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Original scientific paper

PROGNOSTIC AND PREDICTIVE IMPORTANCE OF TUMOR BUDDING, LYMPHOVASCULAR AND PERINEURAL INVASION IN COLORECTAL CARCINOMA

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Abstract

Background: Colorectal carcinoma (CRC) remains one of the most common malignant neoplasms worldwide and a leading cause of cancer-related mortality. Accurate identification of prognostic markers is essential for predicting recurrence and stratifying patients for individualized treatment. This study aimed to evaluate the prognostic and predictive significance of tumor budding (TB), lymphovascular invasion (LVI), and perineural invasion (PNI) in colorectal carcinoma.

Methods: This retrospective observational cohort study included 243 patients with histologically confirmed colorectal adenocarcinoma treated at the University Clinical Center of Kosovo (UCCK) between January 2020 and December 2024. Clinical and pathological data were collected, including TB, LVI, and PNI. Statistical analysis included descriptive statistics, chi-square tests, binary logistic regression, receiver operating characteristic (ROC) curve analysis with area under the curve (AUC), and Kaplan–Meier disease-free survival (DFS) analysis with log-rank tests.

Results: High tumor budding (BD3) was present in 29.2% of patients, lymphovascular invasion in 34.1%, and perineural invasion in 26.7%. At least one invasive histopathological factor was identified in 48.6% of patients. All three parameters were significantly associated with tumor recurrence ($p < 0.001$). Logistic regression analysis identified LVI (OR 4.72; 95% CI 2.46–9.03), PNI (OR 3.91; 95% CI 1.98–7.72), and tumor budding (OR 3.24; 95% CI 1.67–6.29) as independent predictors of recurrence. ROC analysis demonstrated the highest predictive value for LVI (AUC = 0.781). Patients with more than one invasive factor showed significantly reduced disease-free survival (21.6 vs. 33.2 months; $p < 0.001$).

Conclusion: Lymphovascular invasion, perineural invasion, and tumor budding represent independent prognostic histopathological factors in colorectal carcinoma. LVI demonstrated the strongest predictive value for recurrence. These parameters should be routinely included in pathological reports and integrated into prognostic models to improve individualized risk assessment and therapeutic decision-making.

Keywords:colorectal carcinoma; tumor budding; lymphovascular invasion; perineural invasion; disease-free survival; ROC analysis

Introduction

Colorectal carcinoma (CRC) is among the most common malignancies worldwide and represents one of the leading causes of cancer-related mortality. According to global cancer statistics, more than 1.9 million new cases were reported worldwide in 2023. Despite improvements in surgical techniques, chemotherapy, and radiotherapy, recurrence rates remain significant and long-term survival largely depends on tumor biology. Histopathological characteristics play a crucial role in predicting disease progression and recurrence. Among the most important invasive morphological features are tumor budding (TB), lymphovascular invasion (LVI), and perineural invasion (PNI). These parameters are increasingly recognized as indicators of tumor aggressiveness and metastatic potential. Tumor budding refers to the presence of single tumor cells or small clusters at the invasive tumor front and is associated with epithelial-mesenchymal transition and metastatic potential. Lymphovascular invasion represents the presence of tumor cells within lymphatic or vascular channels, facilitating systemic dissemination. Perineural invasion describes tumor infiltration of neural structures and is associated with aggressive disease and increased recurrence risk. Although the TNM staging

system remains the cornerstone of prognostic assessment, it does not fully reflect the biological heterogeneity of colorectal cancer. Advanced statistical models such as logistic regression and ROC analysis may help identify the most relevant prognostic variables and improve clinical risk stratification.

Aim of the Study

The aim of this study was to evaluate the **prognostic and predictive value of tumor budding, lymphovascular invasion, and perineural invasion** in patients undergoing surgical treatment for colorectal carcinoma at the **University Clinical Center of Kosovo (UCCK)**. Specifically, the study aimed to determine the relationship of these histopathological factors with tumor stage, disease recurrence, and disease-free survival.

Materials and methods

Study Design

This study was designed as a **retrospective observational cohort study** conducted at the University Clinical Center of Kosovo.

Patient Selection

Patients treated surgically for colorectal adenocarcinoma between **January 2020 and December 2024** were included. Inclusion criteria were histologically confirmed colorectal carcinoma and complete clinical-pathological documentation.

Histopathological Evaluation

Tumor budding was assessed according to the **International Tumor Budding Consensus Conference (ITBCC) 2016** guidelines. Lymphovascular and perineural invasion were evaluated according to the **College of American Pathologists (CAP) 8th edition guidelines**.

