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The histopathological features and their prognostic impact in the postoperative follow-up of colorectal cancer patients

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Abstract

The validation of histological prognostic markers in colorectal cancer not only for survival parameters but also for early postoperative outcomes is of paramount importance. The aim of our study was to search for the tumor histopathological (HP) characteristics that may influence the postoperative morbidity, especially the occurrence of anastomotic leakage. Our results indicated that peritumoral inflammatory cell infiltrate appeared to correlate with both anastomotic fistula and overall postoperative complications. Likewise, high-grade and undifferentiated colorectal tumors seemed to be correlated with a higher incidence of postoperative leakage and complications. No relation could be established between the other HP features and the postoperative untoward outcomes.

Keywords: anastomotic leakage, risk factors, colon cancer, colorectal anastomosis.

Introduction

Colorectal cancer (CRC) is the most common type of abdominal malignancy and the third most lethal malignancy in the world [1]. Given the high mortality and increasing incidence, especially in young adults, numerous efforts are made to better understand the pathogenesis and to find new tools for fighting this disease [2, 3]. Tumor micro-environment plays a decisive role in its progression, acting as a mounting pad for local progression, but also as a potential modulating agent for the local inflammatory response [4]. Although the relation between inflammation and cancer is well recognized, the role of the colorectal tumor inflammatory environment on various therapeutic strategies remains obscure [5]. Likewise, other tumoral characteristics such as grading, and vascular and perineural invasion, may play a bigger role than expected in tumoral and host response in relation to the wide array of treatment options.

Surgery remains the cornerstone of CRC treatment, but it is associated with a high rate of postoperative complications, with anastomotic leakage (AL) being one of the most dreaded one. The morbidity associated to AL remained a crucial determinant for the early surgical outcomes in CRC surgery, with numerous attempts being made to better predict the risk for AL by analyzing a vast array of pre- and intraoperative variables [6–8]. Most of these attempts are either too complex or time consuming, and require additional resources and extended competences, making them unsuitable for current surgical practice. On the other hand, the role of the tumor, node, metastasis (TNM) system, as a simple and practical instrument for treatment planning and prognosis may be overrated, as there are many more local factors involved in tumor progression and therapeutic response. However, surprisingly little effort was made to analyze the impact of the basic histopathological (HP) characteristics of the tumor, such as local immune response, grading, perineural or vascular invasion. In most cases, these HP data are well documented and make a mandatory condition before any oncological surgical procedure.

Aim

The aim of this study was to identify the potential tumor HP characteristics that may hinder the anastomotic healing process and lead to postoperative fistula formation in patients undergoing surgery for left colon and rectal cancer. Additionally, we analyzed the impact of these HP features on the surgical-related postoperative complications.

Patients, Materials and Methods

Study population and design

This retrospective study included all cases with a confirmed diagnosis of left colon or rectal cancer that underwent colorectal anastomosis in a tertiary hospital between January 2017 and December 2021. The relevant

demographic and clinical data as well as the pathology reports were obtained from the electronic medical database of the Emergency County Hospital of Craiova, Romania. The study was conducted according to the Guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova (Protocol Code 71/28.04.2022). Informed consent was obtained from all subjects involved in the study.

The clinico-demographic variables consisted of aspects such as age, sex, comorbidities, type of admission, and clinical symptoms. Tumor variables included relevant data about primary site, HP subtype, dimensions, grading, local tumor extension, lymph node invasion, metastases, vascular and perineural invasion, and tumor inflammatory cell infiltrate. Surgical variables analysis mentioned essential intra- and postoperative aspects, including the surgical procedure, the type of anastomosis, surgical margins, and the occurrence of postoperative complications or AL.

The exclusion criteria referred to patients with clinically confirmed active infection or chronic inflammatory condition, those admitted in the emergency setting, and all cases undergoing colostomy or ileostomy. Postoperative complications were distributed into five groups according to the Clavien–Dindo classification, while the ALs were defined in accordance with the recommendations of the *International Study Group of Rectal Cancer* (ISREC), based on specific clinical or radiological findings [9, 10].

