RESEARCH PAPER

Tumour Budding as a Prognostic Indicator in Colorectal Carcinoma: A Cross-Sectional Study of Primary Colorectal Carcinoma Cases in a South Indian Tertiary Care Centre

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ABSTRACT

Colorectal carcinoma (CRC) is the third most common cancer worldwide. Although many established prognostic factors exist for CRC, tumour budding is emerging as a valuable morphological marker of tumour aggressiveness. Tumour budding is not yet universally incorporated into routine CRC reporting, but it has been recommended for systematic assessment and grading by the International Tumour Budding Consensus Conference (ITBCC) 2016. The aim of the present study is to evaluate the distribution of tumour budding in primary CRC specimens using the ITBCC 2016 standardised criteria and to correlate budding grade with clinicopathological parameters including tumour staging, lymphovascular invasion, perineural invasion, tumour grade, microvessel density (MVD), and CD44 expression.

Sixty-four cases of primary CRC resection specimens were studied from the Department of Pathology, SRM Medical College Hospital and Research Centre, Kattankulathur, Tamil Nadu. Tumour budding was assessed on haematoxylin and eosin (H&E)-stained sections per ITBCC 2016 criteria and graded as low (BD1: <5 buds/HPF), intermediate (BD2: 5–9 buds/HPF), or high (BD3: ≥10 buds/HPF). Low budding (BD1) was found in 84.4% (n=54), intermediate (BD2) in 7.8% (n=5), and high (BD3) in 7.8% (n=5) of cases. High and intermediate budding were exclusively confined to the high MVD group, with a borderline significant association (p=0.054). No significant association was identified between tumour budding and CD44 expression (p=0.579), histological grade, LVI, PNI, or TNM staging in this cohort. Tumour budding is a valuable morphological parameter that merits routine reporting in CRC and, in combination with other prognostic markers, can enhance biological risk stratification.

Keywords: *tumour budding; colorectal carcinoma; ITBCC; prognostic marker; microvessel density; CD44; clinicopathological correlation*

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INTRODUCTION

Colorectal carcinoma (CRC) is the third most common malignancy worldwide and the second leading cause of cancer-related mortality, with over 1.9 million new cases reported globally in 2020. The standard pathological reporting of CRC follows guidelines from the College of American Pathologists (CAP, 8th edition) and the Royal College of Pathologists (UK), incorporating parameters such as tumour site, extent, histological type, grade, margins, lymphovascular invasion, and perineural invasion. Despite the strength of TNM-based staging, conventional histopathological parameters alone do not fully capture the biological heterogeneity of CRC, particularly in early-stage disease where the distinction between patients requiring adjuvant therapy and those who can safely undergo observation remains clinically contested.

Tumour budding is defined as the presence of isolated single tumour cells or small clusters of fewer than five tumour cells at the invasive front of the tumour. It was first described by Imai in 1954 who referred to it as 'sprouting' and associated it with rapid disease progression. The term 'budding' was subsequently coined by Morodomi et al. in 1989, who demonstrated its association with lymph node metastasis. Since then, a large body of evidence has established tumour budding as an independent morphological marker of poor prognosis in CRC.

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Biologically, tumour budding reflects epithelial-to-mesenchymal transition (EMT) at the invasive front: tumour cells downregulate E-cadherin and upregulate vimentin, N-cadherin, and fibronectin, acquiring migratory and invasive properties. High-grade budding (BD3) has been significantly associated with lymph node metastasis even in pT1 lesions, advanced TNM stage, lymphovascular invasion, and poor survival across multiple independent studies.

The International Tumour Budding Consensus Conference (ITBCC), held in 2016, standardised tumour budding assessment methodology and proposed a three-tier grading system (BD1: 0–4 buds; BD2: 5–9 buds; BD3: ≥10 buds) per hotspot field of 0.785 mm² at ×20 objective using H&E-stained sections. This consensus framework has subsequently been recommended for incorporation into CRC reporting datasets by the Royal College of Pathologists and College of American Pathologists as an optional element. Its consistent applicability across diverse cohorts underscores its potential as a universally reportable prognostic biomarker.