Statistical Analysis

Statistical analyses were performed using **IBM SPSS Statistics v26.0**. Descriptive statistics summarized clinical characteristics. Comparative analyses were performed using chi-square tests and Student's t-tests where appropriate. Independent predictors of recurrence were assessed using **binary logistic regression analysis**. ROC curves were used to evaluate predictive performance. Disease-free survival was analyzed using **Kaplan–Meier curves** and compared using the **log-rank test**.

Ethical Approval

This retrospective study was approved by the **Ethics Committee of the University Clinical Center of Kosovo (UCCK)**. The study was conducted in accordance with the principles of the **Declaration of Helsinki**. Due to the retrospective nature of the study and anonymized data analysis, informed consent was waived.

Results

The study included **243 patients**, of whom **138 (56.8%) were male and 105 (43.2%) were female**, with a mean age of **63.4 years**. Tumor localization was identified in the **rectum in 96 cases (39.5%)**, the **left colon in 87 patients (35.8%)**, and the **right colon in 60 patients (24.7%)**.

The surgical procedures performed included **low anterior resection (32.1%)**, **left hemicolectomy (29.6%)**, **right hemicolectomy (24.3%)**, and **abdominoperineal resection (14.0%)**. Histopathological analysis revealed **high tumor budding (BD3) in 71 patients (29.2%)**, **lymphovascular invasion (LVI) in 83 patients (34.1%)**, and **perineural invasion (PNI) in 65 patients (26.7%)**. Overall, **118 patients (48.6%) presented at least one invasive histopathological marker (TB, LVI, or PNI).** Histological grading showed **G1 in 22 patients (9.1%)**, **G2 in 156 patients (64.2%)**, and **G3 in 65 patients (26.7%)**. According to the **TNM classification**, **174 patients (71.6%) were diagnosed with stage II–III disease**, while **21 patients (8.6%) were classified as stage IV**.During a **mean follow-up period of 30.4 months (range 6–48 months)**, **64 patients (26.3%) developed local or distant recurrence**. The presence of **lymphovascular invasion, perineural invasion, and high tumor budding was significantly more frequent in patients with recurrence ($p < 0.001$ for all three factors).**

Table 1. Association between histopathological factors and disease recurrence

Histopathological factors	Recurrence (+) n = 64	Recurrence (–) n = 179	p-value (Chi-square)
LVI positive	47 (73.4%)	36 (20.1%)	<0.001
PNI positive	39 (60.9%)	26 (14.5%)	<0.001
High tumor budding (BD3)	41 (64.1%)	30 (16.8%)	<0.001

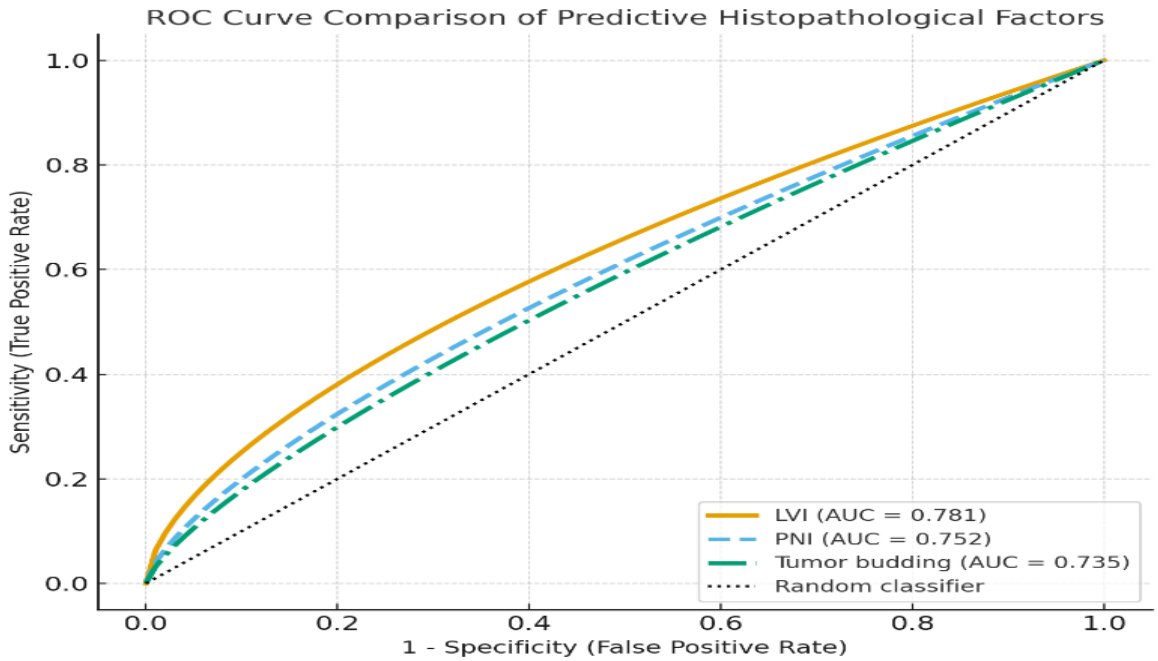
Table 2. Binary logistic regression analysis

In the logistic regression model, **LVI**, **PNI**, and **tumor budding** emerged as **independent prognostic predictors** of disease recurrence.

