Original paper

# The potential prognostic role of peritumoral eosinophils within whole tumor-associated inflammatory cells and stromal histological characteristics in colorectal cancer

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We aimed to determine the prognostic role of whole tumor-associated inflammatory cells, especially eosinophils, and stromal histological characteristics in relation to other prognostic parameters in patients with colorectal carcinoma (CRC). A total of 122 patients who underwent an operation for CRC were included in this retrospective study. Conventional (tumor grade, TNM stage and venous invasion [VI]) and other histopathological (intratumoral/peritumoral budding [ITB/PTB], desmoplasia) tumor parameters were recorded and classified by density, as were the tumor-associated inflammatory parameters (intratumoral/peritumoral lymphocytes [ITL/PTL], eosinophils [IE/PTE], overall inflammation [ITI/PTI], Crohn-like inflammation [CLI]). Cancer-specific survival data were analyzed with respect to all tumor parameters. High ITB and PTB were significantly correlated with a higher rate of pT4, VI and desmoplasia ($p < 0.05$). An association of moderate ITL and extensive PTL with lesser likelihood of VI and metastasis; an association of extensive CLI with a significantly lower rate of metastasis and TNM stage IV; and minimal PTE with a significantly higher rate of pT4 stage, metastasis and ITB were detected ($p < 0.05$ for each). Our findings revealed that low score tumoral budding and an increase in tumor-related inflammation were associated with lesser likelihood of poor prognostic tumor parameters. Nonetheless, given the association of an increase in PTE with lesser likelihood of ITB, pT4, metastasis, and with non-significantly for better survival rates, our findings emphasize the potential role of peritumoral eosinophils as an additional prognostic parameter in CRC.

**Key words:** colorectal cancer, survival, tumor-related inflammation, tumor budding.

## Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide and the third leading cause of cancer-related mortality, despite better understanding of its pathogenesis and advances in therapy. Colorectal cancer incidence has been increasing steadily, especially in developing countries. Obesity, sedentary life style, red meat consumption, alcohol and tobacco are the major risk factors behind the growth of CRC [1].

Colon cancer and rectal cancer have similar histopathologic risk factors and similar survival rates [1, 2].

Colorectal cancer comprises a group of diseases driven by several mutations and mutagens. Hereditary CRCs account for 7-10% of all cases and include Lynch syndrome, adenomatous and hamartomatous syndromes [3]. Up to 30% of CRC patients have a family history of the neoplasm, meaning there are probably predisposing germ-line mutations. To date, several nuclear DNA variants have been shown to be associated with increased risk of CRC. However, there are conflicting results about the role of inherited mitochondrial DNA (mtDNA) mutations in CRC carcinogenesis. Previous studies suggested that certain polymorphic mtDNA positions were associated with increased risk of CRC in different nations [4, 5] but a British study [6] and a recent Polish [7] study did not support the hypothesis that mtDNA variants contribute to inherited predisposition of CRC.

Currently, post-surgical staging according to the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC), tumor node metastasis (TNM) system is a standard reference for prognosis and a guide for therapeutic decision making in CRC [8]. However, TNM classification reflects an anatomical extent rather than biological tumor characteristics [9]. Alongside considerable variation in the clinical course of disease among patients with an identical stage [8, 10], unexpected progression during the follow-up of early stage, lymph node negative patients causes difficulties to decide the therapeutic modalities [9, 10, 11, 12]. Tumor budding seems important in this regard, as a strong adverse prognostic factor to select patients for adjuvant therapy particularly in those with early stage, lymph node negative disease [12, 13].

However, given the heterogeneity of tumor biology within each stage, there is a need for additional stage-independent prognostic histopathological parameters to identify patients with more aggressive clinical behavior. This will guide clinical decisions in terms of follow-up scheduling and provision of adjuvant therapy tailored to individualized risk of progression [8, 10, 12].

In accordance with the increasingly apparent recognition of CRC outcome to be dependent not only on tumor characteristics but also on the interactions between host immune/inflammatory response and tumor [14], assessment of tumor-related local inflammation has been proposed as a novel prognostic parameter [10, 14].

Several studies to date have confirmed the prognostic value of the tumor-related inflammatory or immune cell infiltrate especially the adaptive immune response (i.e. infiltration of T cells) in patients with CRC [14, 15, 16]. For example, Galon *et al*. found a positive correlation between the presence of markers for Th1 polarization of cytotoxic and memory T cells and a low incidence of tumor recurrence [15]. An international concortium suggested the Immunoscore assay to assess the prognostic value of total tumor-infiltrating T-cell counts and cytotoxic tumor-infiltrating T-cell counts in patients with stage I-III colon cancer. They found that the Immunoscore provides a reliable estimate of the risk of recurrence in patients with colon cancer [16]. There are a few studies that have focused on the innate immune cells (i.e. eosinophils) with a standardized assessment regarding type, density and location of inflammatory infiltrate [17, 18, 19]. A simplified histopathology-based scoring method described by Klintrup *et al*. [18] is important in this regard, since it represents standard criteria for the prognostic value of tumor-related inflammatory infiltrate assessments.

This study was therefore designed to investigate the prognostic role of tumor-associated inflammatory parameters and tumor budding in relation to other prognostic tumor parameters in patients operated on CRC. Tumor-associated inflammatory infiltrates were assessed based on standardized criteria being stratified by location (intratumoral, peritumoral), inflammatory cell subtype (lymphocyte, eosinophil, overall inflammation) and density.

## Material and methods

### Study population

A total of 122 patients operated on for colorectal cancer (mean (SD) age: 59.9 (12.4) years, 59.0% were men) were included in this retrospective study that was conducted at a tertiary care center between 2013 and 2014 (Table I). Rare histologic subtypes such as medullary and squamous cell carcinoma were excluded from the study and patients with insufficient disease information were excluded from data analysis.

The study was conducted in full accordance with local good clinical practice guidelines and current legislations, while permission was obtained from our institutional ethics committee for the use of patient data for publication purposes. The need for consent from the patients was waived because of the retrospective nature of this study.

### Study parameters

Data on patient demographics (age, gender), tumor grade and pT and pN stages, and neoadjuvant therapy history were retrospectively obtained from patient charts. Metastases were recorded both at the time of diagnosis and during follow-up. Intratumoral (ITB), peritumoral budding (PTB), venous invasion and desmoplasia were evaluated in addition to tumor-associated inflammatory parameters (intratumoral

lymphocyte {ITL}, peritumoral lymphocyte {PTL}, Crohn-like inflammation {CLI}, intratumoral eosinophils {IE}, peritumoral eosinophils {PTE}, intratumoral overall inflammation {ITI}, peritumoral overall inflammation {PTI}). Survival data were obtained from patients' medical records or via phone call interviews.

The prognostic role of tumor-associated inflammatory parameters and desmoplasia in relation to conventional prognostic parameters were evaluated, along with 5-year cancer-specific survival (CSS) rate and mean CSS time. The relation of tumor budding with conventional prognostic parameters, tumor-related inflammatory parameters and desmoplasia was also analyzed along with the concordance between ITB and PTB.