Tumor stage was classified according to the 7th edition of the *American Joint Committee on Cancer* (AJCC), while the analysis of the grading was based on the *World Health Organization* (WHO) Classification of Tumors [11–13].

Histological technique

The biological material harvested during surgery, consisting of the colon or rectal tumor together with the adjacent safety margins, underwent 10% (*v/v*) neutral buffered formalin tissue fixation, followed by washing with running tap water for one hour. Afterwards, the samples underwent successive passing of increasing concentrations of alcohol (70%, 90%, 96%, and 100%), three hours long xylene incubations, and paraffin impregnation at 56°C. The paraffin blocks were cut to a thickness of 4 μm using a HMB350 rotary microtome (ThermoScientific), and the sections were applied to poly-L-lysine treated slides followed by 24 hours drying in the thermostat set at 37°C. Subsequently, classical Hematoxylin–Eosin (HE) staining protocol was applied, and the slides were analyzed using light microscopy (Panthera L; Motic). The classification of the tumoral inflammatory infiltrate was performed in accordance to the Klintrup–Mäkinen (K–M) criteria on a scale from 0 to 3, where K–M class of "0" expressed no inflammatory cell infiltrate, class "1" corresponded to a reduced or patchy infiltrate, class "2" defined an important inflammatory cell infiltrate potentially associated with cancer cell destruction, while class "3" corresponded to "cup-like" florid peritumoral cell infiltrate [14]. Based on this initial evaluation of the pathology team, the peritumoral inflammatory reaction was further classified into low-score (K–M 0 and 1) and high-score (K–M 2 and 3). In randomly selected cases, the peritumoral inflammatory reaction was confirmed by immunohistochemical (IHC) staining for T- and B-type lymphocytes, as well as for macrophage markers [cluster of differentiation (CD)3, CD20, and CD68, respectively].

Statistical analysis

Microsoft Excel and IBM Statistical Package for Social Sciences (SPSS) version 20.0 software were used for data analysis. The categorical variables displayed as absolute and percent values, were analyzed using cross-tabulation and Fisher's exact test or χ^2 (*chi*-squared) test. The grouping variables (<anastomotic leakage> and <postoperative complications>) were analyzed as dichotomous variables. For ordinal values, Spearman's *rho* (ρ) was used to evaluate the strength of the relationship between variables. Statistically significant results were considered if $p<0.05$ corresponding to a 95% confidence interval (CI).

Results

Clinical characteristics of the enrolled patients

We selected 197 consecutive cases of left colon and rectal cancers that fit the diagnosis criteria and were admitted to our Surgical Department between 2017 and 2022, out of which 95 had a colorectal anastomosis performed. After applying the exclusion criteria, data on 91 patients was included (54 males and 38 females), with a median of 67 years (range 27–88 years).

End-to-end hand-sewn anastomosis was performed in 64 (70.33%) cases, while mechanical colorectal anastomosis was used in 27 (29.67%) patients. Postoperatively, 27 patients developed surgical-related complications, with 16 patients being diagnosed with AL.

The analysis of the clinical characteristics of our group indicated a high incidence of cardiovascular comorbidities (54.9%), followed by obesity (14.2%) and diabetes (7.6%).

Histopathological features

All cases taken into analysis were adenocarcinomas, most of them being the conventional/not otherwise specified (NOS) type. In 17 cases, cribriform comedo-type adenocarcinoma was observed, with tumors displaying mucinous features (mucin <50%) accounting for 10 cases, while mucinous adenocarcinoma (mucin >50%) was present in four cases.

The analysis of the grading showed that most cases were low-grade, formerly well-differentiated (G1 – 54.9%) or moderately differentiated tumors (G2 – 36.2%), with poorly or undifferentiated tumors accounting for 8.8% of cases (six cases of G3 and two cases of G4, respectively) (Figure 1A).