In Stage II CRC, tumour budding has been shown to be an independent predictor of survival, guiding treatment strategy and adjuvant therapy decisions. High bud score has also been associated with other parameters of poor prognosis such as higher tumour grade and lymphovascular permeation. The present study evaluates tumour budding distribution and its clinicopathological associations in a cohort of 64 surgically resected CRC specimens from a South Indian tertiary care centre, using the standardised ITBCC 2016 methodology.

MATERIALS AND METHODS

Study Design and Patient Selection

A cross-sectional study incorporating both prospective and retrospective resected CRC specimens was conducted between January 2020 and January 2025 at the Department of Pathology, SRM Medical College Hospital and Research Centre, Kattankulathur, Tamil Nadu, India. Institutional Ethics Committee approval was obtained prior to the commencement of the study. A total of 64 histopathologically confirmed CRC resection specimens were included.

Cases of confirmed colorectal adenocarcinoma (all subtypes) on resection specimens — hemicolectomy, total colectomy, low anterior resection, and abdominoperineal resection — were included. Exclusion criteria comprised colonic biopsies, specimens with inadequate material for histological assessment, cases with inflammatory bowel disease or familial adenomatous polyposis, and specimens received following pre-operative radiotherapy or chemotherapy.

Histopathological Assessment

All specimens were fixed in 10% neutral buffered formalin. Sections of 4–5 µm thickness were processed by standard paraffin embedding and stained with haematoxylin and eosin (H&E). Each case was assessed for tumour site, laterality, gross morphology, histological type, histological grade (per WHO classification), depth of invasion, lymphovascular invasion (LVI), perineural invasion (PNI), tumour infiltrating lymphocytes (TILs), tumour budding, and TNM staging per AJCC 8th edition guidelines.

Assessment of Tumour Budding

Tumour budding was defined as isolated single tumour cells or small clusters of fewer than five tumour cells at the invasive front of the tumour, consistent with ITBCC 2016 criteria. Assessment was performed on H&E-stained sections by two independent pathologists. All slides were screened at ×10 objective to identify fields with the highest peritumoral bud density at the tumour–stroma interface. The hotspot field with the highest budding density at ×20 objective (corresponding to a field area of 0.785 mm²) was identified per ITBCC methodology. Tumour budding was graded using the validated ITBCC 2016 three-tier system:

- BD1 (Low): 0–4 buds per hotspot field
- BD2 (Intermediate): 5–9 buds per hotspot field
- BD3 (High): ≥10 buds per hotspot field

In mucinous carcinomas, only tumour cells meeting the strict criteria for tumour budding were counted; tumour cells or clusters floating in pools of mucin were excluded. For poorly differentiated carcinomas, tumour cell clusters of five or more cells were not counted as budding foci.

Immunohistochemical Assessment

All cases additionally underwent immunohistochemical (IHC) staining for CD44 (cancer stem cell marker) and CD34 (endothelial marker for microvessel density assessment). CD44 expression was scored as low (<10% positive tumour cells), moderate (10–50%), or strong (>50%). CD34-assessed microvessel density (MVD) was quantified by the Weidner hotspot counting method at ×400 magnification, using the median value of 24.5 vessels/HPF to categorise cases as high MVD (≥24.5) or low MVD (<24.5). The PAP method was used for antigen detection with monoclonal antibodies from PATHINSITU.

Statistical Analysis

Descriptive analysis was expressed as frequencies and percentages for categorical variables and mean ± standard deviation (SD) for continuous variables. Associations between tumour budding grade and other clinicopathological parameters were assessed using the Chi-square test or Fisher's exact test, as appropriate. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics Version 22.

RESULTS

Clinicopathological Profile

The study comprised 64 surgically resected CRC specimens. The mean age of the study population was 62.14 ± 10.04 years, with a range of 41–78 years. A male

predominance was observed, with 38 (59.4%) male and 26 (40.6%) female patients. Left-sided tumours accounted for 54.7% (n=35) of cases and right-sided for 45.3% (n=29). The ascending colon was the most common single tumour site at 23.4% (n=15), followed by the rectosigmoid junction at 18.8% (n=12) and the sigmoid colon at 15.6% (n=10).