## Histopathology

For the classification of TNM, AJCC/UICC 2017 criteria were used {20}, while histological tumor type and differentiation (grade) were assessed based on the 2010 edition of the World Health Organization classification {21}.

We analyzed venous invasion on hematoxylin and eosin (HE) slides with attention to the orphan arteriole and protruding tongue signs. An orphan arteriole sign is described as a circumscribed tumor nodule adjacent to a muscularized artery without an obvious accompanying vein. The protruding tongue sign is described as a smooth bordered protrusion of a tumor into pericolic fat adjacent to an artery {22}. Besides these morphologic findings, many studies have demonstrated the superiority of elastin staining compared to (HE) alone in the detection of VI {22, 23}. Since determination of the true prevalence of VI is difficult based on HE, especially when the muscular wall of the vein is obliterated, elastin stain was also performed on all tumor blocks. Venous invasion was considered positive when a tumor was observed in an endothelium-lined space {22}.
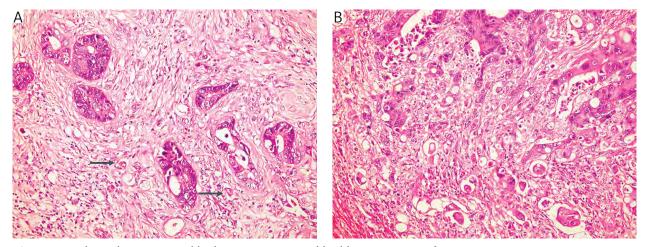
Both intratumoral and peritumoral budding were assessed on HE slides as described previously by Gra-

Table I. Demographic and clinical characteristics of the study group

| AGE, YEARS (MEAN, RANGE) | 60 | 28-87 |
|---|---|---|
| | N | % |
| Gender | | |
| Male | 72 | 59 |
| Female | 50 | 41 |
| Grade | | |
| Low | 107 | 87.7 |
| High | 15 | 12.3 |
| T | | |
| 1-2 | 23 | 18.8 |
| 3 | 70 | 53.4 |
| 4 | 29 | 27.8 |
| N | | |
| 0 | 72 | 59.0 |
| 1 | 33 | 26.9 |
| 2 | 17 | 14.1 |
| Metastasis | | |
| No | 97 | 79.5 |
| Yes | 25 | 20.5 |
| TNM Stage | | |
| I | 21 | 17.2 |
| II | 44 | 36.1 |
| III | 35 | 28.7 |
| IV | 22 | 18.0 |

ham *et al.* {24}. The intensity of budding was scored accordingly as no budding, low (1-9 budding foci), and high (≥ 10 foci) (Figs. 1, 2). Desmoplasia was scored semi-quantitatively as minimal, moderate and severe.



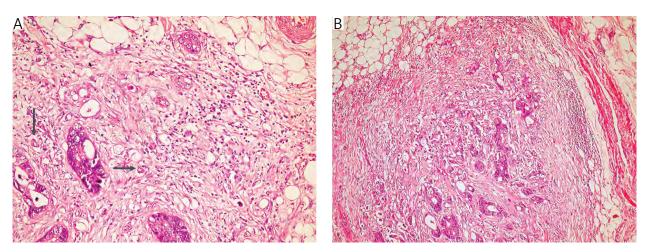**Fig. 1.** Low (shown by arrows) and high score intratumoral budding (HE, magnification 200×)

**Fig. 2.** Low (shown by arrows) and high score peritumoral budding (HE, magnification 200×)
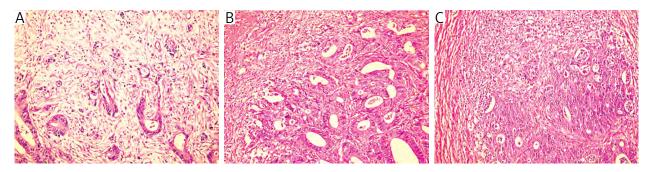


**Fig. 3.** The intensity of overall inflammatory cell reaction in order to minimal, moderate and severe (HE, magnification 200×)
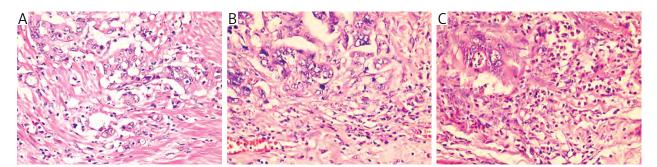


**Fig. 4.** The intensity of eosinophil infiltration in order to minimal, moderate and severe (HE, magnification 400×)

## Tumor-associated inflammation

The intensity of overall inflammatory cell reaction was assessed separately within the tumor center (intratumoral) and in the stroma at the invasive tumor margin (peritumoral) with a modified method described by Klintrup *et al.* [18] and scored from 1 to 3 based on a mild or patchy increase (score 1), a prominent inflammatory reaction (score 2), and a florid "cuplike" inflammatory infiltrate (score 3; Fig. 3).

The numbers of intratumoral and peritumoral eosinophils were evaluated separately on HE stained slides, using a 40× objective lens in a high power field (HPF) measuring 0,24 mm$^2$ (Olympus BX45).

IE and PTE were categorized according to modified Fernandez-Acenero criteria, considering of < 10 cells/ 0.24 mm$^2$ area as minimal, 10-50 cells/0.24 mm$^2$ area as moderate and > 50 cells/0.24 mm$^2$ area as extensive infiltration [25] (Fig. 4).

Crohn-like response was scored as none or mild-to-moderate and marked according to College of American Pathologists (CAP) criteria [26]. Intratumoral lymphocytes were evaluated based on CAP criteria and graded as none, mild-to-moderate (1-2 per HPF) and marked (≥ 3 per HPF), (Fig. 5).

Peritumoral lymphocytes were evaluated according to modified Huh *et al.* [27] criteria on HE stained slides and categorized as minimal, moderate and severe (Fig. 6).
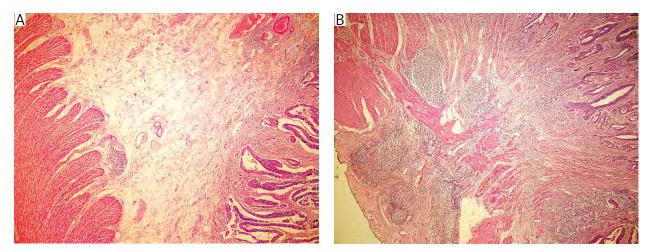
**Fig. 5.** Crohn-like response: minimal and marked (HE, magnification 40×)
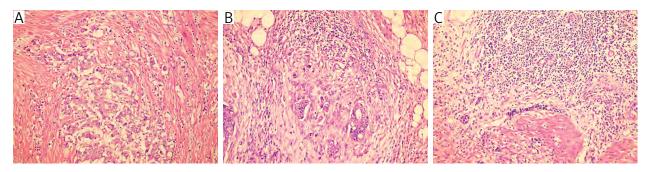


**Fig. 6.** Peritumoral lymphocyte infiltration in order to minimal, moderate and severe (HE, magnification 200×)

## Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY). The $\chi^2$ test was used for the comparison of categorical data, while the one way ANOVA test was used for the parametric variables. The κ concordance index was used to determine the concordance between intratumoral and peritumoral budding. Cancer-specific survival was assessed using the Kaplan-Meier method and the significance among patient groups was calculated using the log-rank test. Cox proportional hazards regression model was used to determine hazard ratios and data were expressed as "mean (standard deviation; SD)", minimum-maximum, 95% confidence interval (CI) and percent (%) where appropriate. $P < 0.05$ was considered statistically significant.

