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ORIGINAL RESEARCH

The Role of Pan-Cytokeratin In Tumor Budding Upgrading In Malignant Colorectal Polyps At Stage pT1

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Purpose: Tumor budding (TB) is an established prognostic factor in early colorectal cancer. However, its evaluation on hematoxylin–eosin (H&E) slides may underestimate the true budding activity. This study aimed to investigate the role of pan-cytokeratin (Pan-CK) immunohistochemistry in upgrading TB assessment in malignant colorectal polyps at the pT1 stage and to determine clinicopathological factors associated with TB upgrading.

Patients and Methods: We retrospectively reviewed 104 malignant colorectal polyps at the pT1 stage diagnosed between January 2015 and June 2024 at the University Medical Center, Ho Chi Minh City. TB was assessed on H&E and Pan-CK stained slides according to the 2016 International Tumor Budding Consensus Conference (ITBCC) criteria. Cases were classified as Bd1 (0–4 buds), Bd2 (5–9 buds), or Bd3 (≥10 buds). Upgrading was defined as an increase in TB grade on Pan-CK compared with H&E, particularly evaluated among cases classified as Bd1 on H&E. Clinicopathological features associated with upgrading were analyzed using univariate and multivariate models.

Results: The number of tumor buds was significantly higher on Pan-CK compared with H&E (median [IQR] 1.0 [0.0–7.0] vs 0.5 [0.0–3.0]; $p < 0.001$). Among 90 cases classified as Bd1 on H&E, 25 (27.78%) were upgraded to Bd2/3 after Pan-CK staining. In univariate analysis, higher Haggitt/Kikuchi level and the presence of synchronous polyps were significantly associated with upgrading, whereas tumor grade 2 demonstrated a borderline association. Multivariate analysis identified synchronous polyps as the only independent predictor (OR = 3.00, 95% CI: 1.03–8.76; $p = 0.045$).

Conclusion: Pan-CK substantially increases TB detection and grading in pT1 malignant colorectal polyps. Synchronous polyps were identified as the sole independent predictor of TB upgrading, representing a novel finding. Selective Pan-CK use in these cases may optimize resources and guide management.

Keywords: immunohistochemistry, early colorectal carcinoma, submucosal invasion, tumor budding

Introduction

Colorectal cancer (CRC) is one of the most common and lethal malignancies worldwide, particularly in developing countries. According to GLOBOCAN 2022, more than 1.93 million new CRC cases were diagnosed and approximately 904,000 deaths were recorded.[1] With the increasing implementation of colorectal cancer screening programs, a greater number of lesions are now being detected at an early stage, including malignant colorectal polyps.[2]

A malignant colorectal polyp is defined as a polypoid lesion in which cancer cells invade the submucosa but do not extend into the muscularis propria, corresponding to pathological stage pT1.[3] The detection of such lesions raises the need to balance two treatment strategies: endoscopic polypectomy versus segmental colectomy.[4] This growing number of early-stage diagnoses highlights the urgent need for accurate assessment of prognostic factors to guide optimal treatment decisions. Although malignant polyps represent an early stage of colorectal cancer, previous studies have reported that the rate of lymph node metastasis ranges from 5.6% to 15.2% of cases are associated with lymph node metastasis,

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underscoring the critical role of prognostic evaluation in determining the risk of recurrence and the need for definitive surgical management.[5–9] Several clinicopathological factors have been reported to be associated with lymph node metastasis in pT1 colorectal cancer, including tumor differentiation, lymphovascular invasion, depth of submucosal invasion, tumor budding, and tumor location.[10,11]

In the context of early-stage disease, tumor budding (TB)—defined as the presence of single cancer cells or small clusters of fewer than five cells at the invasive front—has been recognized as a significant independent prognostic factor.[12] Several studies have demonstrated that in pT1 colorectal cancer, high-grade TB (≥5 buds per 0.785 mm^2, corresponding to grade 2 or higher according to the International Tumor Budding Consensus Conference (ITBCC) criteria) is strongly associated with lymph node metastasis.[8,12,13] Biologically, this phenomenon is closely linked to the epithelial–mesenchymal transition (EMT), a process that enables cancer cells to detach from the primary tumor and invade the surrounding stroma.[14,15] Accordingly, international guidelines, including those from CAP and NCCN, have recommended the reporting of TB in pathology assessments of pT1 colorectal cancer to support prognostication and treatment decision-making.[16,17]

