Modern Pathology (2013) 26, 295–301

Proposal for a 10-high-power-fields scoring method for the assessment of tumor budding in colorectal cancer

Eva Karamitopoulou[1,2], Inti Zlobec[1], Viktor Kölzer[1], Agathi Kondi-Pafiti[2], Efstratios S Patsouris[2], Konstantin Gennatas[3] and Alessandro Lugli[1]

[1]*Institute of Pathology, University of Bern, Bern, Switzerland;* [2]*1st Department of Pathology, University of Athens, Athens, Greece and* [3]*Department of Oncology, Aretaieion University Hospital, Athens, Greece*

Although tumor budding is linked to adverse prognosis in colorectal cancer, it remains largely unreported in daily diagnostic work due to the absence of a standardized scoring method. Our aim was to assess the inter-observer agreement of a novel 10-high-power-fields method for assessment of tumor budding at the invasive front and to confirm the prognostic value of tumor budding in our setting of colorectal cancers. Whole tissue sections of 215 colorectal cancers with full clinico-pathological and follow-up information were stained with cytokeratin AE1/AE3 antibody. Presence of buds was scored across 10-high-power fields at the invasive front by two pathologists and two additional observers were asked to score 50 cases of tumor budding randomly selected from the larger cohort. The measurements were correlated to the patient and tumor characteristics. Inter-observer agreement and correlation between observers' scores were excellent ($P<0.0001$; intraclass correlation coefficient = 0.96). A test subgroup of 65 patients (30%) was used to define a valid cutoff score for high-grade tumor budding and the remaining 70% of the patients were entered into the analysis. High-grade budding was defined as an average of $\geq$10 buds across 10-high-power fields. High-grade budding was associated with a higher tumor grade ($P<0.0001$), higher TNM stage ($P=0.0003$), vascular invasion ($P<0.0001$), infiltrating tumor border configuration ($P<0.0001$) and reduced survival ($P<0.0001$). Multivariate analysis confirmed its independent prognostic effect ($P=0.007$) when adjusting for TNM stage and adjuvant therapy. Using 10-high-power fields for evaluating tumor budding has independent prognostic value and shows excellent inter-observer agreement. Like the BRE and Gleason scores in breast and prostate cancers, respectively, tumor budding could be a basis for a prognostic score in colorectal cancer.
Modern Pathology (2013) **26,** 295–301; doi:10.1038/modpathol.2012.155; published online 28 September 2012

Keywords: colorectal cancer; prognosis; score; tumor budding

Tumor budding corresponds to a type of diffusely infiltrative growth observed in many colorectal cancers and is defined as the presence of detached isolated single cells or small cell clusters (up to 5 cells) scattered in the stroma at the invasive tumor margin.[1] Budding cells have been shown to share some properties of malignant stem cells, suggesting that their histomorphological and immunophenotypic features are dynamic and reversible.[2,3] Tumor budding may reflect the process of epithelial–mesenchymal transition, which allows neoplastic epithelial cells to acquire a mesenchymal phenotype, thus increasing their capacity for migration and invasion and may help them become more resistant to apoptotic signals.[4]

Tumor budding has been associated with an adverse prognosis in colorectal cancer and according to the third edition of 'Prognostic Factors in Cancer' published by the International Union for Cancer Control in 2006 is considered to be an additional prognostic factor.[5] More recently, it has been included in the category IIB (shown to be promising in multiple studies but insufficient for inclusion in category I or IIA) of colorectal cancer prognostic factors.[6] In more detail, tumor budding has been shown to be associated with poor differentiation, presence of vascular and lymphatic invasion, local tumor recurrence and lymph node and/or distant metastasis.[7–14] Moreover, tumor budding has repeatedly been linked to unfavorable disease outcome and has been shown to have an independent

Correspondence: Dr E Karamitopoulou, MD, Division of Clinical Pathology, Institute of Pathology, University of Bern, Murtenstrasse 31, Bern 3010, Switzerland.
E-mail: eva.diamantis@pathology.unibe.ch
Received 20 April 2012; revised 23 July 2012; accepted 23 July 2012; published online 28 September 2012

adverse effect on disease-free and overall survival.[1–13]