TNM staging indicated that 45% of the enrolled patients were staged as T3 (41 patients), while in 27.5% of cases the tumor penetrated the surface of the visceral peritoneum (T4a – 15 patients) or invaded the nearby organs (T4b – 10 patients). The lymph node involvement was uniformly distributed between N0 and N1 (43 *vs* 39 cases), with cancer cells identified in more than three lymph nodes (N3) in eight cases.

Out of the 946 lymph nodes that were analyzed (average 10.39), a total number of 138 (average 1.51) displayed sign of neoplastic invasion.

Metastatic disease was identified in 11% of cases (Table 1). None of the 91 cases had tumoral invasion in the distal or in the circumferential resection margin.

Table 1 – ***Clinico-demographic and HP features of the enrolled cases***

Variable	Subtypes	*N* (91)	Percentage
Age [years]	Mean ± SD	66.40±11.00	
	Range	27–96	
Sex	Male	53	58.2%
	Female	38	41.8%
Comorbidities	Cardiovascular diseases	50	54.9%
	Diabetes mellitus	7	7.6%
	Obesity	13	14.2%
Staging (TNM)	T_1	5	5.5%
	T_2	20	22%
	T_3	41	45%
	T_4	25	27.5%
	N_0	43	47.2%
	N_1	39	42.9%
	N_2	9	9.9%
	M_1	10	11%
Tumor inflammatory cell infiltrate	0	16	17.6%
	1	27	29.7%
	2	31	34%
	3	17	18.7%
Grading	G1	50	54.9%
	G2	33	36.2%
	G3/G4	8	8.8%
Postoperative complications	Overall	*27*	*29.6%*
	AL	16	17.6%
	Deaths	5	5.5%

AL: Anastomotic leakage; HP: Histopathological; *N*: No. of cases; SD: Standard deviation; TNM: Tumor, node, metastasis.

Based on the K–M criteria, the tumoral inflammatory cell reaction was estimated as mild in 27 cases (K–M1; 29.6%), intermediate in 31 cases (K–M2; 34%), and prominent in 17 cases (K–M3; 18.6%) (Figure 1B). In 16 cases (K–M0; 17.5%), no increase in inflammatory cell infiltrate was noticed. In five cases, microabscesses were observed, all of which displaying an intense peritumoral inflammatory cell infiltrate.

Perineural invasion, considered as the presence of neoplastic cells in the perineural space covering over 30% of the nerve circumference or within any layer of the nerve sheath, was documented in 16 (17.58%) cases (Figure 2A). Vascular invasion in the primary tumor was identified in a similar number of patients (Figure 2B).

No statistical association was observed between perineural and vascular invasion, and grading, inflammatory cell infiltrate or the TNM staging characteristics. However, perineural invasion occurred more frequently in cases with vascular invasion [p<0.001, odds ratio (OR) 12.49, 95% CI 3.55–43.91]. The IHC staining confirmed lymphocyte (CD3, CD20) and macrophage (CD68) reaction at the invasive front area (Figure 3, A–C).

Histopathological impact on surgical outcome

We classified the extent of tumoral inflammatory cell infiltrate into <low density>, corresponding to K–M classes of 0 and 1, and <high density> for K–M class of 2 and 3. Likewise, the grading was also divided into two subgroups: <low-grade> consisting of tumor grading 1 and 2 (G1, G2), and <high-grade> for tumor grading of 3 and 4 (G3, G4).

The overall peritumoral inflammatory reaction score was positively correlated with the occurrence of AL (ρ=0.446), while only a moderate relationship was observed between tumor grading and postoperative leakage (ρ=0.388) (Table 2). In case of postoperative morbidity, tumor inflammatory cell infiltrate moderately correlated with the overall post-operative complications (ρ=0.375), with tumor differentiations displaying a weak relationship with this grouping variable (ρ=0.257) (Table 3).