Adenocarcinoma constituted 98.4% (n=63) of cases, with a single case (1.6%) of signet ring cell adenocarcinoma. Grade 2 (moderately differentiated) tumours were most frequent at 73.4% (n=47), followed by Grade 1 (well differentiated) at 21.9% (n=14) and Grade 3 (poorly differentiated) at 4.7% (n=3). LVI was identified in 28.1% (n=18) of cases and PNI in 12.5% (n=8). High TILs were present in 7.8% (n=5) and low TILs in 92.2% (n=59) of cases. TNM staging distribution: pT2N0Mx 34.4% (n=22), pT3N1Mx 21.9% (n=14), pT3N0Mx 20.3% (n=13), pT1N0Mx 14.1% (n=9), and pT2N1Mx 9.4% (n=6). AJCC Stage I was the most common group at 48.4% (n=31), followed by Stage IIIB at 21.9% (n=14) and Stage IIA at 20.3% (n=13). A detailed clinicopathological profile is summarised in Table 1.

Parameter	Category	Frequency (N=64)	Percentage (%)
Sex	Male	38	59.4
	Female	26	40.6
Tumour side	Left	35	54.7
	Right	29	45.3
Histological type	Adenocarcinoma	63	98.4
	Signet ring cell type	1	1.6
Grade	Grade 1 (Well differentiated)	14	21.9
	Grade 2 (Moderately differentiated)	47	73.4
	Grade 3 (Poorly differentiated)	3	4.7
LVI	Present	18	28.1
	Not identified	46	71.9
PNI	Present	8	12.5
	Not identified	56	87.5
TILs	High	5	7.8
	Low	59	92.2
AJCC Stage	Stage I	31	48.4
	Stage IIA	13	20.3
	Stage IIIA	6	9.4
	Stage IIIB	14	21.9

Table 1: Clinicopathological profile of the study population (N=64)