## Results

### Tumor-associated inflammatory parameters and tumor budding according to grade, TNM stage and venous invasion

Tumor-associated inflammatory parameters and tumor budding according to TNM stage are shown in Table II.

High ITB as compared with the absence of ITB and low ITB was associated with significantly higher rate of pT4 (42.0% vs. 6.3% and 12.5%, respectively, p = 0.002) and venous invasion (54.0% vs. 18.7% and 33.9%, respectively, p = 0.019).

High PTB as compared with the absence of PTB and low PTB was associated with significantly higher rate of pT4 (41.5% vs. 6.3% and 11.3%, respectively, p = 0.001) and TNM stage IV (28.3% vs. 12.5% and 9.4%, respectively, p = 0.043) alongside higher likelihood of venous invasion (52.8% vs. 18.8% and 34.0%, respectively, p = 0.024).

Most of our cases were low grade (87.7%). We only found a statistically significant positive correlation with grade and ITL. Extensive ITL was strongly associated with a higher likelihood of high grade tumor rather than mild-to-moderate ITL (26.9% vs. 7.2%, p = 0.033). However extensive ITL was inversely correlated with venous invasion (p = 0.048).

The presence of metastasis was more likely to occur in cases of minimal (28.1%) and moderate (17.9%) PTL in comparison to extensive (0.0%) PTL (p = 0.025).

Marked CLI as compared to the absence of CLI and mild-to-moderate CLI was associated with a significantly lower rate of metastasis and TNM stage IV (p = 0.005).

Table II. Tumor budding and tumor-associated inflammatory parameters with respect to tumor grade, TNM stage and venous invasion

| | | GRADE | | T | | | N | | | METASTASIS | | TNM STAGE | | | | VENOUS INVASION | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Low | High | 1-2 | 3 | 4 | 0 | 1 | 2 | No | Yes | 1 | 2 | 3 | 4 | No | Yes |
| **ITB** | | | | | | | | | | | | | | | | | |
| Absent | | 13 (81.3) | 3 (18.8) | 5 (31.3) | 10 (62.5) | 1 (6.3) | 11 (68.8) | 3 (18.8) | 2 (12.5) | 13 (81.3) | 3 (18.8) | 5 (0.3) | 4 (0.3) | 5 (0.3) | 2 (0.1) | 13 (81.3) | 3 (18.7) |
| Low | | 53 (94.6) | 3 (5.4) | 13 (23.2) | 36 (64.3) | 7 (12.5) | 36 (64.3) | 15 (26.8) | 5 (8.9) | 49 (87.5) | 7 (12.5) | 11 (0.2) | 23 (0.4) | 16 (0.3) | 6 (0.1) | 37 (66.1) | 19 (33.9) |
| High | | 41 (82.0) | 9 (18.0) | 5 (10.0) | 24 (48.0) | 21 (42.0)[a] | 25 (50.0) | 15 (30.0) | 10 (20.0) | 35 (70.0) | 15 (30.0) | 5 (0.1) | 17 (0.3) | 14 (0.3) | 14 (0.3) | 23 (46.0) | 27 (54.0)[a] |
| $p^{ITB}$ groups | | 0.099 | | 0.002 | | | 0.389 | | | 0.082 | | 0.159 | | | | 0.019 | |
| **PTB** | | | | | | | | | | | | | | | | | |
| Absent | | 14 (87.5) | 2 (12.5) | 6 (37.5) | 9 (56.3) | 1 (6.3) | 12 (75.0) | 2 (12.5) | 2 (12.5) | 13 (81.3) | 3 (18.8) | 6 (37.5) | 4 (25.0) | 4 (25.0) | 2 (12.5) | 13 (81.3) | 3 (18.8) |
| Low | | 48 (90.6) | 5 (9.4) | 11 (20.8) | 36 (67.9) | 6 (11.3) | 35 (66.0) | 15 (28.3) | 3 (5.7) | 47 (88.7) | 6 (11.3) | 10 (18.9) | 23 (43.4) | 15 (28.3) | 5 (9.4) | 35 (66.0) | 18 (34.0) |
| High | | 45 (84.9) | 8 (15.1) | 6 (11.3) | 25 (47.2) | 22 (41.5)[b] | 25 (47.2) | 16 (30.2) | 12 (22.6) | 37 (69.8) | 16 (30.2) | 5 (9.4) | 17 (32.1) | 16 (30.2) | 15 (28.3)[b] | 25 (47.2) | 28 (52.8)[b] |
| $p^{PTB}$ groups | | 0.674 | | 0.001 | | | 0.052 | | | 0.054 | | 0.043 | | | | 0.024 | |
| **ITL** | | | | | | | | | | | | | | | | | |
| Absent | | 24 (88.9) | 3 (11.1) | 6 (22.2) | 16 (59.3) | 5 (18.5) | 15 (55.6) | 9 (33.3) | 3 (11.1) | 19 (70.4) | 8 (29.6) | 6 (22.2) | 7 (25.9) | 8 (29.6) | 6 (22.2) | 15 (55.6) | 12 (44.4) |
| Mild-moderate | | 64 (92.8) | 5 (7.2) | 10 (14.5) | 41 (59.4) | 18 (26.1) | 43 (62.3) | 18 (26.1) | 8 (11.6) | 55 (79.7) | 14 (20.3) | 8 (11.6) | 31 (44.9) | 17 (24.6) | 13 (18.8) | 37 (53.6) | 32 (46.4) |
| Extensive | | 19 (73.1) | 7 (26.9)[c] | 7 (26.9) | 13 (50.0) | 6 (23.1) | 14 (53.8) | 6 (23.1) | 6 (23.1) | 23 (88.5) | 3 (11.5) | 7 (26.9) | 6 (23.1) | 10 (38.5) | 3 (11.5) | 21 (80.8) | 5 (19.2)[d] |
| $p^{ITL}$ groups | | 0.033 | | 0.637 | | | 0.587 | | | 0.264 | | 0.189 | | | | 0.048 | |
| **PTL** | | | | | | | | | | | | | | | | | |
| Minimal | | 58 (90.6) | 6 (9.4) | 11 (17.2) | 36 (56.3) | 17 (26.6) | 34 (53.1) | 22 (34.4) | 8 (12.5) | 46 (71.9) | 18 (28.1) | 10 (15.6) | 19 (29.7) | 20 (31.3) | 15 (23.4) | 33 (51.6) | 31 (48.4) |
| Moderate | | 34 (87.2) | 5 (12.8) | 8 (20.5) | 23 (59.0) | 8 (20.5) | 26 (66.7) | 9 (23.1) | 4 (10.3) | 32 (82.1) | 7 (17.9) | 7 (17.9) | 17 (43.6) | 8 (20.5) | 7 (17.9) | 26 (66.7) | 13 (33.3) |
| Extensive | | 15 (78.9) | 4 (21.1) | 4 (21.1) | 11 (57.9) | 4 (21.1) | 12 (63.2) | 2 (10.5) | 5 (26.3) | 19 (100.0) | 0 (0.0)[e] | 4 (21.1) | 8 (42.1) | 7 (36.8) | 0 (0.0) | 14 (73.7) | 5 (26.3) |
| $p^{PTL}$ groups | | 0.393 | | 0.954 | | | 0.147 | | | 0.025 | | 0.257 | | | | 0.129 | |