Neither the histological subtype, nor the perineural and vascular invasion were associated with increased risk for either AL or surgical-related complications. The TNM staging did not seem to influence the anastomotic outcomes or the overall postoperative morbidity.

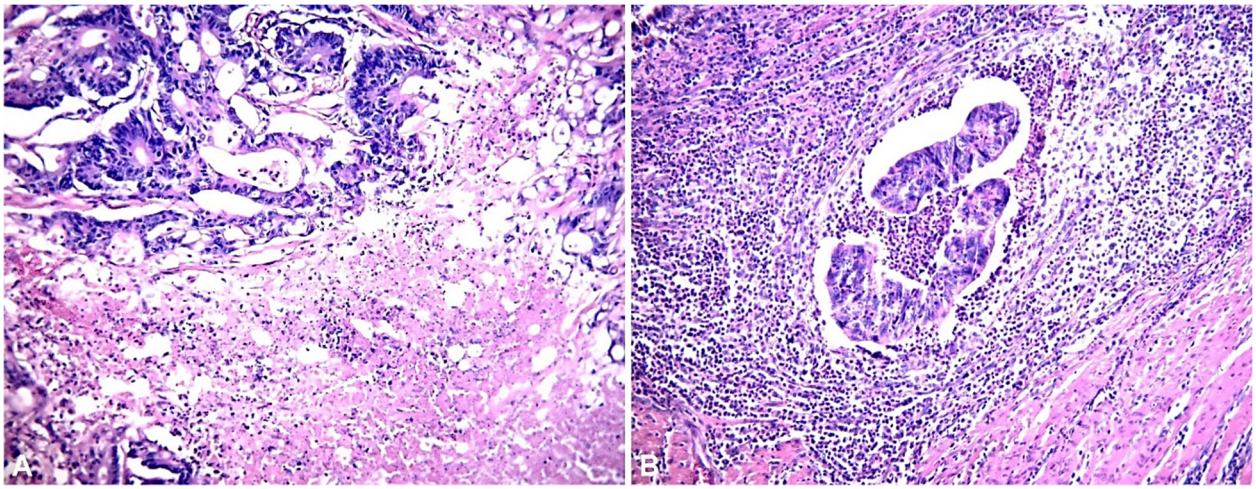

Figure 1 – ***Morphopathological microscopic aspects of colon adenocarcinoma: (A) Colorectal adenocarcinoma, NOS, low-grade, with extensive necrotic areas; (B) Colon adenocarcinoma, NOS, high-grade, abundant acute inflammatory infiltrate. HE staining: (A and B) ×200. HE: Hematoxylin–Eosin; NOS: Not otherwise specified.***