Although tumor budding has been recognized by the International Union for Cancer Control as an additional prognostic factor in colorectal cancer, it remains largely unreported in daily diagnostic work. In contrast to other cancers, including breast and prostate cancer where scores are used in daily routine, there has not been real progress with respect to additional prognostic factors or scoring systems in colorectal cancer. Many different scoring systems have been proposed by different authors including categorical[7,11,15] as well as quantitative systems.[8,12–14,16] In a previous study by our group,[17] we compared and evaluated six scoring methods for tumor budding after pan-cytokeratin staining, including those proposed by Hase, Nakamura, Ueno, and Wang, the evaluation of the densest high-power field (1 HPF) and also 10-high-power fields (10 HPFs) at the invasive margin, in correlation with clinicopathological features in stage II colorectal cancer patients. We have thus identified the 1 HPF and 10 HPFs methods as promising and reproducible way of assessing tumor budding. Moreover, we showed that the 10 HPFs method accounts for heterogeneity by maintaining a strong predictive value for the adverse effect on outcome. Based on this, the principal aim of the present study was to validate the 10 HPFs method of assessing tumor budding in a different, well characterized cohort of colorectal cancers, including patients of all stages. For this purpose we examined 10 HPFs at the area of the invasive front in whole tissue sections of 215 colorectal cancer cases after immunohistochemical staining with a pan-cytokeratin antibody (AE1/AE3) to facilitate the recognition of buds. The relationship between tumor budding and other prognostic factors as well as survival of the patients was also assessed. Our study aims to achieve a basis for a future consensus for the use of a standardized scoring system that will facilitate the interpretation of our results toward a more optimal categorization of the colorectal cancer patients.

Materials and methods

Patients and Specimens

Two hundred fifteen non-consecutive colorectal cancer patients were randomly selected from the archives of the Department of Pathology, University of Athens Medical School, Greece. Patients were treated between 2004 and 2007. All histomorphological data were reviewed from the corresponding hematoxylin and eosin stained slides, while clinical data were obtained from corresponding reports. Patient characteristics are listed in Table 1. Clinico-pathological information included gender, age, tumor diameter, histological subtype, tumor location, TNM stage, vascular invasion, and lymphatic invasion. Information on post-operative therapy was available for all patients. The mean age of the patient cohort was 68 years and ranged from 35 to 93 years. Clinical outcome of interest was disease-specific survival time. Five-year survival rate was 46.7% (95% CI (confidence interval): 35–58).

Table 1 Patient characteristics ($n = 215$)

Feature	*Frequency* N *(%)*
Age (years)	
Median (range)	71 (35–93)
Tumor size (cm)	
Median (range)	4.5 (1–12)
Gender	
Female	109 (51)
Male	104 (49)
Histological subtype	
Mucinous	21 (10)
Other	194 (90)
Tumor grade	
G1-2 (low grade)	136 (63)
G3 (high grade)	79 (37)
Tumor location	
Left-sided	129 (61)
Right-sided	28 (13)
Rectum	56 (26)
pT classification	
pT1-2	53 (25)
pT3-4	160 (75)
pN classification	
pN0	107 (50)
pN1-2	106 (50)
pM classification	
pM0	192 (90)
pM1	21 (10)
TNM stage	
I	46 (22)
II	57 (27)
III	88 (41)
IV	21 (10)
Vessel invasion	
L + V +	30 (14)
L + V −	56 (26)
L − V +	8 (4)
L − V −	121 (56)
Tumor border	
Infiltrating	151 (70)
Pushing	43 (20)
Mixed	21 (10)
Adjuvant therapy	
None	82 (38)
Treated	133 (62)
Survival rate	
5-year (95% CI)	47 (35–58)

Abbreviations: CI, confidence interval; L, lymphatic invasion; V, venous invasion.

Immunohistochemistry

Paraffin-embedded tissue blocks from all 215 patients were retrieved, cut at 4 μm and immunostained for AE1/AE3 antibody, a marker of epithelial cells that serves to highlight areas of tumor budding. This marker is routinely used in our laboratory for diagnostic purposes. Whole tissue sections were de-waxed and re-hydrated in dH2O. Following pressure cooker-mediated antigen retrieval in 0.001 mol/l ethylenediaminetetraacetic acid pH 8.0, endogenous peroxidise activity was blocked using 0.5% H_2O_2. Sections were incubated with 10% normal goat serum for 20 min. After incubation with primary antibody (AE1/AE3 mAb, 1:100, Monosan), sections were incubated with HRP-conjugated secondary antibody (DakoCytomation, Glostrup, Denmark) for 30 min at room temperature, immersed in 3-amino-9-ethylcarbazole + substrate-chromogen (DakoCytomation) for 30 min, and counterstained with haematoxylin.