Table II. Cont.

| | GRADE | | T | | | N | | | METASTASIS | | TNM STAGE | | | | VENOUS INVASION | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Low | High | 1-2 | 3 | 4 | 0 | 1 | 2 | No | Yes | 1 | 2 | 3 | 4 | No | Yes |
| **CLI** | | | | | | | | | | | | | | | | |
| Absent | 43 (93.5) | 3 (6.5) | 13 (28.3) | 26 (56.5) | 7 (15.2) | 29 (63) | 12 (26.1) | 5 (10.9) | 32 (69.6) | 14 (30.4) | 13 (28.3) | 10 (21.7) | 11 (23.9) | 12 (26.1) | 29 (63) | 17 (37.0) |
| Mild-moderate | 34 (87.2) | 5 (12.8) | 5 (12.8) | 21 (53.8) | 13 (33.3) | 21 (53.8) | 11 (28.2) | 7 (17.9) | 29 (74.4) | 10 (25.6) | 4 (10.3) | 16 (41.0) | 10 (25.6) | 9 (23.1) | 19 (48.7) | 20 (51.3) |
| Marked | 30 (81.1) | 7 (18.9) | 5 (13.5) | 23 (62.2) | 9 (24.3) | 22 (59.5) | 10 (27) | 5 (13.5) | 36 (97.3) | 1 (2.7)f | 4 (10.8) | 18 (48.6) | 14 (37.8) | 1 (2.7)f | 25 (67.6) | 12 (32.4) |
| p CLI groups | 0.230 | | 0.157 | | | 0.893 | | | 0.005 | | 0.005 | | | | 0.210 | |
| **IE** | | | | | | | | | | | | | | | | |
| Minimal | 71 (85.5) | 12 (14.5) | 13 (15.7) | 49 (59.0) | 21 (25.3) | 45 (54.2) | 26 (31.3) | 12 (14.5) | 63 (75.9) | 20 (24.1) | 12 (14.5) | 28 (33.7) | 26 (31.3) | 17 (20.5) | 45 (54.2) | 38 (45.8) |
| Moderate | 20 (100.0) | 0 (0.0) | 4 (20.0) | 12 (60.0) | 4 (20.0) | 15 (75.0) | 3 (15.0) | 2 (10.0) | 17 (85.0) | 3 (15.0) | 3 (15.0) | 11 (55.0) | 3 (15.0) | 3 (15.0) | 15 (75.0) | 5 (25.0) |
| Extensive | 16 (84.2) | 3 (15.8) | 6 (31.6) | 9 (47.4) | 4 (21.1) | 12 (63.2) | 4 (21.1) | 3 (15.8) | 17 (89.5) | 2 (10.5) | 6 (31.6) | 5 (26.3) | 6 (31.6) | 2 (10.5) | 13 (68.4) | 6 (31.6) |
| p IE groups | 0.185 | | 0.600 | | | 0.491 | | | 0.334 | | 0.249 | | | | 0.166 | |
| **PTE** | | | | | | | | | | | | | | | | |
| Minimal | 53 (82.8) | 11 (17.2) | 10 (15.6) | 34 (53.1) | 20 (31.3) | 32 (50.0) | 21 (32.8) | 11 (17.2) | 45 (70.3) | 19 (29.7) | 9 (14.1) | 18 (28.1) | 21 (32.8) | 16 (25.0) | 33 (51.6) | 31 (48.4) |
| Moderate | 29 (93.5) | 2 (6.5) | 4 (12.9) | 25 (80.6) | 2 (6.5)g | 21 (67.7) | 8 (25.8) | 2 (6.5) | 28 (90.3) | 3 (9.7)h | 4 (12.9) | 16 (51.6) | 8 (25.8) | 3 (9.7) | 21 (67.7) | 10 (32.3) |
| Extensive | 25 (92.6) | 2 (7.4) | 9 (33.3) | 11 (40.7) | 7 (25.9) | 19 (70.4) | 4 (14.8) | 4 (14.8) | 24 (88.9) | 3 (11.1) | 8 (29.6) | 10 (37.0) | 6 (22.2) | 3 (11.1) | 19 (70.4) | 8 (29.6) |
| p PTE groups | 0.223 | | 0.008 | | | 0.206 | | | 0.030 | | 0.094 | | | | 0.144 | |
| **ITI** | | | | | | | | | | | | | | | | |
| Minimal | 57 (86.4) | 9 (13.6) | 12 (18.2) | 38 (57.6) | 16 (24.2) | 39 (59.1) | 18 (27.3) | 9 (13.6) | 48 (72.7) | 18 (27.3) | 11 (16.7) | 22 (33.3) | 18 (27.3) | 15 (22.7) | 36 (54.5) | 30 (45.5) |
| Moderate | 29 (87.9) | 4 (12.1) | 4 (12.1) | 22 (66.7) | 7 (21.2) | 19 (57.6) | 10 (30.3) | 4 (12.1) | 28 (84.8) | 5 (15.2) | 4 (12.1) | 14 (42.4) | 10 (30.3) | 5 (15.2) | 20 (60.6) | 13 (39.4) |
| Extensive | 21 (91.3) | 2 (8.7) | 7 (30.4) | 10 (43.5) | 6 (26.1) | 14 (60.9) | 5 (21.7) | 4 (17.4) | 21 (91.3) | 2 (8.7) | 6 (26.1) | 8 (34.8) | 7 (30.4) | 2 (8.7) | 17 (73.9) | 6 (26.1) |
| p ITI groups | 0.824 | | 0.424 | | | 0.954 | | | 0.111 | | 0.643 | | | | 0.263 | |

Table II. Cont.