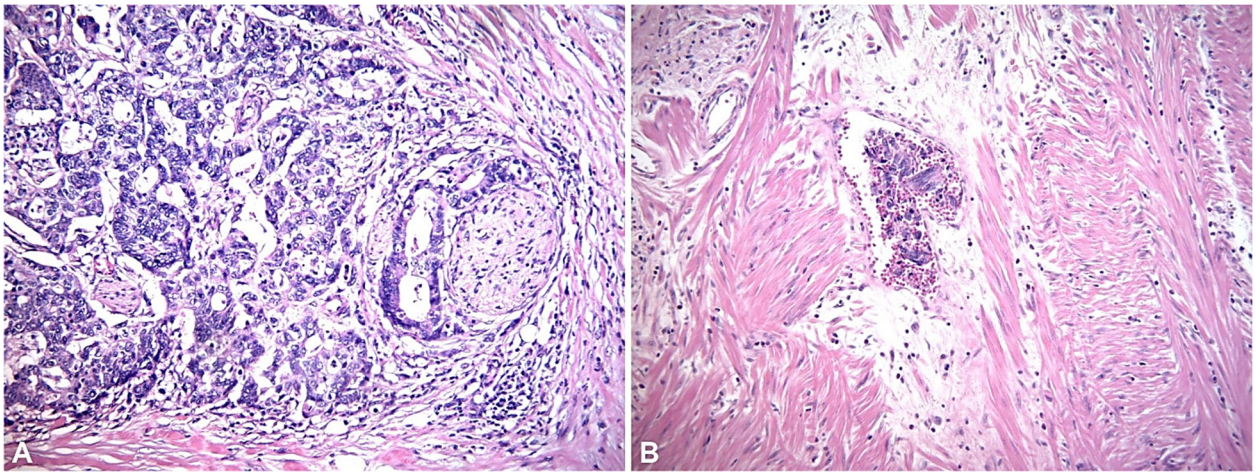

Figure 2 – ***Morphopathological microscopic aspects of colon adenocarcinoma: (A) Colon adenocarcinoma, NOS, low-grade, with perineural invasion; (B) Colon adenocarcinoma, NOS, low-grade, vascular invasion. HE staining: (A and B) ×200. HE: Hematoxylin–Eosin; NOS: Not otherwise specified.***

