approved by the Ethics Committee of the "SF. Ap. Andrei" Clinical Emergency Hospital from Galati, Romania (no. 25196/14.11.2023). Written informed consents were obtained from the patients.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

REFERENCES