| | Grade Low | Grade High | T 1-2 | T 3 | T 4 | N 0 | N 1 | N 2 | Metastasis No | Metastasis Yes | TNM 1 | TNM 2 | TNM 3 | TNM 4 | Venous No | Venous Yes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PTI** | | | | | | | | | | | | | | | | |
| Minimal | 57 (87.7) | 8 (12.3) | 11 (16.9) | 37 (56.9) | 17 (26.2) | 36 (55.4) | 20 (30.8) | 9 (13.8) | 48 (73.8) | 17 (26.2) | 10 (15.4) | 20 (30.8) | 20 (30.8) | 15 (23.1) | 37 (56.9) | 28 (43.1) |
| Moderate | 28 (82.4) | 6 (17.6) | 7 (20.6) | 21 (61.8) | 6 (17.6) | 21 (61.8) | 10 (29.4) | 3 (8.8) | 28 (82.4) | 6 (17.6) | 6 (17.6) | 14 (41.2) | 9 (26.5) | 5 (14.7) | 20 (58.8) | 14 (41.2) |
| Extensive | 22 (95.7) | 1 (4.3) | 5 (21.7) | 12 (52.2) | 6 (26.1) | 15 (65.2) | 3 (13.0) | 5 (21.7) | 21 (91.3) | 2 (8.7) | 5 (21.7) | 10 (43.5) | 6 (26.1) | 2 (8.7) | 16 (69.6) | 7 (30.4) |
| p^PTI groups | 0.325 | | 0.869 | | | 0.399 | | | 0.182 | | 0.683 | | | | 0.563 | |
| **Desmoplasia** | | | | | | | | | | | | | | | | |
| Minimal | 53 (85.5) | 9 (14.5) | 13 (21.0) | 39 (62.9) | 10 (16.1) | 39 (62.9) | 14 (22.6) | 9 (14.5) | 51 (82.3) | 11 (17.7) | 12 (19.4) | 21 (33.9) | 20 (32.3) | 9 (14.5) | 39 (62.9) | 23 (37.1) |
| Moderate | 31 (91.2) | 3 (8.8) | 6 (17.6) | 18 (52.9) | 10 (29.4) | 21 (61.8) | 10 (29.4) | 3 (8.8) | 23 (67.6) | 11 (32.4) | 5 (14.7) | 15 (44.1) | 4 (11.8) | 10 (29.4) | 19 (55.9) | 15 (44.1) |
| Extensive | 23 (88.5) | 3 (11.5) | 4 (15.4) | 13 (50.0) | 9 (34.6) | 12 (46.2) | 9 (34.6) | 5 (19.2) | 23 (88.5) | 3 (11.5) | 4 (15.4) | 8 (30.8) | 11 (42.3) | 3 (11.5) | 15 (57.7) | 11 (42.3) |
| p^desmopl. groups | 0.713 | | 0.367 | | | 0.527 | | | 0.105 | | 0.122 | | | | 0.774 | |
| **Neoadj tx.** | | | | | | | | | | | | | | | | |
| Absent | 83 (88.3) | 11 (11.7) | 11 (11.7) | 58 (61.7) | 25 (26.6) | 55 (58.5) | 23 (24.5) | 16 (17.0) | 76 (80.9) | 18 (19.1) | 10 (10.6) | 39 (41.5) | 27 (28.7) | 18 (19.1) | 53 (56.4) | 4 (43.6) |
| Present | 24 (85.7) | 4 (14.3) | 12 (42.9) | 12 (42.9) | 4 (14.3)^i | 17 (60.7) | 10 (35.7) | 1 (3.6) | 21 (75.0) | 7 (25.0) | 11 (39.3)^i | 5 (17.9)^i | 8 (28.6) | 4 (14.3) | 20 (71.4) | 8 (28.6) |
| p^tx groups | 0.746 | | 0.001 | | | 0.148 | | | 0.501 | | 0.003 | | | | 0.154 | |

ITB – intratumoral budding; PTB – peritumoral budding; ITL – intratumoral lymphocyte; PTL – peritumoral lymphocyte; CLI – Crohn-like inflammation; IE – intratumoral eosinophils; PTE – peritumoral eosinophils; ITI – intratumoral overall inflammation; PTI – peritumoral overall inflammation

p < 0.05; compared to ^a low and absent ITB groups, ^b low and absent PTB groups, ^c minimal and moderate ITL groups, ^d absent and minimal ITL group, ^e minimal and extensive PTE groups, ^f absent and mild-moderate CLI groups, ^g minimal ITL groups, ^h minimal and moderate PTL groups, ^i minimal PTE group and ^j absence of neoadjuvant therapy (χ² test)

