Content available at: https://www.ipinnovative.com/open-access-journals

IP Archives of Cytology and Histopathology Research

Journal homepage: https://www.achr.co.in/

Original Research Article

Tumour budding in colorectal carcinoma: Association with other histopathological prognostic parameters

Pragnya P Mishra[1,*], K Madan[1], Siddhartha Biswas[2], Anuradha Calicut Kini Rao[3], Rohan Shetty[2], Premanand Panda[4]

[1]*Dept. of Oncopathology, Yenepoya Medical College, Mangalore, Karnataka, India*
[2]*Dept. of Oncology, Yenepoya Medical College, Mangalore, India*
[3]*Dept. of Pathology, Yenepoya Medical College, Mangalore, Karnataka, India*
[4]*Dept. of Radiology, JP Hospital, Rourkela, Odisha, India*

ARTICLE INFO

Article history:
Received 25-01-2022
Accepted 28-01-2022
Available online 04-03-2022

Keywords:
Colorectal carcinoma
Outcome
Prognosis
Tumour budding

ABSTRACT

Background: Colorectal carcinoma (CRC) is the third leading cancer in India. Pathologists play a crucial role in assessing stage, analyzing surgical margins and documenting the histopathologic prognostic parameters. Tumour budding is one such prognostic parameter, defined as single cells or small groups of tumour cells (up to 4 cell clusters) at the invasive front of the tumour.
The aim of this study is to examine the association of tumour budding with other histopathological prognostic parameters in patients with colorectal carcinoma.
Materials and Methods: The Hematoxylin & Eosin (H &E) stained slides of 52 histopathologically diagnosed CRC resection specimens were reviewed and tumour budding (BD) was assessed into four grades under 200x power. Other histopathological prognostic parameters like tumour size, site, grade, laterality, lymphovascular invasion, perineural invasion, T and N stage were analyzed using descriptive statistics and Chi-square test with Software SPSS version 23.
Results: A higher BD score is seen to be more often associated with grade 3 tumour morphology, presence of perineural invasion, tumour size of 5cm or more and tumours located in the sigmoid colon or rectum. No association of tumour budding is seen with TIL's or tumour of size < 5cm.
Conclusion: Tumour budding is a practical and significant histological index for identification of high malignant potential and poor outcome in CRC patients with rectal or sigmoid colon location, size more than 5cm, perineural invasion and higher histological grade. Tumour budding may help identify patients who need a more intensive postoperative follow up and the possibility of adjuvant therapy.

1. Introduction

Colorectal cancer (CRC) is the third most deadly and fourth most commonly diagnosed cancer in the world as per 2018 data GLOBOCAN. CRC arises when certain cells of the epithelium acquire a series of genetic and/or epigenetic events.[1] The risk of CRC is influenced by endogenous (constitutional) and exogenous (environmental) factors.[2] Colorectal carcinoma is morphologically diverse with several histological types, Adenocarcinoma is the most common, amounting to 98% of all malignancies in colorectal region. Most of these adenocarcinomas occur sporadically in about 75-85% whereas 25-30% are familial. When diagnosed in an early stage, the prognosis is good, with the 5- year relative survival rate of 91% for localized cancers and 70% for those with loco-regional invasion. A

* *Corresponding author*.
E-mail address: pparamita1982@gmail.com (P. P. Mishra).

https://doi.org/10.18231/j.achr.2022.006

lack of a reliable method for the early detection of CRC is impacting patient prognosis, early diagnosis and cure.[3]

The most powerful tool for assessing prognosis following curative surgery is the pathologic analysis of the resected specimens. Although factors determining the pathologic stage are the strongest predictors of postoperative outcome, other clinical, molecular, and histologic features may influence prognosis independent of stage.