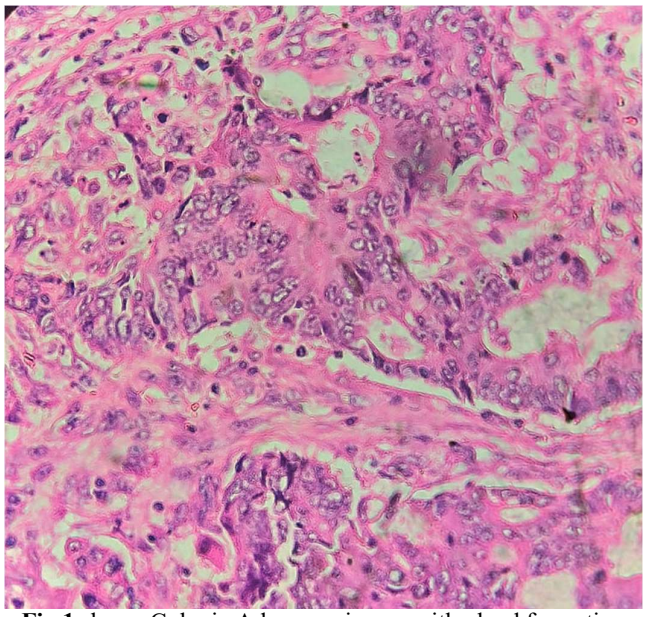

Fig 1 shows Colonic Adenocarcinoma with gland formation

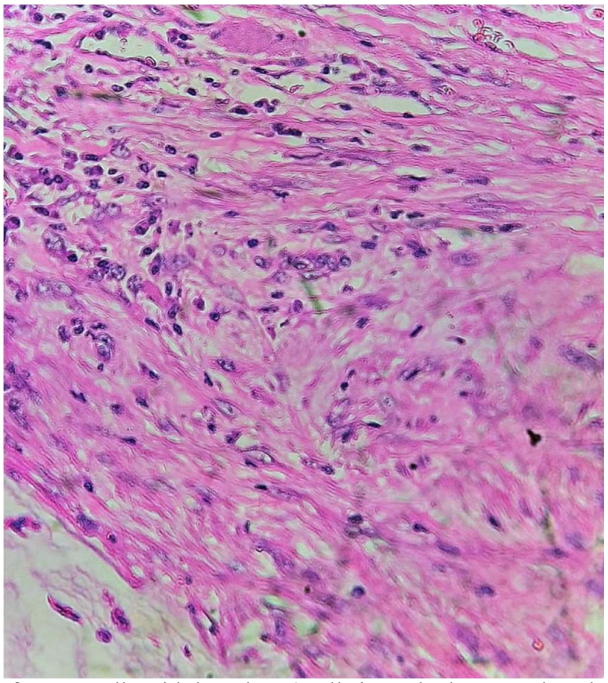

Fig 2 shows clusters of tumor cells with less than 4 cells in each cluster at the advancing edge of tumor

Distribution of Tumour Budding

Tumour budding was assessed on H&E-stained sections per ITBCC 2016 criteria by two independent pathologists. Low tumour budding (BD1) was the most frequent category, observed in 84.4% (n=54) of cases. Intermediate budding (BD2) and high budding (BD3) were each identified in 7.8% (n=5) of cases (Table 2). The high inter-observer reproducibility of the standardised ITBCC method was maintained, with consensus reached for all cases.

Tumour Budding Grade	Definition	Frequency (N=64)	Percentage (%)
BD1 (Low)	0–4 buds/HPF	54	84.4
BD2 (Intermediate)	5–9 buds/HPF	5	7.8

Tumour Budding Grade	Definition	Frequency (N=64)	Percentage (%)
BD3 (High)	≥10 buds/HPF	5	7.8
Total		64	100.0

Table 2: Distribution of tumour budding in the study population (N=64) per ITBCC 2016 criteria

Correlation of Tumour Budding with Microvessel Density (CD34-assessed MVD)

The mean CD34-assessed MVD in the study was 24.84 ± 4.25 vessels/HPF (range 12.10–33.10), with a median of 24.5. Using the median as the cut-off, high MVD was identified in 62.5% (n=40) and low MVD in 37.5% (n=24) of cases. The distribution of tumour budding grades across MVD categories is presented in Table 3.

High and intermediate tumour budding were present exclusively within the high MVD group (12.5% each for BD3 and BD2 vs 0% in both categories in the low MVD group). All cases in the low MVD group demonstrated low budding (100%), while 75.0% of the high MVD group showed low budding. This pattern is biologically compelling: high budding represents EMT at the invasive front, a process that generates pro-angiogenic signals, while high MVD provides the vascular infrastructure required for haematogenous tumour dissemination. Although this association approached but did not reach statistical significance (p=0.054), the biological directionality is clear and consistent with published evidence.

Tumour Budding	High MVD (N=40)	Low MVD (N=24)	p-value
BD3 (High)	5 (12.5%)	0 (0%)	0.054
BD2 (Intermediate)	5 (12.5%)	0 (0%)	
BD1 (Low)	30 (75.0%)	24 (100%)	

Table 3: Correlation of tumour budding with CD34-assessed MVD (N=64)

Correlation of Tumour Budding with CD44 Expression

CD44 immunohistochemical expression was categorised as low (<10% positive tumour cells), moderate (10–50%), and strong (>50%). The distribution of tumour budding grades across CD44 expression categories is summarised in Table 4. In the low CD44 expression group, all tumours (100%) showed low budding with no cases of intermediate or high budding. In moderate CD44 expression, low budding was observed in 78.6%, intermediate in 14.3%, and high in 7.1% of tumours. In strong CD44 expression, low budding accounted for 84.6%, intermediate for 3.8%, and high for 11.5% of cases. The association between CD44 expression and tumour budding was not statistically significant (p=0.579).