In routine practice, the assessment of TB on hematoxylin–eosin (H&E) slides is challenging. Tumor buds are often very small, easily mistaken for stromal elements, or obscured by inflammatory infiltrates and disrupted glandular fragments at the invasive front, leading to underestimation. Moreover, interobserver agreement in evaluating TB on H&E is generally only moderate to low.[18–20] To address these limitations, several authors have proposed the use of immunohistochemistry with pan-cytokeratin (Pan-CK), which highlights isolated tumor cells and increases sensitivity in TB detection.[21–23] Data from both research and clinical practice have shown that Pan-CK can reveal three- to four-fold more tumor buds compared with H&E,[7] while also significantly improving reproducibility (with ICC increasing from moderate on H&E to high on Pan-CK).[18,20,24,25] Although Pan-CK offers clear advantages in early-stage CRC and has been recommended by some authors for broader application, its routine use in all cases is not feasible, particularly in resource-limited settings, due to constraints of cost, time, and technical capacity. This raises an important question as to which factors determine the necessity of Pan-CK staining in order for TB upgrading to carry prognostic significance. Based on this rationale, the present study was designed to clarify the role of Pan-CK in evaluating TB in malignant colorectal polyps (pT1) and to identify independent predictors of TB upgrading after Pan-CK staining. We anticipate that our findings will provide scientific evidence to support selective use of Pan-CK in routine practice. This selective approach is particularly relevant for low-resource settings, thereby contributing to global oncology equity.

Materials and Methods

Patient Selection and Data Collection

This retrospective study included 265 patients who were initially diagnosed with colorectal adenocarcinoma at the pT1 stage between January 2015 and June 2024 at the University Medical Center Ho Chi Minh City, Vietnam. After applying inclusion and exclusion criteria, 104 patients were eligible and included in the final analysis (Figure 1). Demographic characteristics (age, sex), clinical and endoscopic findings (tumor site and number of polyps), and laboratory data (serum CEA, lipid profile, total protein, albumin) were retrieved from electronic medical records.

Inclusion criteria were: (1) histopathological confirmation of invasive adenocarcinoma confined to the submucosa (pT1 stage), (2) availability of adequate H&E-stained slides for TB assessment, and (3) complete and clearly documented clinical and pathological records. Exclusion criteria were: (1) history or synchronous of another primary malignancy, (2) prior chemotherapy, and (3) technically inadequate specimens (eg, unreadable H&E slides or severely damaged paraffin blocks).

Pathological Assessment

Histopathological variables assessed on H&E slides included histological subtype, tumor grade, depth of submucosal invasion, distance to resection margin, precursor lesion, Haggitt/Kikuchi classification, lymphovascular invasion, perineural invasion, tumor necrosis, tumor-infiltrating lymphocytes (TILs), and nodal status (for surgical specimens). Depth of submucosal invasion was assessed according to standardized criteria. Pedunculated malignant polyps were evaluated using the Haggitt classification (levels 1–4), whereas sessile lesions were assessed using the Kikuchi classification (SM1–SM3).

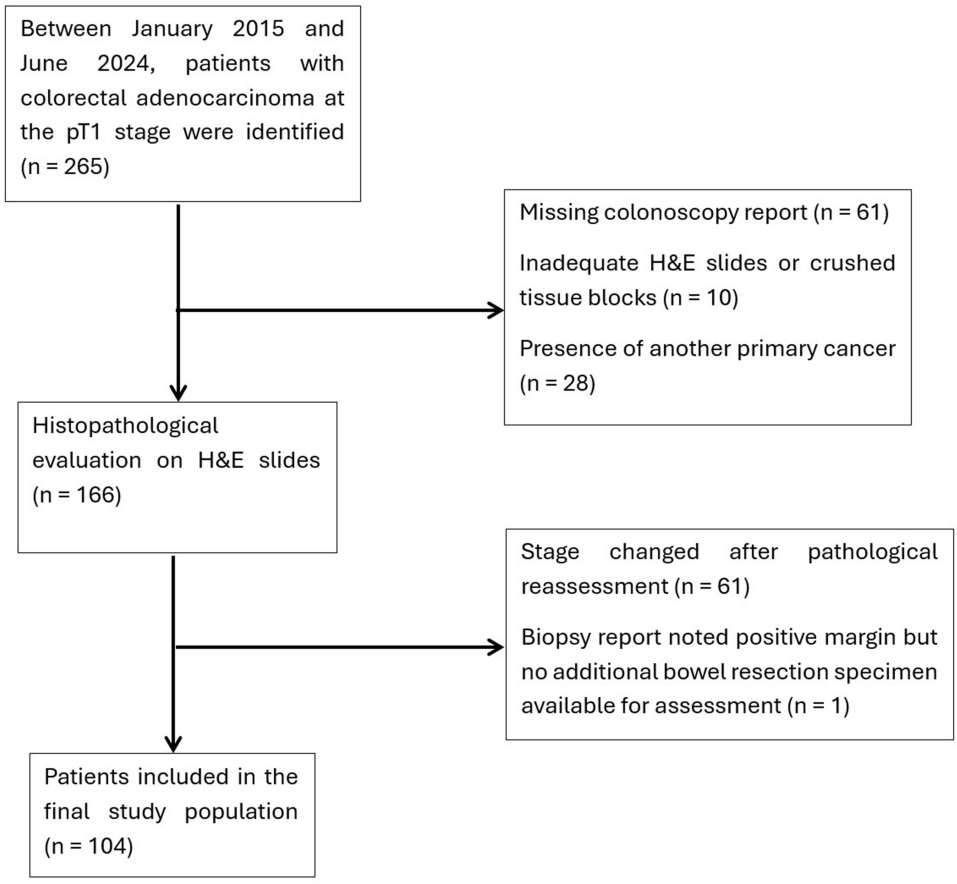

Figure 1 Flow diagram of patient selection.