The histologic parameters include pathologic stage and stage-independent prognostic factors such as tumour site, histologic grade, laterality, lymphovascular invasion, perineural invasion, margin, size and tumour budding. The same holds good for molecular markers; few of them being, Beta-catenin, VEGF, E-cadherin, MSI, BRAF, PDL1, HER-2 etc.[4]

Tumour budding (TB) is closely related to the epithelial-mesenchymal transition (EMT) and represents an important histomorphological indicator of tumour invasion and metastasis.[5] Tumour budding is observed at the invasive front of the tumour, where single or small clusters of tumour cells (up to four cells) are detached from the neoplastic epithelium and migrate into the surrounding stroma.[6] Tumour budding is considered as an adverse prognostic factor.

Tumour budding can be appreciated in conventional slides when it is prominent, but careful observation is still required. A more complete assessment of tumour budding is achieved more easily if the neoplastic epithelium is highlighted by pan-cytokeratin immunostains.

The aim of this study was to assess the intensity of tumour budding and investigate its associations with other histopathological parameters in CRC patients.

2. Materials and Methods

This is a cross-sectional study consisting of 52 CRC, treated with surgical resection and with the available histopathological data of patients diagnosed in the Department of Oncopathology, Yenepoya Medical College, Mangalore, between January 2020 and June 2021. Ethical approval for this study was taken from the hospital Ethical committee board.

4 micron thick sections were taken on non-coated slides and were stained with H&E, on an autostainer. All the tumour sections were examined and studied thoroughly by our panel of Oncopathologists, twice.

Scoring of TB and peritumoral inflammation: TB was assessed on H & E stained sections in the invasive front of the tumour, scanned at low power (100x) to identify the region with the highest density of TB. Tumour Buds were then counted in one 200x power field. The degree of tumour budding was classified into four grades: termed BD0, BD1, BD2, and BD3, which consist of 0, 1–4, 5–9, and 10 or more buds in a hotspot of 200x, respectively.[7] Tumour budding was further categorized into low bud (BD0 and BD1) and high bud. (BD2 and BD3)

Peritumoral inflammation is defined as a reaction with lymphocytic infiltration. It was estimated employing a semiquantitative three tiered system: no or mild inflammation with no or only scattered inflammatory cells, moderate with a marked inflammatory reaction, and severe inflammation with a dense accumulation of lymphocytes.[8]

Correlation of tumour budding, specifically low and high bud, with other prognostic histopathological parameters like tumour site, grade, size, laterality, lymphovascular invasion, perineural invasion, tumour stage and lymphnode stage were studied.

2.1. Statistical analysis

• Sample size of 52 was calculated using the formula;
$n = Z1- \alpha/2 \times P \times (1-P)/ d^2$, with level of significance α = 5%,
Power = 1 - P = 80% with 95% confidence interval.

SPSS Version 23.0 was used for data analysis. Basic descriptive statistics were used to find the association of prognostic factors. Descriptive statistics were reported in frequencies and percentage. Chi-square test was used to find the association of tumour budding with other histopathological parameters.

• Sampling technique: Simple random sampling technique

3. Results

A total of 52 cases of colorectal carcinoma diagnosed on excised specimens were included in the study. The age distribution of cases was 32 to 84 years with a peak incidence in the age range of 41-50 years (25%). The patient and tumour characteristics are shown in Table 1.

The most frequent site of involvement was the rectum and sigmoid colon (59.6%) followed by caecum, ascending colon (26.9%), descending colon (7.6%) and transverse colon (5.7%) as shown in Table 1. The majority of the cases (61.5%) were of grade 2 (Figure 2) and in 63.5% of cases, the tumour size was more than 5cm in maximum diameter. 42.3% of cases showed the presence of lymphovascular invasion, whereas perineural invasion was noted in 15.3% of cases.

The tumour budding was identified in 40 (78.8%) of the cases, which was further classified into 4 grades i.e., BD1 – BD3 as mentioned. Most of the cases were of grade 3 tumour bud (BD3) which constituted for 46% of the total cases with tumour budding. (Figures 1, 2, 3 and 4) Tumour budding as seen is depicted in Figures 1, 2, 3 and 4.