Tumour Budding	Low CD44 (N=10)	Moderate CD44 (N=28)	Strong CD44 (N=26)	p-value
BD1 (Low)	10 (100%)	22 (78.6%)	22 (84.6%)	0.579
BD2 (Intermediate)	0 (0%)	4 (14.3%)	1 (3.8%)	
BD3 (High)	0 (0%)	2 (7.1%)	3 (11.5%)	

Table 4: Correlation of tumour budding with CD44 expression (N=64)

Correlation of Tumour Budding with Clinicopathological Parameters

The distribution of tumour budding grades across all major clinicopathological parameters is presented in Table 5. In this cohort, the predominance of early-stage disease (48.4% AJCC Stage I) and correspondingly low proportions of advanced-stage tumours resulted in limited numbers of BD2 and BD3 cases, which constrained formal statistical analysis. The observed trends are summarised below and discussed in context of the published literature.

Histological Grade: Low budding (BD1) was the dominant pattern across all three histological grades. In the low budding group, Grade 2 tumours comprised 85% of cases. In the high budding group, Grade 2 tumours were most frequent (60%) followed by Grade 1 (20%). The small numbers in the BD2 and BD3 groups precluded meaningful statistical testing, but no clear grade-budding gradient was apparent.

Lymphovascular Invasion (LVI): Among cases with LVI present (n=18), 12 (66.7%) showed low budding, 4 (22.2%) intermediate, and 2 (11.1%) high budding. Among cases without LVI (n=46), all (100%) showed low budding. A directional trend toward higher budding in LVI-positive cases was identified, consistent with the known biological

relationship between EMT-driven budding and vascular intravasation.

Perineural Invasion (PNI): Among cases with PNI (n=8), 5 (62.5%) showed low budding and 3 (37.5%) high budding. Among PNI-absent cases (n=56), 49 (87.5%) showed low budding. This directional trend toward higher budding in PNI-positive cases, while numerically compelling, did not reach statistical significance given the small number of PNI-positive cases.

TNM Staging: Among Stage I cases (n=31), all (100%) showed low budding. Among Stage IIIB cases (n=14), 6 (42.9%) showed low budding, 3 (21.4%) intermediate, and 5 (35.7%) high budding. A clear directional association was observed between advancing stage and higher budding grade, consistent with the established biological role of tumour budding in driving nodal dissemination and invasive progression.

Parameter	Category	BD1 Low	BD2 Intermediate	BD3 High
Sex	Male (N=38)	32 (84.2%)	4 (10.5%)	2 (5.3%)
	Female (N=26)	22 (84.6%)	1 (3.8%)	3 (11.5%)
Tumour side	Left (N=35)	29 (82.9%)	4 (11.4%)	2 (5.7%)
	Right (N=29)	25 (86.2%)	1 (3.4%)	3 (10.3%)
Grade	Grade 1 (N=14)	12 (85.7%)	1 (7.1%)	1 (7.1%)
	Grade 2 (N=47)	40 (85.1%)	4 (8.5%)	3 (6.4%)
	Grade 3 (N=3)	2 (66.7%)	0 (0%)	1 (33.3%)
LVI	Present (N=18)	12 (66.7%)	4 (22.2%)	2 (11.1%)
	Absent (N=46)	42 (91.3%)	1 (2.2%)	3 (6.5%)
PNI	Present (N=8)	5 (62.5%)	0 (0%)	3 (37.5%)
	Absent (N=56)	49 (87.5%)	5 (8.9%)	2 (3.6%)
TILs	High (N=5)	3 (60%)	1 (20%)	1 (20%)
	Low (N=59)	51 (86.4%)	4 (6.8%)	4 (6.8%)
AJCC Stage	Stage I (N=31)	31 (100%)	0 (0%)	0 (0%)
	Stage IIA (N=13)	12 (92.3%)	1 (7.7%)	0 (0%)
	Stage IIIA (N=6)	5 (83.3%)	1 (16.7%)	0 (0%)
	Stage IIIB (N=14)	6 (42.9%)	3 (21.4%)	5 (35.7%)
MVD	High MVD (N=40)	30 (75.0%)	5 (12.5%)	5 (12.5%)
	Low MVD (N=24)	24 (100%)	0 (0%)	0 (0%)
CD44	Low expression (N=10)	10 (100%)	0 (0%)	0 (0%)
	Moderate expression (N=28)	22 (78.6%)	4 (14.3%)	2 (7.1%)
	Strong expression (N=26)	22 (84.6%)	1 (3.8%)	3 (11.5%)