The use of material for this study was approved by the local research Ethics committee.

Assessment of Tumor Budding

Tumor budding was defined as dedifferentiated single cells or clusters of <5 cells at the invasive tumor front. All tumor blocks were first examined at low magnification and the most representative block with the highest number of budding foci was chosen for the analysis. From the AE1/AE3-stained whole tissue sections two experienced pathologists (EK and AKP) selected at a low magnification ($\times 5$) the area with the highest density of peritumoral budding.

Afterwards the number of buds was counted in 10 HPFs ($40\times$) and the average number was used for analysis. In addition to the two pathologists in this study who evaluated all 215 cases, two additional observers were asked to score 50 cases of tumor budding randomly selected from the larger cohort. Evaluation was performed blinded to clinical end points.

Statistical Analysis

The strength of the linear correlation between the two observers' tumor budding counts was assessed using the Pearson correlation coefficient (r). The intraclass correlation coefficient (ICC), a measure of inter-observer agreement for continuous variables and ranging from 0.0 (no agreement) to 1.0 (perfect agreement) was used. The mean and standard deviation (s.d.) were investigated. In order to obtain an unbiased cutoff with which to evaluate the association of tumor budding and clinico-pathological features, the cohort of 215 patients was randomly divided into two subgroups. The first, containing 30% of the data ($n=65$ patients) was used to determine the optimal cutoff value for declaring a case as 'high-grade'. Cutoff score determination was performed by receiver operating characteristic curve analysis with the end point overall survival (Figure 1). The cutoff was validated using 200 bootstrapped replications of the data. Using this method, the optimal cutoff was determined to be 10 buds. Then, on the second group containing the remaining 70% of the data ($n=150$ patients), the 10 buds cutoff was applied and associations with outcome tested. Multivariate survival time models were analyzed with the hazard ratio (HR) of 1.0 as baseline and 95% CI. These analyses were performed on the entire cohort due to the limited number of events in the analysis group. The χ^2- or Fisher's Exact tests were used, where appropriate. The Kaplan–Meier method was used to represent survival curves and the log-rank test was used to test significant survival time differences. Multivariate survival time models were analyzed with the HR of 1.0 as baseline and 95% CI. All analyses were carried out using SAS V9.2 (The SAS Institute, NC, Cary). P-values <0.05 were considered statistically significant.

Results

Examples of low- and high-grade budding are shown in Figure 2.

Inter-observer Agreement of Tumor Budding Scores

In a first step, the average number of tumor buds in 10 HPFs was assessed for the two initial observers (EK and AKP) and the correlation between the scores determined to be excellent ($r=0.98$; $P<0.0001$) (Figure 3). The average ± s.d. number of buds was 11.1 ± 12.0 and 11.3 ± 12.4, respectively. The ICC = 0.97 indicates excellent agreement

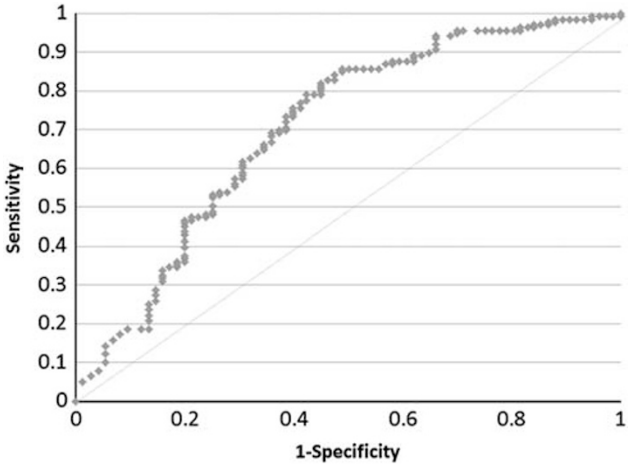

Figure 1 Cutoff score determination was performed by receiver operating characteristic curve analysis with the end point overall survival.