**Table III.** Concordance between ITB and PTB and association of tumor budding with study parameters

| | PTB | | | | ITB | | | |
|---|---|---|---|---|---|---|---|---|
| | **ABSENT** | **LOW** | **HIGH** | **P VALUE** | **ABSENT** | **LOW** | **HIGH** | **P VALUE** |
| Age, mean (SD) | 57.8 (15.5) | 59.5 (12.7) | 61.0 (11.3) | 0.628[1] | 58.3 (15.0) | 59.6 (13.0) | 60.7 (11.0) | 0.774[1] |
| **Gender, n (%)** | | | | | | | | |
| Male | 9 (56.3) | 34 (64.2) | 29 (54.7) | 0.596[2] | 10 (62.5) | 33 (58.9) | 29 (58.0) | 0.950[2] |
| Female | 7 (43.8) | 19 (35.8) | 24 (45.3) | | 6 (37.5) | 23 (41.1) | 21 (42.0) | |
| **ITL, n (%)** | | | | | | | | |
| Absent | 4 (25.0) | 10 (18.9) | 13 (24.5) | 0.838[2] | 4 (25.0) | 11 (19.6) | 12 (24.0) | 0.767[2] |
| Mild-moderate | 8 (50.0) | 30 (56.6) | 31 (58.5) | | 7 (43.8) | 33 (58.9) | 29 (58.0) | |
| Extensive | 4 (25.0) | 13 (24.5) | 9 (17.0) | | 5 (31.2) | 12 (21.4) | 9 (18.0) | |
| **PTL, n (%)** | | | | | | | | |
| Minimal | 10 (62.5) | 24 (45.3) | 30 (56.6) | 0.283[2] | 9 (56.3) | 27 (48.2) | 28 (56.0) | 0.590[2] |
| Moderate | 2 (12.5) | 20 (37.7) | 17 (32.1) | | 3 (18.8) | 20 (35.7) | 16 (32.0) | |
| Extensive | 4 (25.0) | 9 (17.0) | 6 (11.3) | | 4 (25.0) | 9 (16.1) | 6 (12.0) | |
| **CLI, n (%)** | | | | | | | | |
| Absent | 8 (50.0) | 18 (34.0) | 20 (37.7) | 0.487[2] | 7 (43.8) | 21 (37.5) | 18 (36.0) | 0.821[2] |
| Mild-moderate | 3 (18.8) | 16 (30.2) | 20 (37.7) | | 3 (18.8) | 19 (33.9) | 17 (34.0) | |
| Marked | 5 (31.3) | 19 (35.8) | 13 (24.5) | | 6 (37.5) | 16 (28.6) | 15 (30.0) | |
| **IE, n (%)** | | | | | | | | |
| Minimal | 9 (56.3) | 36 (67.9) | 38 (71.7) | 0.443[2] | 10 (62.5) | 35 (62.5) | 38 (76.0) | 0.138[2] |
| Moderate | 2 (12.5) | 10 (18.9) | 8 (15.1) | | 1 (6.3) | 13 (23.2) | 6 (12.0) | |
| Extensive | 5 (31.3) | 7 (13.2) | 7 (13.2) | | 5 (31.3) | 8 (14.3) | 6 (12.0) | |
| **PTE, n (%)** | | | | | | | | |
| Minimal | 8 (50.0) | 21 (39.6) | 35 (66.0) | 0.075[2] | 8 (50.0) | 21 (37.5) | 35 (70.0)* | **0.014[2]** |
| Moderate | 4 (25.0) | 19 (35.8) | 8 (15.1) | | 5 (31.3) | 20 (35.7) | 6 (12.0) | |
| Extensive | 4 (25.0) | 13 (24.5) | 10 (18.9) | | 3 (18.8) | 15 (26.8) | 9 (18.0) | |
| **ITI, n (%)** | | | | | | | | |
| Minimal | 7 (43.8) | 30 (56.6) | 29 (54.7) | 0.921[2] | 7 (43.8) | 30 (53.6) | 29 (58.0) | 0.888[2] |
| Moderate | 5 (31.3) | 14 (26.4) | 14 (26.4) | | 5 (31.3) | 15 (26.8) | 13 (26.0) | |
| Extensive | 4 (25.0) | 9 (17) | 10 (18.9) | | 4 (25.0) | 11 (19.6) | 8 (16.0) | |
| **PTI, n (%)** | | | | | | | | |
| Minimal | 8 (50.0) | 24 (45.3) | 33 (62.3) | 0.468[2] | 8 (50.0) | 25 (44.6) | 32 (64.0) | 0.304[2] |
| Moderate | 4 (25.0) | 18 (34.0) | 12 (22.6) | | 4 (25.0) | 20 (35.7) | 10 (20.0) | |
| Extensive | 4 (25.0) | 11 (20.8) | 8 (15.1) | | 4 (25.0) | 11 (19.6) | 8 (16.0) | |
| **Desmopl., n (%)** | | | | | | | | |
| Minimal | 14 (87.5)q | 33 (62.3) | 15 (28.3) | **< 0.001[2]** | 15 (93.8)q | 34 (60.7) | 13 (26.0) | **< 0.001[2]** |
| Moderate | 2 (12.5) | 11 (20.8) | 21 (39.6) | | 1 (6.3) | 14 (25.0) | 19 (38.0) | |
| Extensive | 0 (0.0) | 9 (17.0) | 17 (32.1) | | 0 (0.0) | 8 (14.3) | 18 (36.0) | |
| **Neoadj tx., n (%)** | | | | | | | | |
| Absent | 13 (81.3) | 39 (73.6) | 42 (79.2) | 0.718[2] | 13 (81.3) | 43 (76.8) | 38 (76.0) | 0.908[2] |
| Present | 3 (18.8) | 14 (26.4) | 11 (20.8) | | 3 (18.7) | 13 (23.2) | 12 (24.0) | |

*ITB – intratumoral budding; PTB – peritumoral budding; ITL – intratumoral lymphocyte; PTL – peritumoral lymphocyte; CLI – Crohn-like inflammation; IE – intratumoral eosinophils; PTE – peritumoral eosinophils; ITI – intratumoral overall inflammation; PTI – peritumoral overall inflammation; Desmopl. – desmoplasia; tx – therapy*

[1] *Oneway ANOVA test, χ² test*

*\*p < 0.05 compared moderate and extensive PTE, qp < 0.05 compared moderate and extensive desmoplasia*

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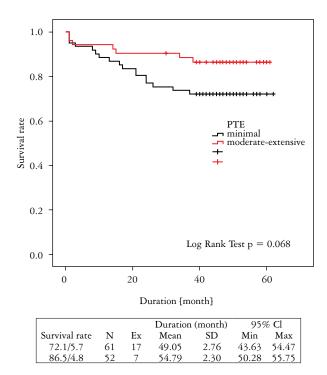| Survival rate | N | Ex | Duration (month) | | 95% Cl | |
|---|---|---|---|---|---|---|
| | | | Mean | SD | Min | Max |
| 72.1/5.7 | 61 | 17 | 49.05 | 2.76 | 43.63 | 54.47 |
| 86.5/4.8 | 52 | 7 | 54.79 | 2.30 | 50.28 | 55.75 |

**Fig. 7.** Survival rate in minimal vs. moderate-extensive peritumoral eosinophils

Minimal PTE was associated with a significantly higher rate of metastasis and pT4 when compared with moderate and extensive PTE (p = 0.03 and 0.008, respectively).

## Concordance between ITB and PTB and their association with study parameters

ITB and PTB were positive totally in 86.9% of patients with high concordance between ITB and PTB (92.8%, p = 0.000). The absence of ITB (87.5% vs. 12.5% and 0.0%, respectively, p < 0.001) and PTB (93.8% vs. 6.3% and 0.0%, respectively, p < 0.001) were both associated with having a higher probability of minimal desmoplasia. The presence of high ITB was associated with a higher rate of minimal PTE (70.0% vs. 12.0 and 18.0%, respectively, p = 0.014). No other significant association of PTB or ITB was noted within the study parameters other than those mentioned above (Table III).

## Survival data

Five-year CSS rate was 78.7%, mean (SE) duration of survival was 52.09 (1.87) months. The absence of metastasis when compared to the presence of metastasis was associated with significantly higher 5-year survival rate (86.6% vs. 47.8%) and a longer duration of survival (mean [SE] 56.0 [1.69] vs. 35.83 [5.06] months) (p < 0.001). TNM stage, tumor grade, venous invasion, desmoplasia, neoadjuvant therapy, budding or other tumor-related inflamma-

tory parameters had no significant impact on CSS. Albeit not statistically significant, there was a higher rate of 5-year CSS (86.5% vs. 72.15%) and a longer duration of survival mean [SE] 54.79 [2.30] vs. 49.05 [2.76] months) in the presence of a moderate-extensive rather than a minimal PTE (p = 0.068) (Table IV, Fig. 7).

## Cox proportional hazards regression model assessing the prognostic significance of study parameters

In Cox proportional hazards regression analyses, an increasing age (hazard ratio 1.076, 95% CI: 1.025-1.130, p = 0.003) and the presence of metastasis (hazard ratio: 4.591, 95% CI: 1.814-11.619, p = 0.001) were independently associated with poor CSS.