Prognostic factors	OR	95% CI	p-value
LVI (yes vs no)	4.72	2.46–9.03	<0.001
PNI (yes vs no)	3.91	1.98–7.72	<0.001
Tumor budding BD3 vs BD1–2	3.24	1.67–6.29	0.002
TNM Stage III–IV vs I–II	2.31	1.19–4.47	0.016

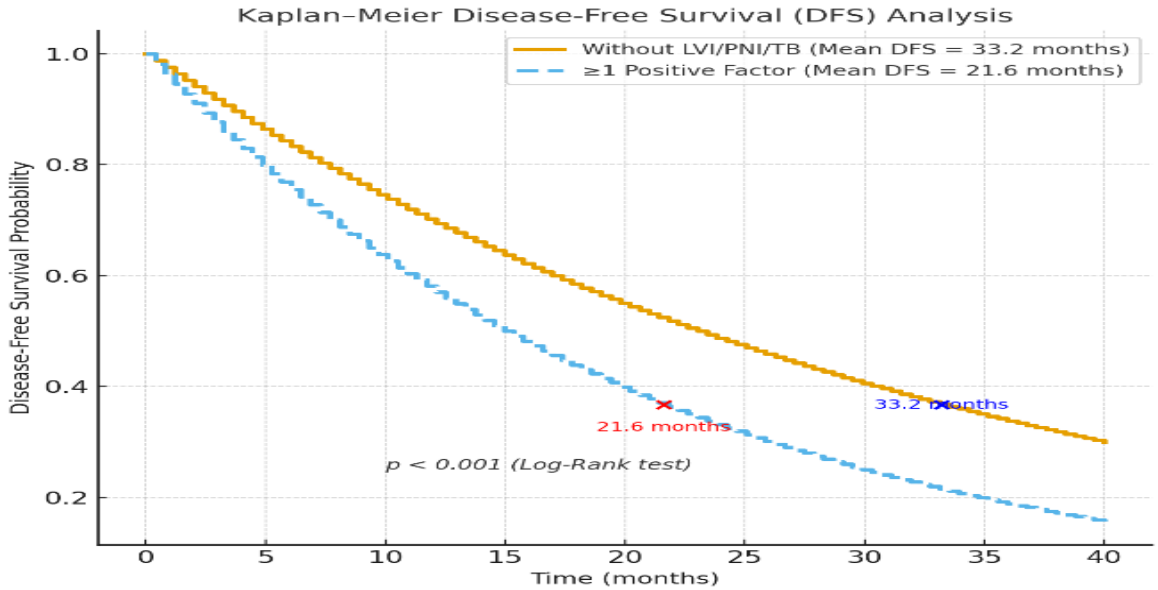
Summary

Overall, **48.6% of the cohort presented at least one invasive histopathological factor**. **Lymphovascular invasion (LVI)** demonstrated the strongest predictive value for disease recurrence, with an **area under the ROC curve (AUC) of 0.781**. **Disease-free survival (DFS)** was significantly shorter in patients with **lymphovascular invasion, perineural invasion (PNI), or tumor budding (TB)**. These findings suggest that the combined evaluation of these histopathological markers may contribute to the development of **clinicopathological prognostic models for improved risk stratification of recurrence in colorectal carcinoma.**



Graph. 1. ROC curve analysis of histopathological predictors of recurrence.

ROC curve analysis demonstrated a comparison among the three histopathological factors. Lymphovascular invasion (LVI) showed the highest area under the curve (AUC = 0.781), indicating the strongest predictive accuracy for disease recurrence compared with perineural invasion (PNI) and tumor budding (TB).



Graph. 2. Kaplan–Meier disease-free survival curves according to invasive histopathological factors.

Kaplan–Meier survival analysis demonstrated that the mean disease-free survival (DFS) was 33.2 months in patients without invasive histopathological factors (LVI, PNI, or TB), compared with 21.6 months in patients presenting at least one of these factors. The difference between the groups was statistically significant ($p < 0.001$, log-rank test), indicating that the presence of invasive histopathological features is associated with earlier recurrence and poorer disease-free survival.