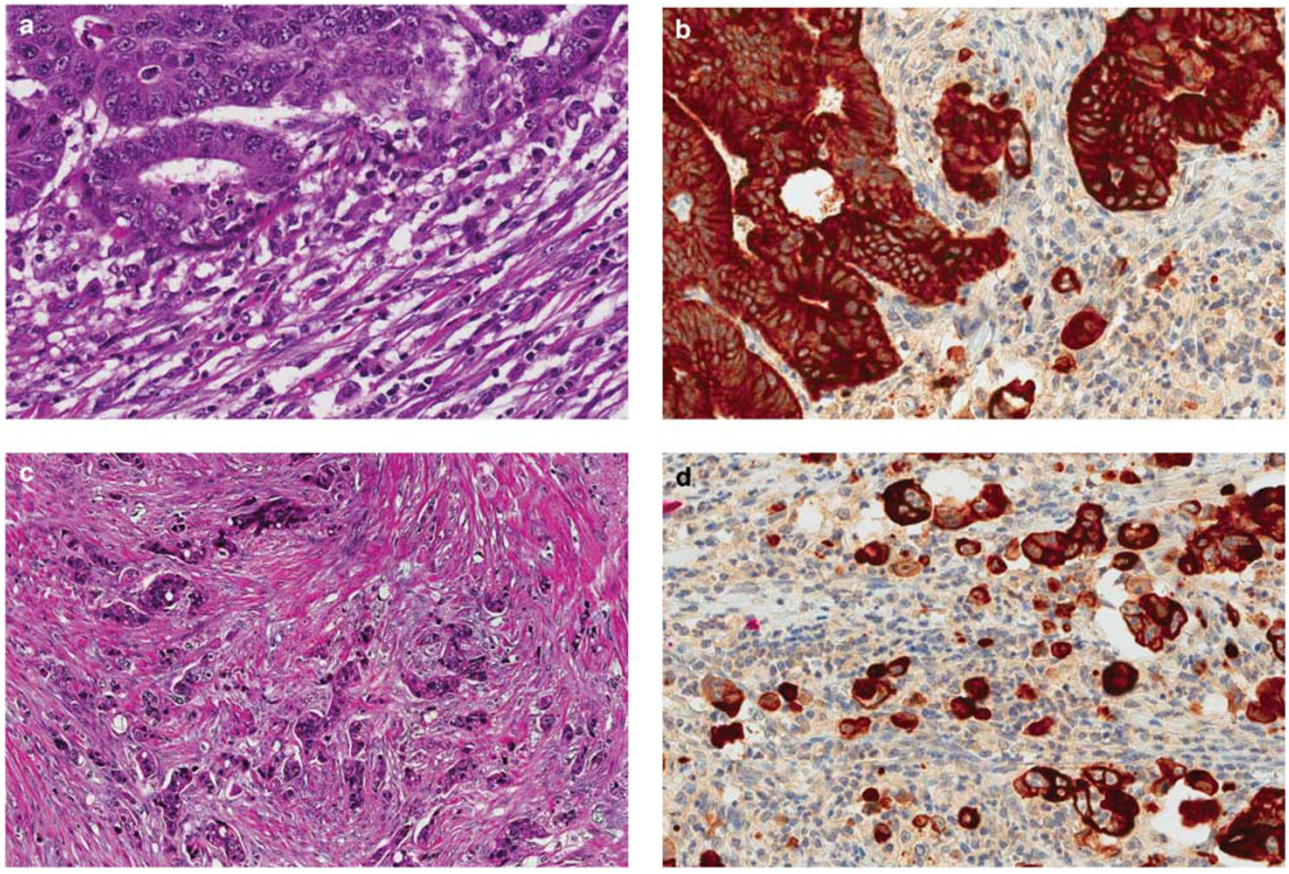

Figure 2 Examples of low- (**a,** hematoxylin and eosin, **b,** cytokeratin AE1/AE3), and high-grade budding (**c**, hematoxylin and eosin, **d**, cytokeratin AE1/AE3) in CRC (× 200).

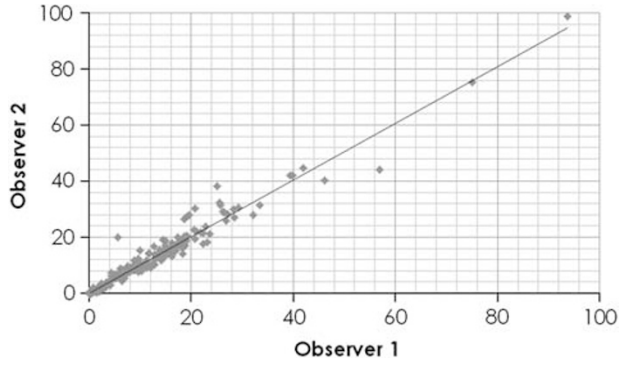

Figure 3 Scatter-plot showing the strong correlation between the observers' raw tumor budding scores across 10 HPFs.

between the two observers. After evaluation of 50 randomly selected cases by two additional observers (IZ and VK) the intra-class correlation coefficient used to evaluate the raw tumor budding data remained excellent, ICC = 0.96. When scores were categorized around the cutoff value of 10 buds/10 HPFs, average percent-concordance was high at 85.8% and ranged from 77 to 94% between different observers. Kappa values also show strong agreement between observers ($k = 0.71$), ranging between 0.5 and 0.87 (Table 2).