Table 5: Distribution of tumour budding grades across clinicopathological parameters (N=64). BD1=low; BD2=intermediate; BD3=high

DISCUSSION

The results of the present study demonstrate the feasibility and reproducibility of standardised ITBCC 2016 tumour budding assessment in a prospective-retrospective CRC resection cohort from a South Indian tertiary care centre. Tumour budding is a morphologically accessible, cost-

effective, and methodologically reproducible prognostic parameter that can be reliably assessed on routine H&E-stained sections without additional immunohistochemical staining. The data generated provide a clinicopathological framework for interpreting budding grade in the context of complementary biological markers CD44 expression and CD34-assessed MVD in the same cohort.

Distribution of Tumour Budding in the Study Population

Low budding (BD1) was the dominant pattern in the present cohort, observed in 84.4% (n=54) of cases, with intermediate (BD2) and high (BD3) budding each identified in 7.8% (n=5) of cases. This distribution is consistent with published resected CRC cohorts where early-stage disease predominates. Lugli et al. reported 65% BD1 in their ITBCC validation cohort, while Koelzer et al. found 70% low budding across 485 Stage I–III cases, and Giger et al. documented 84% BD1 in a Swiss population-based series. The 84% BD1 prevalence in our cohort sits at the upper range of published values and is directly attributable to the strong enrichment of early-stage disease in our series, with 48% of cases being AJCC Stage I. This is consistent with the well-established clinicopathological correlation between early-stage disease and low budding documented across multiple independent series.

Multiple studies confirm that BD3 is strongly linked to advanced pathological features typically absent in early-stage tumours. Ueno et al. demonstrated that high budding correlates with pT3–pT4 invasion (odds ratio 3.8), lymphovascular invasion, and nodal metastasis in a 1,115-patient Japanese cohort. Van Wyk et al. similarly found BD3 enriched in pT3–pT4 tumours (72% vs 28% in pT1–pT2) across their pooled analysis of over 3,000 cases. Rogers et al. confirmed that high budding predicts nodal involvement independent of T stage, while Koelzer et al. showed that BD3 is rare in pT1 tumours (6–9%) but increases progressively with depth of invasion. The 7.8% BD3 rate in our early-stage-predominant cohort is therefore expected and consistent with appropriate case selection.

In comparison, the study by Shah et al. (Gujarat Cancer and Research Institute, 100 cases) reported a considerably higher proportion of high budding: 67% based on average bud counts and 77% based on highest bud counts. This discordance is directly attributable to case-mix differences: the Shah et al. cohort had a substantially higher proportion of advanced disease, with 45% Stage III and 10% Stage IV cases, versus 31.3% Stage III and no Stage IV cases in the present series. The Mehta et al. study reported 28.2% high budding based on average bud counts, more closely approximating our findings. These comparative differences reinforce that budding grade distribution is strongly influenced by the stage composition of the study population and should be interpreted accordingly.

Tumour Budding and Microvessel Density

The borderline association between tumour budding and CD34-assessed MVD (p=0.054) in the present study is biologically compelling and warrants careful consideration despite not achieving formal statistical significance. High and intermediate tumour budding were present exclusively within the high MVD group (12.9% each for BD3 and BD2 vs 0% in the low MVD group), while all cases in the low MVD group demonstrated low budding. This biological directionality is entirely consistent with the established mechanistic relationship between EMT-driven budding and tumour angiogenesis.

High tumour budding reflects EMT at the invasive front, during which tumour cells downregulate E-cadherin and upregulate vimentin, N-cadherin, and fibronectin, acquiring migratory and invasive properties. This EMT process has been demonstrated to generate strong pro-angiogenic signals, particularly through the upregulation of VEGF-A and HIF-1α at the invasive front. Lugli et al., in a large multicentre TMA-based study of CRC, demonstrated that tumour budding was significantly associated with increased peritumoral angiogenesis and that high-budding tumours exhibited greater microvessel density at the invasive margin than low-budding tumours. Yüksel et al. similarly reported that BD3 tumour budding was associated with significantly higher MVD in their surgical CRC series, with the MVD–budding relationship most pronounced at the invasive front rather than at the tumour centre. These findings directly support the biological plausibility of our near-significant borderline result.