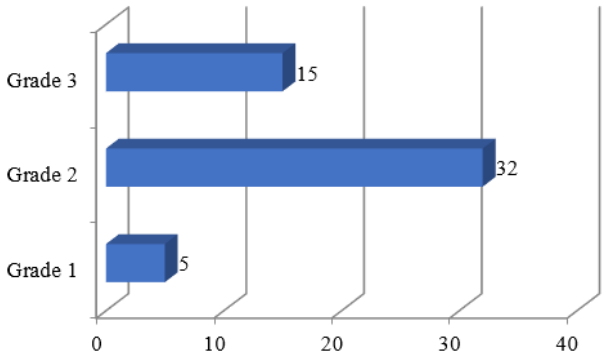
When compared low tumour budding (< 5 bud per 200x) to high tumour budding (>5 bud per 200x) it is observed that tumour size of >5cm is associated more with the highest number of tumour buds. Tumour location has an association with high tumour budding (BD2 and BD3) in the sigmoid

Table 1: Patient and tumour characteristics

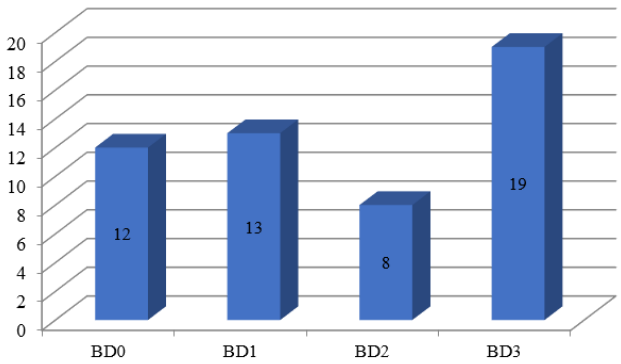
Characteristics	Number (n) = 52
Tumour size	
Less than 5cm	19 (37.5%)
More than 5cm	33 (63.5%)
Tumour location	
Caecum & Ascending colon	14 (26.9%)
Descending colon	4 (7.6%)
Transverse colon	3 (5.7%)
Sigmoid colon & rectum	31 (59.6%)
Lymphovascular invasion	
Absent	30 (57.7%)
Present	22 (42.3%)
Perineural invasion	
Identified	8 (15.3%)
Not identified	44 (84.7%)
Tumour infiltrating lymphocytes	
Absent	37 (71%)
Present	15 (29%)

Table 2: Correlation between tumour budding andclinico-pathological prognostic parameters

Parameter	Tumour size		Location of tumour			
Tumour bud	**< 5cm (n= 15)**	**>5cm (n=37)**	**Caecum & A. colon (n=14)**	**D. colon (n=4)**	**T. colon (n=3)**	**Sigmoid & rectum (n=31)**
BD 0 (12)	02	10	02	02	00	07
BD 1 (13)	04	09	04	02	01	05
BD 2 (08)	03	05	01	00	00	07
BD 3 (19)	06	13	07	00	02	12
P value	0.122		0.067			



Graph 1: Tumour grade



Graph 2: Tumour budding

colon and rectum, which means that the left-sided tumours have an association with high tumour budding.

When compared low tumour budding (< 5 bud per 200x) to high tumour budding (>5 bud per 200x) it is observed that high tumour budding is noted in the presence of lymphovascular invasion (LVI), perineural invasion (PNI) and is seen to be associated with high tumour grade (G2 & G3). Tumour infiltrating lymphocytes lacked correlation with tumour budding.

4. Discussion

Tumour budding is also known as "sprouting" and was originally described by Imai in 1950[9] as "sprouting" leading to invasiveness of the tumour. The first detailed description of tumour buds was put forth by Gabbert et al[10] who described this as 'tumour dedifferentiation' at the invasive edge of colorectal carcinomas. This is the concept behind a substantial number of tumours behaving poorly despite being categorized as low risk based on their TNM stage.