## Discussion

Colorectal cancer is classically seen in aged people and more common in males [1]. In accordance with this, the mean age of our study group was 60 and the majority (59%) were male.

Our findings revealed that there was an association of an increase in tumor-related inflammatory parameters with lesser likelihood of certain conventional poor prognostic tumor parameters such as pT4, TNM stage IV, venous invasion and metastasis, and that of higher PTE with lower ITB. This seems in agreement with the consistently reported association of a high-grade peritumoral or intratumoral local inflammatory response with an effective anti-tumor host immune responses and an improved prognosis following a potentially curative resection for CRC [14, 15, 16, 17].

Our findings emphasize the potential role of PTE in prognostic outcome in operated CRC patients given the association of an increase in PTE with a lesser likelihood of ITB and metastasis as well as with a non-significantly for higher 5-year CSS rate and longer duration of CSS.

High IE and PTE counts were shown to be correlated with improved survival in operable CRC in previous studies [10, 14, 25, 28, 29], being independent of AJCC/UICC stage [14, 25, 28] and more common for tumors lacking lymph node or distant metastases [30, 31]. However, consistent with our findings non-significantly better survival rather than a significant relationship was also reported for PTE in patients with CRC [18].

In a recent study by Harbaum et al. [10] on retrospective analysis of peri- and intratumoral eosinophil counts in 381 CRC patients, the presence or an increasing number of eosinophils at the tumor margin was reported to be strongly associated with a favorable tumor phenotype in terms of TNM stage, tumor grade, vascular invasion, and tumor budding as

**Table IV.** Survival data in relation to study parameters

| | N | Ex | 5-year survival rate (%/SE) | Duration of survival (month) Mean (SE) | 95% CI LB | UB | P value |
|---|---|---|---|---|---|---|---|
| Overall | 113 | 24 | 78.7/3.9 | 52.09 (1.87) | 48.43 | 55.75 | – |
| **Grade** | | | | | | | |
| Low | 98 | 19 | 80.6/4.0 | 52.53 (2.01) | 48.60 | 56.47 | 0.270 |
| High | 15 | 5 | 66.7/12.0 | 43.87 (4.11) | 35.81 | 51.92 | |
| **Gender** | | | | | | | |
| Male | 67 | 12 | 82.1/4.7 | 53.36 (2.35) | 48.75 | 57.97 | 0.312 |
| Female | 46 | 12 | 73.8/6.5 | 49.49 ( 2.97) | 43.67 | 55.31 | |
| **Metastasis** | | | | | | | |
| Absent | 90 | 12 | 86.6/3.6 | 56.00 (1.69) | 52.69 | 59.31 | < 0.001 |
| Present | 23 | 12 | 47.8/10.4 | 35.83 (5.06) | 25.90 | 45.75 | |
| **Venous invasion** | | | | | | | |
| Absent | 71 | 13 | 81.7/4.6 | 52.59 (2.20) | 48.28 | 56.90 | 0.330 |
| Present | 42 | 11 | 73.7/6.8 | 49.86 (3.27) | 43.45 | 56.27 | |
| **ITB** | | | | | | | |
| Absent-low | 68 | 11 | 83.8/4.5 | 53.12 (2.23) | 48.74 | 57.50 | 0.123 |
| High | 45 | 13 | 70.9/6.8 | 49.24 (3.14) | 43.08 | 55.40 | |
| **PTB** | | | | | | | |
| Absent-low | 65 | 11 | 83.1/4.7 | 52.65 (2.36) | 48.02 | 57.27 | 0.223 |
| High | 48 | 13 | 72.7/6.5 | 50.09 (2.93) | 44.44 | 55.93 | |
| **ITL** | | | | | | | |
| Absent | 26 | 5 | 80.8/7.7 | 53.04 (3.67) | 45.84 | 60.24 | 0.602 |
| Mild-moderate | 62 | 15 | 75.8/5.4 | 49.17 (2.73) | 43.82 | 54.51 | |
| Extensive | 25 | 4 | 84.0/7.3 | 46.40 (2.06) | 42.37 | 50.43 | |
| **PTL** | | | | | | | |
| Minimal | 59 | 14 | 76.3/5.5 | 51.14 (2.60) | 46.04 | 56.23 | 0.604 |
| Moderate | 38 | 8 | 78.9/6.6 | 49.92 (3.57) | 42.93 | 56.91 | |
| Extensive | 16 | 2 | 86.7/8.8 | 57.00 (1.98) | 53.13 | 60.87 | |
| **CLI** | | | | | | | |
| Absent | 40 | 11 | 72.5/7.1 | 46.87 (3.70) | 39.62 | 54.13 | 0.176 |
| Present | 73 | 13 | 82.1/4.5 | 54.54 (1.97) | 50.69 | 58.39 | |
| **IE** | | | | | | | |
| Minimal | 39 | 17 | 78.5/4.6 | 51.70 (2.28) | 47.23 | 56.16 | 0.896 |
| Moderate-extensive | 74 | 7 | 79.1/7.0 | 51.36 (3.12) | 45.26 | 57.47 | |
| **PTE** | | | | | | | |
| Minimal | 61 | 17 | 72.1/5.7 | 49.05 (2.76) | 43.63 | 54.47 | 0.068 |
| Moderate-extensive | 52 | 7 | 86.5/4.8 | 54.79 (2.30) | 50.28 | 55.75 | |
| **ITI** | | | | | | | |
| Minimal | 61 | 14 | 77.0/5.4 | 51.00 (2.65) | 45.80 | 56.20 | 0.611 |
| Moderate | 30 | 7 | 76.7/7.7 | 50.07 (3.76) | 42.70 | 57.43 | |
| Extensive | 22 | 3 | 85.9/7.5 | 55.03 (2.95) | 49.25 | 60.81 | |

**Table IV.** Cont.

| | N | Ex | 5-year survival rate (%/SE) | Mean (SE) | 95% CI LB | 95% CI UB | P value |
|---|---|---|---|---|---|---|---|
| PTI | | | | | | | |
| Minimal | 61 | 15 | 75.4/5.5 | 50.15 (2.73) | 44.80 | 55.49 | 0.624 |
| Moderate | 30 | 5 | 83.3/6.8 | 52.50 (3.22) | 46.18 | 58.82 | |
| Extensive | 22 | 4 | 81.3/8.4 | 53.74 (3.53) | 46.82 | 60.67 | |
| Desmoplasia | | | | | | | |
| Minimal | 57 | 11 | 80.7/5.2 | 52.44 (2.45) | 47.64 | 57.24 | 0.772 |
| Moderate | 33 | 7 | 78.8/7.1 | 50.36 (3.31) | 43.88 | 56.84 | |
| Extensive | 23 | 6 | 73.7/9.2 | 49.41 (4.59) | 40.42 | 58.39 | |
| Neoadj. Tx | | | | | | | 0.818 |
| Absent | 88 | | 78.4/4.4 | 51.71 (2.17) | 47.45 | 55.98 | |
| Present | 25 | | 80.0/8.0 | 48.60 (3.08) | 42.55 | 54.65 | |