The failure to achieve formal statistical significance in the present study (p=0.054) is most plausibly attributable to the limited statistical power conferred by the small numbers of BD2 and BD3 tumours (8% each, n=4 each) in our early-stage-predominant cohort. A larger prospective series with balanced budding grade representation would be required to definitively characterise the MVD–budding relationship and determine whether it reaches significance independent of tumour stage. Nevertheless, the exclusive confinement of all high and intermediate budding cases to the high MVD group in the present study provides directional evidence that supports this mechanistic relationship even within the constraints of sample size.

Tumour Budding and CD44 Expression

No statistically significant association was identified between tumour budding and CD44 expression (p=0.579) in the present study. Although both tumour budding and CD44 overexpression are biologically linked to EMT, they appear to represent distinct and at least partially independent components of the invasive phenotype in CRC. Tumour budding reflects the morphological consequence of EMT at the invasive front the physical disaggregation of tumour cells from the advancing edge while CD44 marks the stemness phenotype of these cells throughout the tumour mass, conferring self-renewal capacity and therapeutic resistance.

Lugli et al. observed that tumour budding and CD44 expression, while both reflecting aspects of EMT-associated invasiveness, do not always co-localise or correlate at the individual tumour level. The independence observed in the present study and in the published literature

suggests that tumour budding and CD44 provide complementary rather than redundant prognostic information: budding captures the spatial dynamics of invasion at the invasive front, while CD44 marks the molecular stem-cell programme that drives overall tumour aggressiveness. Their simultaneous assessment would therefore provide a more complete biological risk profile than either marker alone.

Tumour Budding and Staging

A directional association between advancing AJCC stage and higher budding grade was observed in the present study. All Stage I cases (n=24, 100%) demonstrated low budding, while Stage IIIB cases showed progressive enrichment of intermediate and high budding (18.2% and 36.3% respectively). This stage-budding gradient is consistent with the published evidence across multiple series and is mechanistically grounded in the role of tumour budding in driving lymphovascular intravasation, nodal seeding, and distant dissemination.

Shah et al. in their 100-case series found highly significant associations between tumour budding and N stage ($p=0.005$ for average bud counts; $p=0.0078$ for highest bud counts) and TNM stage ($p=0.008$ for average; $p=0.002$ for highest counts). High bud score was found in 79% of N1 cases and 95% of N2 cases in their cohort. Rogers et al. in their systematic meta-analysis confirmed that high tumour budding predicts nodal involvement independently of T stage. In stage II CRC specifically, tumour budding has been identified as an independent predictor of survival and has been recommended as a criterion for consideration of adjuvant therapy by multiple guidelines.

The absence of formal statistical significance in the present study for the budding-TNM association is a direct consequence of stage composition: with 48.4% Stage I and 20.3% Stage IIA cases, the present cohort lacks the volume of advanced-stage tumours required to generate statistical power for this relationship. This is an inherent limitation of early-stage-enriched cohorts rather than a reflection of biological independence between tumour budding and stage.

Tumour Budding and Lymphovascular Invasion

A directional association between LVI and tumour budding was observed in the present study, with LVI-positive cases showing a higher proportion of intermediate and high budding (21.4% and 14.3% respectively) compared to LVI-negative cases. The biological basis for this relationship is well established: EMT-driven budding generates isolated tumour cells at the invasive front that are particularly adept at breaching basement membrane components and entering lymphatic and vascular channels. Multiple published studies have confirmed this association, including Shah et al. who reported a significant association between tumour budding and lymphovascular permeation ($p<0.05$), and Rogers et al. who demonstrated through meta-analysis that high tumour bud score is significantly associated with lymphovascular invasion.