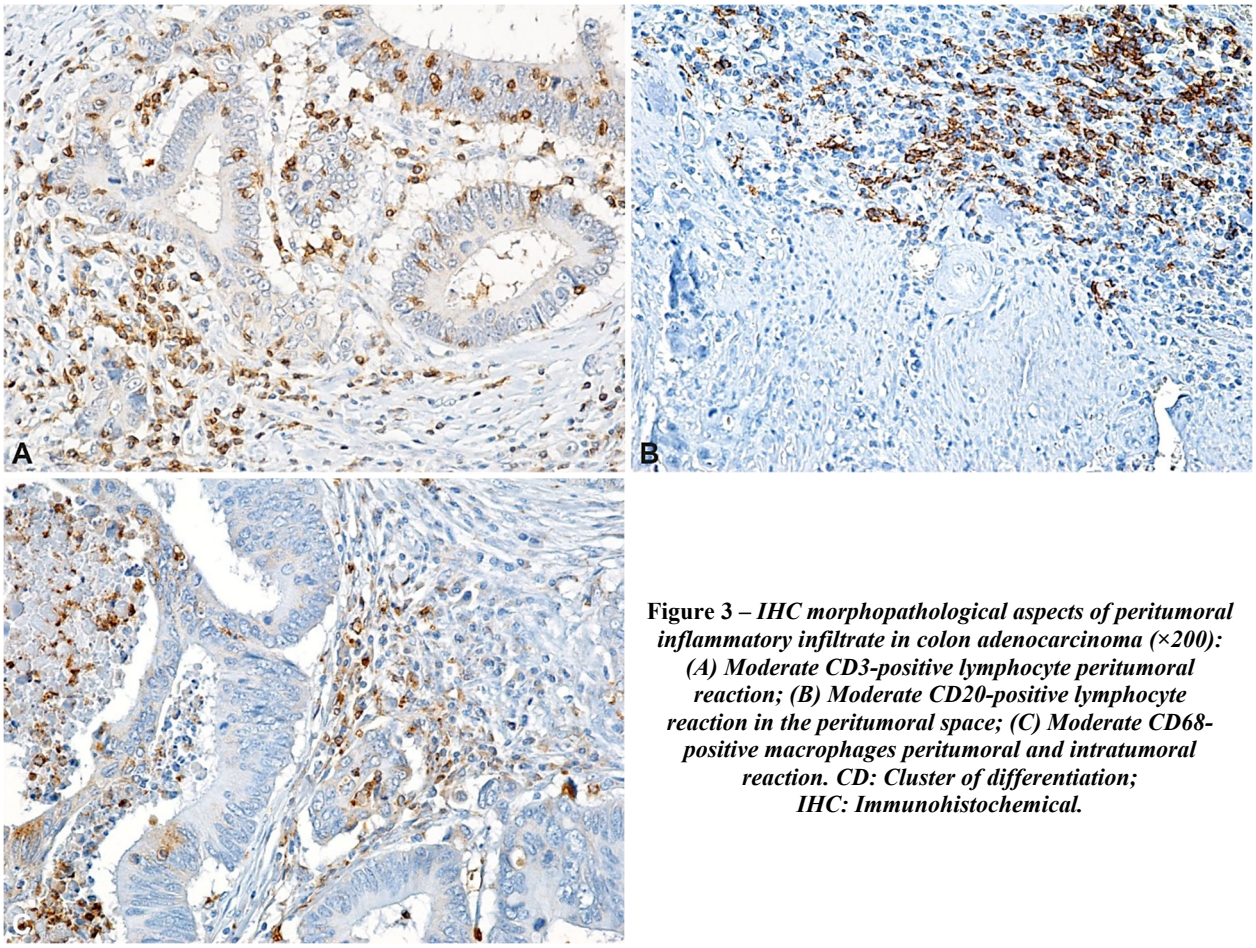

Figure 3 – ***IHC morphopathological aspects of peritumoral inflammatory infiltrate in colon adenocarcinoma (×200): (A) Moderate CD3-positive lymphocyte peritumoral reaction; (B) Moderate CD20-positive lymphocyte reaction in the peritumoral space; (C) Moderate CD68-positive macrophages peritumoral and intratumoral reaction. CD: Cluster of differentiation; IHC: Immunohistochemical.***

Table 2 – ***The analysis of main HP risk factors in patients with AL after CRC surgery***

HP variable	Without AL 75 cases (82.4%)	With AL 16 cases (17.5%)	*p* (Fisher)	OR	95% CI	*Rho* (Spearman)*
Inflammatory infiltrate						
Low density (*K–M 0–1*)	41 (45.05%)	2 (2.19%)	0.002	8.44	1.79–39.76	0.446
High density (*K–M 2–3*)	34 (37.36%)	14 (15.38%)				
Grading						
Low-grade (G1–G2)	70 (76.92%)	11 (12.08%)	0.013	6.36	1.58–25.62	0.388
High-grade (G3–G4)	5 (5.49%)	5 (5.49%)				

HP variable	Without AL 75 cases (82.4%)	With AL 16 cases (17.5%)	*p* (Fisher)	OR	95% CI	*Rho* (Spearman)*
Perineural invasion						
(–)	63 (70.32%)	12 (12.08%)	0.47	1.75	0.48–6.35	–
(+)	12 (12.08%)	4 (5.49%)				
Vascular invasion						
(–)	64 (73.62%)	11 (8.79%)	0.14	2.64	0.76–9.09	–
(+)	11 (12.08%)	5 (5.49%)				

*Spearman's *rho* (*ρ*) was calculated for ordinal variables (inflammatory infiltrate 0–4; tumor grading G1–G4). AL: Anastomotic leakage; CI: Confidence interval; CRC: Colorectal cancer; HP: Histopathological; K–M: Klintrup–Mäkinen score; OR: Odds ratio.

Table 3 – ***The analysis of the main HP risk factors in patients with postoperative complications after CRC surgery***

HP variable	Without SC 64 cases (70.3%)	With SC 27 cases (29.7%)	*p* (Fisher)	OR	95% CI	*Rho* (Spearman)*
Inflammatory infiltrate						
Low density (*K–M 0–1*)	36 (39.56%)	7 (7.69%)	0.011	3.67	1.36–9.90	0.375
High density (*K–M 2–3*)	28 (30.76%)	20 (21.97%)				
Grading						
Low-grade (G1–G2)	59 (64.83%)	22 (24.17%)	0.15	2.68	0.70–10.16	0.257
High-grade (G3–G4)	5 (5.49%)	5 (5.49%)				
Perineural invasion						
(–)	53 (58.24%)	22 (24.17%)	0.54	1.09	0.34–3.52	–
(+)	11 (12.08%)	5 (5.49%)				
Vascular invasion						
(–)	55 (60.43%)	20 (21.97%)	0.22	2.13	0.70–6.50	–
(+)	9 (9.89%)	7 (7.69%)				

*Spearman's *rho* (*ρ*) was calculated for ordinal variables (inflammatory infiltrate 0–4; tumor grading G1–G4). CRC: Colorectal cancer; HP: Histopathological; K–M: Klintrup–Mäkinen score; OR: Odds ratio; SC: Surgical complications.