In addition, invasion <1000 μm from the muscularis mucosae was defined as superficial and ≥1000 μm as deep. TB was initially evaluated on H&E slides according to the ITBCC 2016 criteria,[12] and the counts were recorded as baseline values. After Pan-CK immunostaining, the same invasive front was re-examined to identify the corresponding hotspot (0.785 mm^2 at ×20 objective). Pan-CK staining was used to confirm the epithelial nature of buds and reveal additional discrete clusters obscured by stromal or inflammatory components. The interpretation of budding morphology was based primarily on H&E features, with Pan-CK serving as a complementary tool to enhance TB detection. TB was classified as Bd1 (0–4 buds), Bd2 (5–9 buds), or Bd3 (≥10 buds). For analytical purposes, Bd1 was categorized as "low risk", while Bd2–3 were grouped as "high risk". Upgrading was defined as an increase in TB grade on Pan-CK compared with H&E. TB evaluation was independently performed by two pathologists who were blinded to clinical and outcome information. In cases of disagreement, the slides were jointly reviewed at a multi-headed microscope to reach a consensus. This consensus-based evaluation was intended to enhance reproducibility and ensure methodological rigor.

Immunohistochemistry Staining

For each case, serial sections were cut from formalin-fixed paraffin-embedded blocks and immunostained for cytokeratin using the Pan-CK antibody (clone AE1/AE3, Ventana, Roche Diagnostics, USA) on a fully automated BenchMark XT platform. The protocol included deparaffinization with EZ Prep solution, antigen retrieval using Cell Conditioning 1 buffer (CC1, pH 8.5, 95°C, 30 minutes), and visualization via the OptiView DAB IHC Detection Kit. Positive staining highlighted epithelial tumor cells, including isolated cells and small clusters, which were then evaluated for tumor budding (TB) according to the ITBCC 2016 criteria as described above. TB evaluation on Pan-CK–stained slides was performed by two pathologists using the same scoring approach as for H&E slides.

Statistical Analysis

All statistical analyses were conducted using Stata version 17 (StataCorp, College Station, TX, USA). Continuous variables were summarized as means ± standard deviations or medians with interquartile ranges, depending on data distribution, while categorical variables were presented as counts and percentages. Group comparisons were performed using Student's *t*-test, chi-square test, or Fisher's exact test, as appropriate. The correlation between TB counts on H&E and Pan-CK slides was assessed using Spearman's rank correlation coefficient. To evaluate factors associated with TB upgrading, logistic regression models were applied. Multivariable logistic regression was performed, including the three variables that showed the lowest p-values in univariate analysis, given the limited number of upgrading events. A two-sided p-value < 0.05 was considered statistically significant.

Results

A total of 104 malignant colorectal polyps at the pT1 stage were included, comprising 58 males and 46 females, with a mean age of 63.88 ± 11.48 years. Of the 104 malignant polyps, 61 lesions (58.7%) were treated by endoscopic resection alone, 38 (36.5%) by surgical resection, and 5 (4.8%) by endoscopic resection followed by additional surgical resection. Most polyps were pedunculated (101 cases, 97.12%), with a mean size of 20.6 ± 12.4 mm. The most frequent tumor locations were the sigmoid colon (43 cases, 41.35%) and rectum (40 cases, 38.46%). Histologically, tubulovillous adenoma was the most common precursor lesion (60 cases, 57.69%), and adenocarcinoma not otherwise specified accounted for 94 cases (90.38%). Deep submucosal invasion was present in 87 (83.65%) cases, while lymphovascular invasion was identified in 2 cases (1.92%). Perineural invasion was not observed. Among 43 patients who underwent segmental resection or additional surgical resection, lymph node metastasis was identified in 2 cases (4.65%).

TB Assessment by Sequential H&E and Pan-CK

The number of TB foci was significantly higher on Pan-CK immunostained slides compared to H&E slides (median [IQR]: 1.0 [0.0–7.0] vs 0.5 [0.0–3.0]; $p < 0.001$). A strong positive correlation was observed between the two methods (Spearman's $r = 0.90$, $p < 0.001$) (Figure 2). Based on H&E, the majority of cases were classified as Bd1 (86.54%), followed by Bd2 (12.50%) and Bd3 (0.96%). In contrast, Pan-CK staining increased the proportions of Bd2 (26 cases, 25.00%) and Bd3 (13 cases, 12.50%), while Bd1 decreased to 65 cases (62.50%). The overall agreement between H&E and Pan-CK across three Bd categories was only fair ($\kappa = 0.301$). When TB was dichotomized into low- vs high-risk (Bd1 vs Bd2/3), agreement slightly improved ($\kappa = 0.411$). Representative histological images illustrating TB on H&E and Pan-CK immunostaining are shown in Figure 3.