Discussion

The present study conducted at the **University Clinical Center of Kosovo (UCCK)** between 2020 and 2024 highlights the prognostic significance of invasive histopathological markers—tumor budding, lymphovascular invasion, and perineural invasion—in patients undergoing surgical treatment for colorectal carcinoma. Among the 243 analyzed patients, **48.6% exhibited at least one invasive histopathological feature**, indicating the presence of aggressive biological behavior in a considerable proportion of colorectal tumors. Our results demonstrate that **lymphovascular invasion (LVI), perineural invasion (PNI), and high-grade tumor budding (BD3)** are all independently associated with disease recurrence ($p < 0.001$ for each factor). Multivariate logistic regression analysis confirmed that all three variables remained independent predictors of recurrence, with **LVI showing the strongest association** (odds ratio 4.72; 95% CI 2.46–9.03). These findings are consistent with previous studies reporting that vascular and perineural invasion represent key mechanisms of tumor dissemination and recurrence. Studies by **Alessandro Lugli et al. (2022)** and **Jan Betge et al. (2023)** identified LVI as a strong indicator of systemic tumor spread and unfavorable clinical prognosis in colorectal cancer. The predictive performance of these parameters was further supported by **receiver operating characteristic (ROC) analysis**, which demonstrated that LVI had the highest discriminative ability for predicting recurrence (**AUC = 0.781**), followed by PNI (**AUC = 0.752**) and tumor budding (**AUC = 0.735**). These results suggest that vascular invasion may serve as a robust surrogate marker of tumor aggressiveness and micrometastatic potential and should therefore be incorporated into risk-stratification models and postoperative therapeutic decision-making. Kaplan–Meier survival analysis revealed a clear difference in **disease-free survival (DFS)** between patients without invasive histopathological factors (mean DFS 33.2 months) and those presenting at least one such factor (mean DFS 21.6 months; $p < 0.001$, log-rank test). Even the presence of a single invasive parameter was associated with significantly poorer DFS, emphasizing the need for closer postoperative surveillance and consideration of adjuvant therapy in high-risk subgroups. Similar observations have been reported by **Julio Garcia-Aguilar et al. (2021)** and **Massimo Airoldi et al. (2025)**, who confirmed the prognostic relevance of these invasive histopathological markers in colorectal cancer. Biologically, these markers represent complementary mechanisms of tumor progression. **Tumor budding** reflects epithelial–mesenchymal transition and early metastatic potential. **Lymphovascular invasion** indicates dissemination through lymphatic and vascular pathways, while **perineural invasion** reflects tumor neurotropism, frequently associated with locoregional recurrence and poorer clinical outcomes. Together, these parameters define a highly aggressive tumor phenotype that may not be fully captured by the conventional **TNM staging system** alone. The strengths of this study include the relatively large patient cohort, standardized histopathological evaluation, and the application of multiple statistical methods—including logistic regression, ROC analysis, and Kaplan–Meier survival analysis—to objectively assess prognostic significance. Nevertheless, several limitations should be acknowledged. The retrospective design may introduce potential selection bias, and the analysis was conducted at a single institution. Additionally, molecular biomarkers such as **KRAS mutations** or **microsatellite instability (MSI)** were not included, which could further refine predictive models. Overall, the present findings confirm that **lymphovascular invasion, perineural invasion, and tumor budding are reliable histopathological predictors of recurrence and survival in colorectal carcinoma**. Their systematic evaluation should therefore be incorporated into routine pathological reporting and multidisciplinary treatment planning.

Conclusions

The present retrospective cohort study involving **243 patients with colorectal carcinoma treated at the University Clinical Center of Kosovo (UCCK)** confirms the significant prognostic value of invasive histopathological markers, specifically **lymphovascular invasion (LVI), perineural invasion (PNI), and tumor budding**. Each of these parameters was independently associated with an increased risk of disease recurrence, with **LVI demonstrating the highest predictive accuracy (AUC = 0.781)**.Patients presenting at least one invasive histopathological factor exhibited a significantly reduced **disease-free survival (DFS)** compared with patients without such features (mean DFS **21.6 vs. 33.2 months; p < 0.001**). These findings highlight the importance of comprehensive histopathological assessment as an essential component of oncological risk stratification, complementing the conventional **TNM staging system**.The integration of these markers into routine pathological evaluation may improve prognostic accuracy, support individualized therapeutic decision-making, and facilitate more appropriate postoperative surveillance strategies in patients with colorectal carcinoma.

Recommendations

Based on the findings of this study, several recommendations can be proposed:

1. **Routine assessment of tumor budding, lymphovascular invasion, and perineural invasion** should be systematically incorporated into all histopathological reports of colorectal carcinoma.
2. Patients presenting one or more of these invasive histopathological features should be considered **high-risk cases**, requiring closer clinical monitoring and more intensive radiological follow-up.
3. Future prognostic models should integrate **clinical, histopathological, and molecular biomarkers** in order to improve the prediction of recurrence and survival outcomes.
4. These findings should be validated through **prospective multicenter studies**, which would allow a more precise evaluation of their therapeutic implications and potential implementation at national and regional levels.

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