*ITB – intratumoral budding; PTB – peritumoral budding; ITL – intratumoral lymphocyte; PTL – peritumoral lymphocyte; CLI – Crohn-like inflammation; IE – Intratumoral eosinophils; PTE – peritumoral eosinophils; ITI – intratumoral overall inflammation; PTI – peritumoral overall inflammation; tx – therapy; CI – confidence interval; LB – lower bound; UB – upper bound; SE – standard error. Log Rank Test*

well as with improved survival [10]. The authors also noted that although the PTE count correlated with the intensity of the overall inflammatory cell reaction, it was independently associated with the outcome [10]. In our study, although we found that high PTE was strongly associated with better survival rates, probably due to the relatively small sample size, we did not obtain statistically significant p values (p = 0.068). Similarly, in our cohort, although extensive CLI was also associated with a decreased rate of metastasis and TNM stage 4, only PTE was associated significantly with ITB and potential survival. This seems to support the potential role of eosinophil infiltration as an antitumoral mechanism independent of overall host inflammatory cell reaction in CRCs [10, 32].

In a recent study by Briede *et al.* on retrospective analysis of 553 CRC cases, high grade peritumoral inflammation was associated with beneficial morphologic CRC features, including less frequent manifestations of invasion [19]. In our study, we found no association of ITI and PTI with any of the tumor prognostic parameters. This may be due to high density of neutrophil leukocytes in ITI and PTI, since there are controversial findings on the prognostic value of neutrophils in CRC [33, 34]. These controversies may be explained by the duality of neutrophils comprising both a tumor suppressive N1 population and tumor supportive N2 neutrophils [35].

Nonetheless, while the evaluation of PTE seems to be a promising tool with potential to improve risk stratification in CRC patients [10], inconsistency regarding its relationship on overall inflammation or staging of the disease [10, 17, 28, 29] is considered to challenge its incorporation in the routine prognostic assessment in clinical practice [17].

In a study by Nagtegaal *et al.* [28] in 160 CRC patients, an increasing peritumoral, but not intratumoral eosinophil count was reported to be associated with better CSS and lower rates of recurrence. This effect was shown to be dependent on both TNM stage and overall inflammatory cell reaction [28]. In another study carried out by Fisher *et al.* [29] higher numbers of eosinophils were found to be associated with better overall survival, when dependent at the tumor stage but not on the overall inflammatory cell reaction [29].

In a meta-analysis of 30 studies on 2988 patients on the impact of tumor-infiltrating inflammation on survival outcomes in terms of generalized tumor inflammatory infiltrate (n = 12) and T lymphocyte subsets (n = 18), the authors concluded the association of high density of generalized tumor inflammatory infiltrate to be a good prognostic marker for CRC. This was due to its significant association with improved survival, and emphasized a need for further prospective studies on subsets of T lymphocytes due to significant heterogeneity and an insufficient number of studies [36].

In addition, mechanisms of antitumoral activity exhibited by eosinophils also remain not elucidated, while emerging evidence indicates that they may exert their anti-tumor effect not only through their cytotoxicity (such as TNF-α, granzyme, cationic proteins and IL-8), but also via immunomodulatory mechanisms including secretion of T-cell cytokines, activation of dendritic cells or through antigen presentation to T-cells [10, 17, 37, 38]. Additionally, a plethora of factors produced by cancer and immune

cells can attract and/or activate eosinophils in the tumor microenvironment [38].

An association of high-grade tumor infiltrating lymphocytes with a higher likelihood of deriving a survival benefit from adjuvant chemotherapy in patients undergoing curative resection of CRC was reported [17, 39]. Therefore, the increase in ITL with higher tumor grade and lower likelihood of venous invasion in our cohort seems notable. This seems to also emphasize the likelihood of the presence of a distinct lymphocytic infiltrative pattern rather than specific tumor infiltrating lymphocytes to be the most important determinant of survival [14, 40].

In a retrospective analysis of 120 patients with AJCC/UICC stage II colorectal cancer by Betge *et al.*, high score tumor budding was reported to be significantly associated with tumor grade and lymphovascular invasion and thought to be an independent predictor of disease progression and cancer-related death [41]. Our findings revealed a high concordance between ITB and PTB, in parallel to their similar pathogenesis as morphologic manifestations of epithelial-mesenchymal transition [42]. Both high ITB and high PTB were associated with higher rates of pT4 stage, venous invasion and moderate-to-extensive desmoplasia. This indicates that tumor budding is frequently found in tumors with an invasive growth pattern and desmoplastic stromal response. Neither ITB nor PTB was associated with a metastasis rate in our cohort despite the reported association of tumor budding with an increased probability for metastatic dissemination to lymph nodes and distant organs. Our findings were thus compatible with the literature [43].

The presence of higher ITB in patients with minimal as compared to moderate-to-extensive PTE in our cohort seems to support the potential role of eosinophil infiltration as an antitumoral mechanism [10]. It also seems notable given the infrequently encountered tumor budding in patients with a strong peritumoral infiltration caused by CD8+ cytotoxic T cells, indicating the likelihood of an interaction between tumor budding cells and the host immune system as two opposing sides of an attacker-defender model [13, 43].

Our findings emphasized the potential role of additional prognostic parameters related to tumor microenvironment alongside the conventional morphological prognostic parameters in the identification of high-risk pathological features and in guiding provision of adjuvant therapy in CRCs [10, 14, 15].

The retrospective and single center nature of our study was an important limitation and might have influenced our findings in establishing the temporality between cause and effect as well as generalizing our findings to an overall CRC population. The other limitation of this study was the relatively small sample size that might have prevented from obtaining statistical significance concerning prognostic role of tumor inflammatory parameters. The inclusion of patients with and without neoadjuvant therapy together was another limitation of the study. Nevertheless, despite these limitations, given the paucity of the solid information available in this area, our findings represent a valuable contribution to the literature by providing data on assessment of the prognostic role of tumor-associated inflammatory infiltrates based on standardized criteria and being stratified by location (intratumoral, peritumoral) and inflammatory cell subset of both the adaptive and innate immune system (lymphocytes, eosinophils).

In conclusion, our findings revealed that low score tumoral budding and an increase in tumor-related inflammatory parameters were associated with a lesser likelihood of conventional poor prognostic tumor parameters such as pT4, TNM stage IV, venous invasion and metastasis. Nonetheless, given the association of an increase in peritumoral eosinophil infiltrates with a lower probability of intratumoral budding, pT4, metastasis and for better survival rates, our findings emphasize the potential role of peritumoral eosinophils independent of the overall inflammatory reaction. This can be considered as an additional prognostic parameter related to the tumor microenvironment in better stratification of progression and guiding provision of adjuvant therapy tailored to individualized risk.

*The authors declare no conflict of interest.*

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