To investigate factors associated with TB grade upgrading, we focused on cases initially classified as Bd1 on H&E ($n = 90$), in which some were upgraded to Bd2 or Bd3 after Pan-CK immunostaining.

Clinicopathological Features Associated with TB Upgrading

Among the 90 cases initially classified as Bd1 on H&E, 25 (27.78%) were upgraded on Pan-CK (19 to Bd2 and 6 to Bd3). Several clinicopathological factors were associated with TB grade upgrading on Pan-CK staining (Tables 1 and 2). The presence of synchronous polyps defined as the coexistence of one or more additional polyps in the colorectal segment, was significantly associated with TB upgrading (36.73% vs 17.07%; OR = 2.82, 95% CI: 1.04–7.66; $p = 0.042$). Tumor grade 2 showed a borderline association with upgrading compared to grade 1 (33.33% vs 5.88%; OR = 8.00, 95% CI: 1.00–64.12; $p = 0.050$). Similarly, cases with a Haggitt/Kikuchi level ≥ 2 had a higher likelihood of upgrading than those with level 1 (38.64% vs 17.39%; OR = 2.99, 95% CI: 1.13–7.92; $p = 0.028$). In contrast, polyp morphology, tumor subtype, depth of invasion, precursor lesion, distance to resection margin, tumor-infiltrating lymphocytes, and tumor necrosis were not significantly associated with TB upgrading ($p > 0.05$).

Multivariate Analysis

In the multivariable logistic regression model including the three significant histopathological variables from univariate analysis (Figure 4), the presence of synchronous polyps remained independently associated with TB grade upgrading

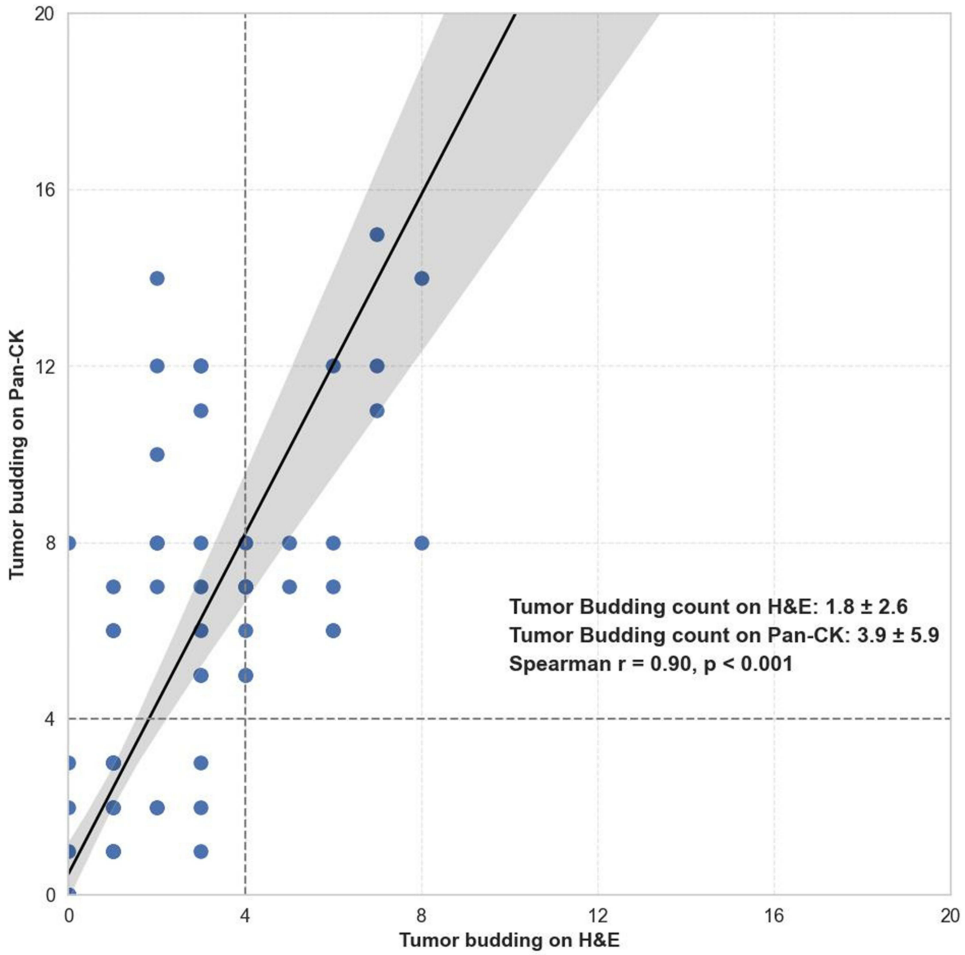

Figure 2 Distribution of tumor budding grades on H&E and Pan-CK immunostaining across study sample.