Table 3: Correlation between tumour bud & other histological parameters:

Parameters	**Tumour grade (n=52)**			**LVI (n=22)**	**PNI (n=8)**	**TIL**		
Tumour Bud	**G1**	**G2**	**G3**			**MILD**	**MOD**	**DENSE**
BD 0 (12)	02	09	01	04	01	02	08	02
BD 1 (13)	02	10	01	05	00	04	07	02
BD 2 (08)	00	08	00	04	01	02	06	00
BD 3 (19)	04	09	06	09	06	07	07	05
P value	0.115	**0.031**	0.031	0.658	**0.045**		0.359	

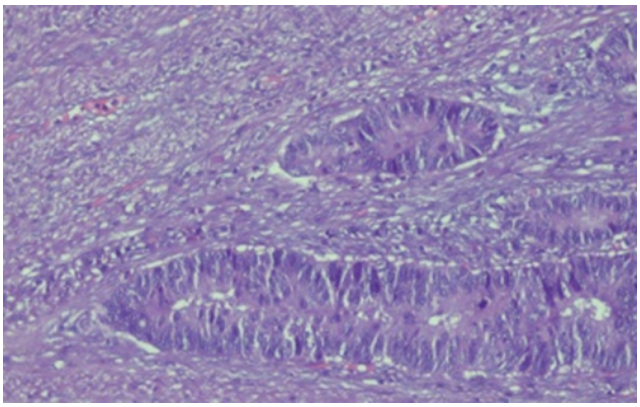


Fig. 1: Photomicrograph: H & E; 200x: No tumourbudding (BD 0)

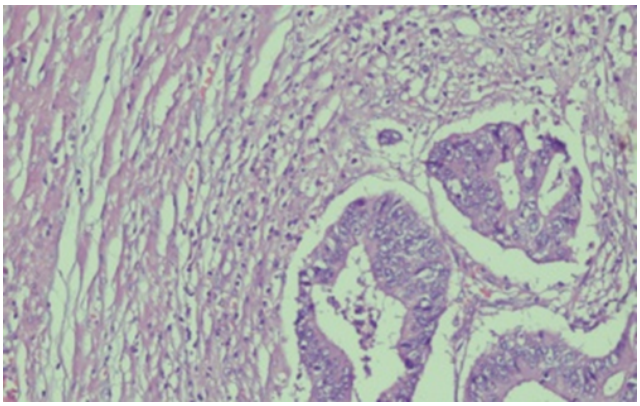


Fig. 2: Photomicrograph:H & E; 200x: Grade 1 tumour budding (BD 1)

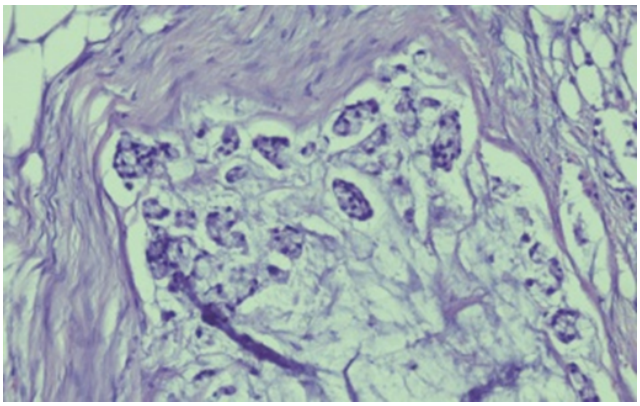


Fig. 3: Photomicrograph: H & E; 200x: Grade 2 tumourbudding (BD 2)

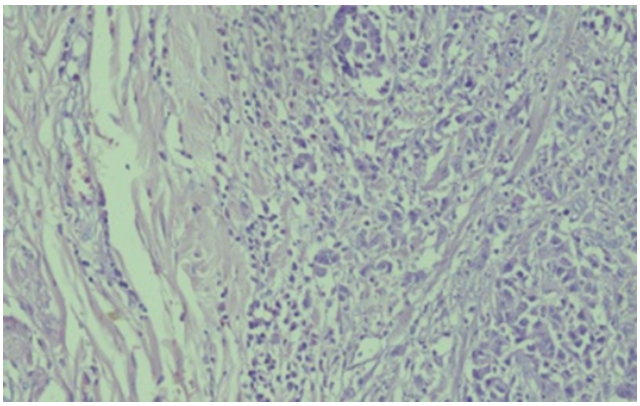


Fig. 4: Photomicrograph: H & E; 200x: Grade 3 tumourbudding (BD 3)