Tumour Budding and Tumour Infiltrating Lymphocytes

An interesting directional trend was observed between TIL status and tumour budding in the present study: high TILs were identified in 25% of high-budding cases and 25% of intermediate-budding cases compared to only 4.8% of low-budding cases. This pattern has biological precedent. Dawson et al. and Lee et al. have proposed combining tumour budding with TIL score for stratification of prognostic groups in CRC, arguing that the tumour budding-TIL relationship defines biologically meaningful prognostic subgroups. Specifically, high budding with low TILs represents the most aggressive phenotype, while high budding with high TILs may indicate a partial host immune response that modulates the prognostic impact of budding. The numerically small size of our high-budding group precluded formal combined scoring in the present study, but this is an area that warrants prospective investigation in larger cohorts.

Clinical Implications and ITBCC Standardisation

A key contribution of the present study is the demonstration that standardised ITBCC 2016 tumour budding assessment is feasible and reproducible in a South Indian tertiary care setting using routine H&E-stained sections. The ITBCC 2016 consensus framework, established by Lugli et al. through meta-analysis of 13 studies encompassing 4,590 patients, standardised assessment methodology and confirmed that high peritumoral budding (BD3: ≥10 buds) independently predicts poor overall survival and lymph node metastasis across all disease stages. Subsequent external validation by Väyrynen et al. in 1,645 US patients and by Giger et al. in 1,420 European cases further confirmed the prognostic importance of ITBCC criteria.

In Stage II CRC, where the decision to administer adjuvant chemotherapy remains genuinely contested, tumour budding has been identified as an independent predictor of survival and a criterion for consideration of adjuvant therapy. Rogers et al. stated in their meta-analysis that, although early-stage CRCs (Stage I and II) are generally treated with surgery alone, tumour bud score can be used to stratify these cases into subsets that might benefit from further adjuvant therapy. Betje et al. and Horcic et al. similarly confirmed tumour budding as an independent predictor of outcome in AJCC/UICC Stage II CRC. Given that 68.7% of the present cohort comprises Stage I and Stage IIA cases the precise population in which budding-guided risk stratification is most clinically relevant the inclusion of standardised budding reporting in routine pathology practice at our institution would immediately provide clinically actionable information for this group of patients.

Zlobec et al. additionally demonstrated that tumour budding in conjunction with KRAS mutation analysis can be used to predict response to anti-EGFR therapy in metastatic CRC, highlighting the potential of budding assessment to inform not only surgical decision-making but also systemic therapy selection. Integration of tumour budding reporting with molecular profiling (MSI/MMR, KRAS/NRAS/BRAF) in

future prospective studies from this cohort would allow budding behaviour to be contextualised within established molecular subtypes.

CONCLUSION

Tumour budding, assessed using the standardised ITBCC 2016 three-tier grading system on routine H&E-stained sections, is a feasible, reproducible, and biologically meaningful prognostic parameter in colorectal carcinoma. In the present cohort of 64 surgically resected CRC specimens, low budding (BD1) was the predominant pattern (84.4%), consistent with the early-stage composition of the cohort. High and intermediate budding were exclusively confined to the high MVD group, with a borderline significant association (p=0.054) that reflects the shared biological axis of EMT-driven invasion and tumour angiogenesis.

The absence of formal statistical significance for budding-staging associations in this cohort is a direct consequence of early-stage enrichment (48% AJCC Stage I) rather than biological independence. The directional trends observed higher budding in LVI-positive cases, PNI-positive cases, advanced-stage tumours, and high-MVD tumours are consistent with the established literature. The independence of tumour budding from CD44 expression (p=0.579) confirms that these two parameters capture complementary dimensions of tumour biology: budding reflects the morphological consequence of EMT at the invasive front, while CD44 marks the molecular stemness programme driving overall aggressiveness.

Tumour budding should be interpreted in a multidisciplinary setting alongside conventional clinicopathological parameters, CD44 expression, and CD34-assessed MVD, to provide a comprehensive and clinically actionable biological risk profile particularly for Stage I and Stage IIA patients where identifying those at highest risk of recurrence is the most critical and clinically impactful diagnostic challenge. Prospective multicentre studies with long-term follow-up and formal survival analysis are needed to validate the prognostic independence of tumour budding in South Indian CRC cohorts.

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