(OR = 3.00, 95% CI: 1.03–8.76, p = 0.045). Tumor grade (OR = 6.42, 95% CI: 0.77–53.41, p = 0.085) and Haggitt/Kikuchi classification (OR = 2.49, 95% CI: 0.86–7.19, p = 0.091) showed strong trends toward association but did not reach statistical significance.

Discussion

TB, a histological manifestation of EMT has been recognized as an important prognostic factor in early-stage colorectal cancer, with high-grade TB closely associated with lymph node metastasis and poor outcomes.[12,14,15] In our study, we observed a strong correlation between H&E and Pan-CK in the assessment of TB (Spearman r = 0.90; p < 0.001). However, the number of TB identified on Pan-CK was significantly higher compared with H&E, with 27.8% (25/90) of cases initially classified as Bd1 on H&E being upgraded to Bd2–3 following Pan-CK staining.

Pan-CK has been consistently shown in multiple studies to substantially increase the number of TB detected compared with H&E. Yamadera et al reported a median TB count of 4 (range 0–20) on H&E versus 8 (range 0–40) on cytokeratin, with a statistically significant difference (p < 0.001).[25] Fisher et al likewise observed that the number of TB identified on Pan-CK was more than four times higher than on H&E when examined on parallel sections.[26] Similarly, Koelzer et al documented a three- to six-fold increase in TB counts on Pan-CK compared with H&E.[20] This discrepancy reflects the inherent limitations of H&E, in which TB recognition can be hampered by inflammatory infiltrates, desmoplastic reactions, or fragmented glands at the invasive front, resulting in subjectivity and suboptimal interobserver agreement. In this context, Pan-CK provides important added value by improving both the sensitivity and reliability of TB assessment in routine pathology practice. Our study also yielded similar findings, with Pan-CK detecting significantly

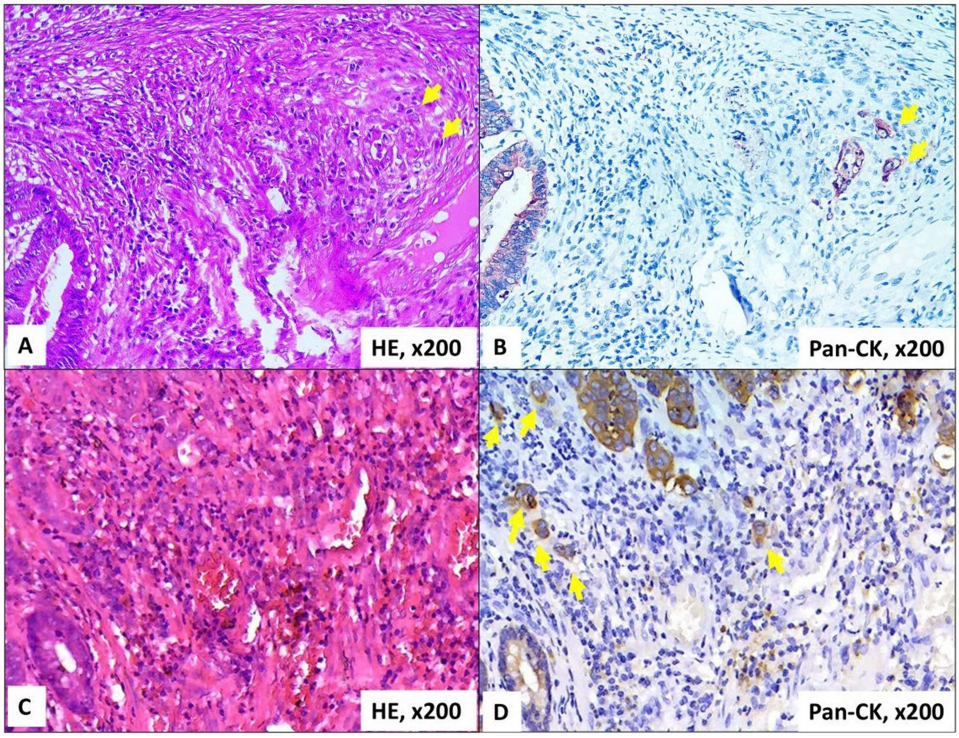

Figure 3 Representative histological images of tumor budding. (**A** and **B**) Cases without change in TB grade after Pan-CK immunostaining (A: H&E, ×200; (**B**) Pan-CK, ×200). (**C** and **D**) Cases showing upgrading from Bd1 on H&E to Bd2 on Pan-CK (C: H&E, ×200; (**D**) Pan-CK, ×200). Yellow arrows indicate tumor buds.

more TB than H&E, further confirming its diagnostic utility. These results support the selective application of Pan-CK in challenging or borderline cases, consistent with CAP and ITBCC 2016 recommendations that emphasize TB reporting in pT1 CRC.[12,16] By integrating Pan-CK staining into diagnostic workflows when H&E assessment is equivocal, pathologists may achieve more reproducible TB scoring, thereby enhancing risk stratification and clinical decision-making.