Discussions

The colorectal anastomotic healing is a complex process involving numerous biological mechanisms, some of which are not yet fully elucidated. This knowledge gap mobilized the efforts of surgical research teams to better understand the factors that interfere with the anastomotic healing process and the physiopathology of postoperative leakage. The complexity of this issue is augmented by the numerous morphopathological changes, including altered inflammatory reaction, tumor-induced angiogenesis, local tissue remodeling, etc., that occur in the context of CRC and may hinder the normal healing process [15–17].

Our study investigated the potential influence of common tumoral histological features on the postoperative results, with special emphasis on anastomotic fistula. We chose those morphopathological characteristics that are readily available prior to surgery, such as peritumoral inflammatory infiltrate, tumoral grading, local tumor extension, and lymph node involvement.

The analysis of the short-term impact of these factors indicated clear and significant correlations between tumoral inflammatory infiltrate and the postoperative outcomes, especially the risk for anastomotic fistula. This finding is surprising, as most studies only emphasized the role of inflammatory cell infiltrate at the invasive margin in solid cancers for long-term survival, while there are no data concerning its influence on the postoperative outcomes, especially in CRC surgery [18, 19].

Cancer-induced inflammation can be a consequence of mutations initiated by the neoplastic process or by intratumoral necrosis and is often potentiated by the overall proinflammatory status caused by extrinsic cancer risk factors, such as obesity, diabetes, smoking, or excessive alcohol consumption [20–22]. The peritumoral inflammatory response, as a marker of the interaction between the neoplastic process and the host, may influence local physiopathological processes in many ways. The most important mechanism is the inflammation-induced procoagulant pathway, which invariably leads to microthromboses and local ischemia [23, 24]. Together with the acidic microenvironment and local ischemia, the peritumoral inflammation can have a detrimental effect on initial postoperative anabolic response phase, including anastomotic healing [25–27].

On the other hand, prolonged inflammation may impede local anastomotic healing by excessive mobilization of macrophages and lymphocytes, with subsequent secretion and regulation of proteases and matrix metalloproteinases, associated with a dysregulation in growth factor synthesis and impeded lymphoblastic migration and growth [28–30].

The presence of peritumoral inflammatory infiltrate, together with the proinflammatory status in the early postoperative period may lead to an increased risk for surgical-related untoward outcomes, the most important one being the AL or dehiscence.

Based on these premises, the analysis the relationship between the tumoral inflammatory infiltrate and routine postoperative endpoints seems reasonable and may allow

to uncover a potential role of cancer-related inflammation on postoperative recovery and, more specifically, on anastomotic healing process.

Surprisingly, another HP factor that influenced the short-term postoperative outcomes was tumor grading. Although a modest level of correlation was observed between the degree of tumor differentiation and the incidence of anastomotic fistula, the results should prompt more efforts to expand the research in this area.

The main drawback of this study was the small number of cases included in the analysis, which may have partially distorted results and prevented them from being extrapolated.

Conclusions

Local tumor cell infiltrate and, to a lesser extent, the grading, seem to play a role in the postoperative outcomes after CRC surgery. Tumor inflammatory cell infiltrate in particular seem to influence anastomotic healing process and the risk for postoperative leakage after CRC surgery.

Conflict of interests

The authors declare no conflict of interests.

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Authors' contribution

Ştefan Pătraşcu and Daniela Marinescu equally contributed to this article.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

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