The biology of tumour budding is not well understood but there are some theories to explain the same. Researchers have attributed this to Epithelial-Mesenchymal transition (EMT), characterized by loss of cell adhesion molecules, cytoskeletal alterations, increased production of extracellular matrix components, resistance to apoptosis, and ability to degrade basement membrane, resulting in a phenotype with increased migratory capacity and invasiveness.[11]

When seen in a single section, tumour buds appear as clusters of cells that have broken off from the main tumour mass. On examining serial sections of high budding tumours stained with anti-cytokeratin antibodies, Prall et al[10] demonstrated that most buds that appear to represent discrete clusters of cells are in fact connected to adjacent larger glands. Indeed, Morodomi et al coined the term 'budding' because the undifferentiated single cells and tubular tumour nests that they counted as buds both appeared to be budding from larger neoplastic glands.

Tumour budding can allow risk stratification of patients into categories and also potentially guide treatment decisions. A high tumour budding is associated with local tumour recurrence and distant metastases hence

indicating worse disease- free survival.[12] Tumour budding predicts a worse prognosis, that is higher tumour grade, infiltrating tumour border, presence of lymphovascular invasion, perineural invasion and lymph node metastases.

There is a strong association between budding and the presence of lymph node metastases and lymphovascular invasion.[13] Kazama et al[14] found no relationship between budding and vascular invasion. In our study, all the 18 cases with lymphovascular invasion are showing budding of all grades and most of them are showing the highest budding (>5 per 200x). However, no stastistical significance is seen.

Lugli et al[15] have demonstrated that a peritumoral lymphocytic reaction is associated with improved prognosis in the setting of tumour budding, suggesting that the immune response might target the tumour buds, but further studies are needed to confirm the reproducibility and prognostic importance of these findings. However, our study showed no correlation between tumour budding, peritumoral lymphocytic reaction and nodal status.

The literature says a higher tumour grade is associated with a higher BD score. Our study showed a maximum high BD score in grade 3 tumours, which was similar in a study by Inti Zlobec et al study.[16] Inti Zlobec et al.[16] study shows, higher tumour grade was associated with mucinous histology, right-sided tumour location, higher pT & pN stage and more frequent distant metastases. Similarly, in our study, out of 8 mucinous carcinomas, 5 cases showed BD3 tumour budding. Similarly, 75% of the cases (6 out of 8 cases) exhibiting perineural invasion were of BD3 tumour budding. Tumour budding in our study is seen to be associated with higher tumour grade (grade 3), perineural invasion, size > 5cm and were located in sigmoid colon & rectum.

Out of 52 cases, 5 cases were post-treatment cases and all of them showed high budding (>5 bud per 200x).

4.1. Survival data

All the patients were followed up to date. 3 patients succumbed to the disease, 15 patients were lost to follow up and the rest are doing well with no recurrence. Out of 03 dead patients, two were exhibiting BD 3 tumour budding and one showing BD 1 tumour budding. When comparing tumour budding with survival data, the P-value was not significant. (0.515)

4.2. Limitation of the study

Our study is of one and half year duration with comparatively smaller sample size. With a larger sample size, a better correlation would have been achieved.

5. Conclusion

Tumour budding is a practical and significant histological index and is associated with worse disease-free survival and poor outcome in CRC cases. Our study revealed an association of tumour budding with CRC of rectal/sigmoid colon location, size >5cm, perineural invasion and a higher histological grade. Tumour budding helps to identify the patients who need more intensive postoperative follow up and a possibility of adjuvant therapy.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

7. Source of Funding

None.