Table 1 Clinical and Laboratory Features Associated with Tumor Budding Upgrading From Bd1 on H&E to Bd2/3 on Cytokeratin Staining

Factors	Tumor Budding Status Change			OR (95% CI)	p
	Total (n=90)	Unchanged (n=65)	Upgraded (n=25)		
Age[¥]	63.57 (11.59)	63.45 (12.11)	63.88 (10.35)	1.00 (0.96–1.04)	0.873
< 65	53 (58.89)	40 (75.47)	13 (24.53)	1	
≥ 65	37 (41.11)	25 (67.57)	12 (32.43)	1.48 (0.58–3.74)	0.411
Sex					
Male	48 (53.33)	34 (70.83)	14 (29.17)	1	
Female	42 (46.67)	31 (73.81)	11 (26.19)	0.86 (0.34–2.18)	0.753
Surgical method (n = 89)					
Resection surgery	29 (32.58)	19 (65.52)	10 (34.48)	1	
Polypectomy	60 (67.42)	46 (76.67)	14 (23.33)	0.58 (0.22–1.53)	0.269
Tumor size (mm)[¥]	19.39 (10.73)	19.35 (10.92)	19.48 (10.42)	1.00 (0.96–1.05)	0.960
≤ 20 mm	62 (68.89)	47 (75.81)	15 (24.19)	1	
> 20 mm	28 (31.11)	18 (64.29)	10 (35.71)	1.74 (0.66–4.58)	0.261
Tumor site					
Colon	57 (63.33)	40 (70.18)	17 (29.82)	1	
Rectum	33 (36.67)	25 (75.76)	8 (24.24)	0.75 (0.28–2.00)	0.569

(*Continued*)

Table 1 (Continued).

Factors	Tumor Budding Status Change			OR (95% CI)	p
	Total (n=90)	Unchanged (n=65)	Upgraded (n=25)		
Number of polyps	3.06 (3.58)	3.02 (3.80)	3.16 (3.01)	1.01 (0.89–1.15)	0.793
Synchronous polyp					
Absent	41 (45.56)	34 (82.93)	7 (17.07)	1	
Present	49 (54.44)	31 (63.27)	18 (36.73)	2.82 (1.04–7.66)	0.042
CEA (ng/mL)[¥] (n = 31)	3.91 (3.86)	3.11 (2.10)	5.37 (5.72)	1.19 (0.92–1.55)	0.185
≤5 ng/mL	27 (87.10)	19 (70.37)	8 (29.63)	1	
>5 ng/mL	4 (12.90)	1 (25.00)	3 (75.00)	7.12 (0.64–79.27)	0.110
Cholesterol total (mmol/L)[¥] (n = 36)	5.42 (1.11)	5.44 (1.25)	5.37 (0.69)	0.95 (0.49–1.84)	0.876
< 5,2 mmol/L	12 (33.33)	9 (75.00)	3 (25.00)	1	
≥ 5,2 mmol/L	24 (66.67)	17 (70.83)	7 (29.17)	1.24 (0.26–5.97)	0.793
HDL (mmol/L) [¥] (n = 36)	1.21 (0.23)	1.21 (0.23)	1.21 (0.23)	0.91 (0.03–26.71)	0.955
≤ 1.2	19 (52.78)	14 (73.68)	5 (26.32)	1	
> 1.2	17 (47.22)	13 (76.47)	4 (23.53)	0.86 (0.19–3.92)	0.847
LDL (mmol/L)[¥] (n = 38)	3.56 (0.83)	3.59 (0.91)	3.48 (0.58)	0.84 (0.35–2.03)	0.706
<3 mmol/L	7 (18.42)	5 (71.43)	2 (28.57)	1	
≥3 mmol/L	31 (81.58)	23 (74.19)	8 (25.81)	0.87 (0.14–5.40)	0.881
Triglyceride (mmol/L)[¥] (n = 38)	6.60 (24.97)	2.60 (2.28)	17.82 (48.56)	1.04 (0.93–1.16)	0.520
<1,7 mmol/L	18 (47.37)	16 (88.89)	2 (11.11)	1	
≥1,7 mmol/L	20 (52.63)	12 (60.00)	8 (40.00)	5.33 (0.95–29.81)	0.057
Serum Protein (g/L)[¥] (n = 26)	70.50 (6.44)	70.22 (7.32)	71.13 (4.17)	1.02 (0.90–1.17)	0.736
≥62 g/L	23 (88.46)	15 (65.22)	8 (34.78)	1	
<62 g/L	3 (11.54)	3 (100)	0 (0)	1	
Serum Albumin (g/L)[¥] (n = 42)	40.82 (7.59)	40.13 (5.00)	42.53 (12.01)	1.04 (0.95–1.14)	0.374
≥35 g/L	37 (88.10)	27 (72.97)	10 (27.03)	1	
<35 g/L	5 (11.90)	3 (60.00)	2 (40.00)	1.80 (0.26–12.41)	0.551

Note: [¥]Reported as mean ± standard deviation.
Abbreviations: CEA, carcinoembryonic antigen; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2 Histopathological Features Associated with Tumor Budding Upgrading From Bd1 on H&E to Bd2/3 on Cytokeratin Staining

Factors	Tumor Budding Status Change			OR (95% CI)	p
	Total (n=90)	Unchanged (n=65)	Upgraded (n=25)		
Polyp morphology					
Sessile polyp	2 (2.22)	1 (50.00)	1 (50.00)	1	
Pedunculated polyp	88 (97.78)	64 (72.73)	24 (27.27)	0.37 (0.02–6.24)	0.494
Subtype					
NOS	85 (94.44)	62 (72.94)	23 (27.06)	1	
Others	5 (5.56)	3 (60.00)	2 (40.00)	1.80 (0.28–11.45)	0.535
Tumor grade (n = 86)					
Grade 1	17 (19.77)	16 (94.12)	1 (5.88)	1	
Grade 2	69 (80.23)	46 (66.67)	23 (33.33)	8.00 (1.00–64.12)	0.050
Depth of Invasion					
Superficial	16 (17.78)	13 (81.25)	3 (18.75)	1	
Deep	74 (82.22)	52 (70.27)	22 (29.73)	1.83 (0.47–7.08)	0.379

(*Continued*)

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Table 2 (Continued).

Factors	Tumor Budding Status Change			OR (95% CI)	p
	Total (n=90)	Unchanged (n=65)	Upgraded (n=25)		
Precursor lesion					
SSLD					
No	88 (97.78)	64 (72.73)	24 (27.27)	1	
Yes	2 (2.22)	1 (50.00)	1 (50.00)	2.67 (0.16–44.35)	0.494
TSA					
No	83 (92.22)	59 (71.08)	24 (28.92)	1	
Yes	7 (7.78)	6 (85.71)	1 (14.29)	0.41 (0.05–3.59)	0.420
Villous adenoma					
No	83 (92.22)	59 (71.08)	24 (28.92)	1	
Yes	7 (7.78)	6 (85.71)	1 (14.29)	0.41 (0.05–3.59)	0.420
Tubulovillous adenoma					
No	68 (75.56)	49 (72.06)	19 (27.94)	1	
Yes	22 (24.44)	16 (72.73)	6 (27.27)	0.97 (0.33–2.84)	0.951
Tubular adenoma					
No	38 (42.22)	27 (71.05)	11 (28.95)	1	
Yes	52 (57.78)	38 (73.08)	14 (26.92)	0.90 (0.36–2.29)	0.832
Distance to Resection Margin (mm) † (n = 57)					
<1	12 (21.05)	7 (58.33)	5 (41.67)	1	
≥1	45 (78.95)	35 (77.78)	10 (22.22)	0.40 (0.10–1.54)	0.182
Haggitt / Kikuchi Classification					
Level 1	46 (51.11)	38 (82.61)	8 (17.39)	1	
Level ≥ 2	44 (48.89)	27 (61.36)	17 (38.64)	2.99 (1.13–7.92)	0.028
TiLs					
Low–Moderate	26 (28.89)	18 (69.23)	8 (30.77)	1	
High	64 (71.11)	47 (73.44)	17 (26.56)	0.81 (0.30–2.21)	0.687
Tumor Necrosis					
< 10%	42 (46.67)	32 (76.19)	10 (23.81)	1	
> 10%	48 (53.33)	33 (68.75)	15 (31.25)	1.45 (0.57–3.71)	0.433

Note: †Assessed only in endoscopic resection specimens.
Abbreviations: NOS, not otherwise specified; TILs, tumor-infiltrating lymphocytes; SSLD, Sessile Serrated Lesion with Dysplasia; TSA, Traditional Serrated Adenoma.