References

1. Ewing I, Hurley JJ, Josephides E, Millar A. The molecular genetics of colorectal cancer. *Frontline Gastroenterol*. 2014;5(1):26–30. doi:10.1136/flgastro-2013-100329.
2. Odze RD, Goldblum JR. Surgical pathology of the GI tract, liver, biliary tract, and pancreas. 3rd Edn. Philadelphia: Elsevier Health Sciences; 2015. p. 737–78.
3. Sjo O. Prognostic factors in colon cancer, Norway. University of Oslo; 2012.
4. Taliano RJ, Legolvan M, Resnick MB. Immunohistochemistry of colorectal carcinoma: current practice and evolving applications. *Hum Pathol*. 2013;44(2):151–63.
5. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T, Opinion, et al. Migrating cancer stem cells ‑ An integrated concept of malignant tumour progression. *Nat Rev Cancer*. 2005;5(9):744–9. doi:10.1038/nrc1694.
6. Lugli A, Karamitopoulou E, Zlobec I. Tumour budding: A promising parameter in colorectal cancer. *Br J Cancer*. 17138209;106(11):1713–7. doi:10.1038/bjc.2012.127.
7. Yusra, Semba S, Yokozaki H. Biological significance of TB at the invasive front of human CRC cells. *Int J Oncol*. 2012;41(1):201–10. doi:10.3892/ijo.2012.1459.
8. Karamitopoulou E. TB cells, cancer stem cells and epithelial‑mesenchymal transition‑type cells in pancreatic cancer. *Front Oncol*. 2012;2:209. doi:10.3389/fonc.2012.00209.
9. Imai T. The growth of human carcinoma: a morphological analysis. *Fukuoka Igaku Zasshi*. 1954;45:30.
10. Gabbert H, Wagner R, Moll R. Tumor dedifferentiation: an important step in tumor invasion. *Clin Exp Metastasis*. 1985;3(4):257–79. doi:10.1007/BF01585081.
11. Kalluri R, Weinberg RA. The basics of epithelial mesenchymal transition. *J Clin Invest*. 2009;119(6):1420–8. doi:10.1172/JCI39104.
12. Okuyama T, Oya M, Ishikawa H. Budding as a useful prognostic marker in pT3 well- or moderately-differentiated rectal adenocarcinoma. *J Surg Oncol*. 2003;83(1):42–7. doi:10.1002/jso.10230.
13. Morodomi T, Isomoto H, Shirouzu K. An index for estimating the probability of lymph node metastasis in rectal cancers. Lymph node metastasis and the histopathology of actively invasive regions of cancer. *Cancer*. 1989;63(3):539–43. doi:10.1002/1097-0142(19890201)63:3<539::aid-cncr2820630323>3.0.co;2-s..
14. Kazama S, Watanabe T, Ajioka Y. Tumour budding at the deepest invasive margin correlates with lymph node metastasis in submucosal colorectal cancer detected by anticytokeratin antibody CAM5.2. *Br J Cancer*. 2006;94(2):293–8. doi:10.1038/sj.bjc.6602927.
15. Lugli A, Karamitopoulou E, Panayiotides I. CD8+ lymphocytes/tumour-budding index: an independent prognostic factor representing a 'pro-/anti-tumour' approach to tumour host

interaction in colorectal cancer. *Br J Cancer*. 2009;101(8):1382–92. doi:10.1038/sj.bjc.6605318.
16. Zlobec I, Dawson H, Blank A, Bokhorst JM, Berger M, Nagtegaal ID, et al. Are tumour grade and tumour budding equivalent in colorectal cancer? A retrospective analysis of 771 patients. *Eur J Cancer*. 2020;130:139–45. doi:10.1016/j.ejca.2020.02.007.

Author biography

Pragnya P Mishra, Fellow

K Madan, Assistant Professor

Siddhartha Biswas, HOD

Anuradha Calicut Kini Rao, Professor

Rohan Shetty, Associate Professor

Premanand Panda, HOD

Cite this article: Mishra PP, Madan K, Biswas S, Rao ACK, Shetty R, Panda P. Tumour budding in colorectal carcinoma: Association with other histopathological prognostic parameters. *IP Arch Cytol Histopathology Res* 2022;7(1):26-31.