In univariate analysis, two histopathological factors—Haggitt/Kikuchi classification, and the presence of synchronous polyps—were significantly associated with the likelihood of TB upgrading after Pan-CK staining. Tumors with moderate differentiation (grade 2) showed a borderline association with upgrading compared with well-differentiated tumors

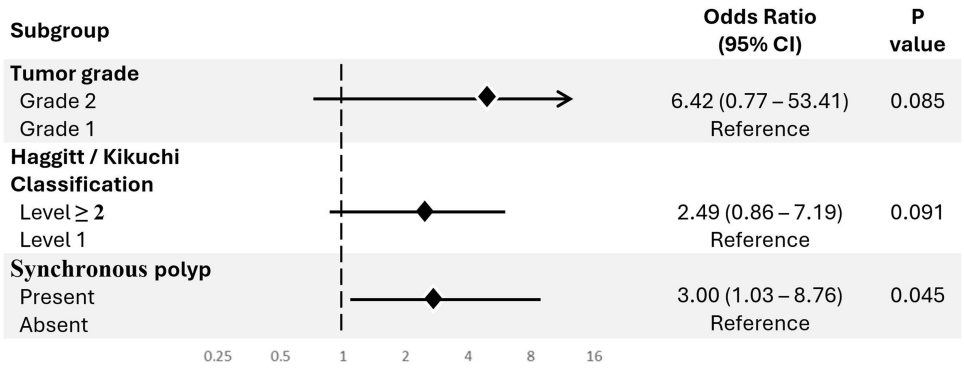

Figure 4 Multivariable logistic regression analysis of clinicopathological factors associated with tumor budding upgrading. Error bars represent 95% confidence intervals.

(grade 1). However, in multivariate analysis, only the presence of synchronous polyps remained independently associated with TB upgrading. This finding may be explained by the "field cancerization" hypothesis, which proposes that multiple neoplastic lesions represent a broader epithelial field that has undergone premalignant molecular alterations. Patel et al provided supporting evidence for this mechanism, demonstrating the presence of cancer stem cell (CSC)-like populations in morphologically normal colonic mucosa of patients with adenomatous polyps. Moreover, CSC markers such as CD44, CD166, and ESA were found to increase with age and were expressed at approximately twice the level in individuals with three to four polyps compared with those with only one to two.[27] These observations suggest that CSC-like cells are present not only within premalignant adenomatous polyps but also in histologically normal colonic mucosa, indicating a broader predisposition to CRC development. Taken together, our findings indicate that synchronous polyps may serve as a practical marker for identifying cases more likely to experience TB upgrading with Pan-CK. To our knowledge, this is the first study to report such an association, although further validation in larger, multicenter cohorts is warranted.

The findings of this study have important clinical implications, demonstrating that Pan-CK should be applied selectively rather than routinely to all pT1 colorectal polyps. The presence of synchronous polyps was identified as an independent predictor associated with TB upgrading. This observation suggests that Pan-CK staining should be considered particularly in cases with synchronous polyps, instead of being performed indiscriminately for all pT1 lesions. Such a selective approach may not only optimize resources and diagnostic workflows but also enhance the effectiveness of Pan-CK in risk stratification, thereby assisting clinicians in making more appropriate follow-up and treatment decisions, especially for patients with tumors harboring aggressive biological features that require definitive surgical intervention.

This study, however, has several limitations. First, it was a retrospective, single-center study with a relatively limited sample size, which reduces the statistical power and generalizability of the findings. Second, TB assessment was performed independently by two pathologists, followed by joint discussion to reach a consensus. While this approach may have improved accuracy through consensus-based interpretation, it limited the ability to assess interobserver variability, which is a critical factor when considering the true impact of Pan-CK in routine practice. Third, as 97.1% of malignant polyps in our cohort were pedunculated and only three were sessile, the findings of this study primarily apply to pedunculated lesions. The small number of sessile or non-pedunculated polyps precluded subgroup analysis; therefore, the generalizability of our results to non-pedunculated lesions should be interpreted with caution. Finally, due to the limited number of lymph node metastasis events in our cohort, we were unable to comprehensively evaluate the prognostic significance of TB upgrading on Pan-CK or determine an optimal cut-off value. These issues should be addressed in future prospective, multicenter studies with larger cohorts and long-term follow-up data.

Conclusion

Pan-CK immunohistochemistry substantially increases TB detection and grading in malignant colorectal polyps at the pT1 stage. Among cases initially classified as low-grade TB on H&E, nearly one-third were upgraded after Pan-CK staining. Notably, the presence of synchronous polyps was identified as the only independent predictor of TB upgrading —suggesting a biologically broader field effect and highlighting its potential role in refining risk stratification.

Therefore, Pan-CK should not be applied routinely to all pT1 lesions, but rather selectively—especially when synchronous polyps are present—to optimize diagnostic cost-effectiveness and support personalized decision-making. This targeted approach is particularly relevant in resource-constrained settings and aligns with the need for more sustainable, evidence-based diagnostic strategies in early colorectal cancer.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are available from Dr. Phat Thi Hong Ho upon reasonable request.

Ethical Approval

The study protocol was approved by the Institutional Review Board of the University of Medicine and Pharmacy at Ho Chi Minh City (approval number: 2855/ĐHYD-HĐĐĐ). The study was conducted in accordance with the Declaration of Helsinki. Because this was a retrospective analysis of anonymized archival material, the requirement for informed consent was waived.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors have no conflicts of interest in the subject of this study.

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