Table 2 Inter-observer agreement data for budding scoring (from four observers on 50 cases)

Kappa values (k)			
Observers	2	3	4
1	0.87	0.6	0.83
2		0.65	0.79
3			0.5
Percent concordance (%)			
Observers	2	3	4
1	93.8	80.4	91.8
2		82.6	89.8
3			76.6
Average buds			
1	10.4 (range: 0.1–39.8)		
2	10.8 (range: 0–42)		
3	11.8 (range: 1.4–39.3)		
4	9.2 (range: 0–35)		

Association of Tumor Budding with Clinico-pathological Features

Using a random sample of 30% of the patient cohort, the optimal cutoff score was determined to be 10 buds (Table 1). This cutoff score was then applied to

remaining 70% of the patient cohort, namely on 150 patients. Results are seen in Table 3. High-grade tumor budding (≥10 buds on average across 10 HPFs) was significantly more frequently associated with higher tumor grade ($P=0.0004$), more advanced pT classification ($P=0.009$), lymph node positivity ($P=0.0004$), more advanced TNM stage ($P=0.0016$), angioinvasion ($P=0.0001$) and with an infiltrating tumor growth pattern ($P<0.0001$). Patients with high-grade tumor budding were more likely to receive adjuvant therapy ($P=0.0009$) and had a considerably poorer outcome at 5-years (63 versus 20% for low grade and high grade, respectively, $P=0.0179$) (Figure 4). This poorer outcome translated into a 2.54 (95% CI: 1.6–4.1) times greater relative risk of death in patients with high- compared with low-grade tumor budding.

Table 3 Association of patient characteristics using cutoff of an average of 10 buds/HPF ($n=150$)

Feature	*Total frequency* (N)	*Frequency,* N *(%)*		P-*value*
		Low (≤10 buds)	*High (>10 buds)*	
Gender				
Female	81	42 (54)	39 (55)	0.8945
Male	68	36 (46)	32 (45)	
Histological subtype				
Mucinous	15	6 (8)	9 (13)	0.3003
Other	135	73 (92)	62 (87)	
Tumor grade				
G1–2 (low grade)	96	61 (77)	35 (49)	0.0004
G3 (high grade)	54	18 (23)	36 (51)	
Tumor location				
Left-sided	92	50 (64)	42 (59)	0.822
Right-sided	16	8 (10)	8 (11)	
Rectum	41	20 (26)	21 (30)	
pT classification				
pT1-2	40	28 (36)	12 (17)	0.009
pT3-4	109	50 (64)	59 (83)	
pN classification				
pN0	71	48 (61)	23 (32)	0.0004
pN1-2	78	30 (39)	48 (68)	
pM classification				
pM0	133	73 (94)	60 (86)	0.113
pM1	15	5 (6)	10 (14)	
TNM stage				
I	34	25 (32)	9 (13)	0.0016
II	35	23 (30)	12 (17)	
III	64	25 (32)	39 (56)	
IV	15	5 (6)	10 (14)	
Vessel invasion				
L+ V+	23	8 (10)	15 (21)	0.0001
L+ V−	39	11 (14)	28 (39)	
L− V+	5	3 (4)	2 (3)	
L− V−	83	57 (72)	26 (37)	
Tumor border				
Infiltrating	107	37 (47)	70 (99)	<0.0001
Pushing	29	29 (37)	0 (0)	
Mixed	14	13 (16)	1 (1)	
Adjuvant therapy				
None	59	41 (52)	18 (25)	0.0009
Treated	91	38 (48)	53 (75)	
Survival rate				
5-year (95% CI)	38.8 (24–53)	63.3 (47–76)	19.5 (5–41)	0.0179

Abbreviations: L, lymphatic invasion; V, venous invasion; 30% of the patient cohort was used to obtain an unbiased cutoff score, while the remaining 70% ($n=150$) presented here were used as analysis cohort.

Multivariate Survival Time Analysis

Multivariate survival time analysis was performed on the entire cohort of 215 patients. High-grade tumor budding maintained its significant and adverse effect on outcome when adjusting for TNM stage and adjuvant therapy (Table 4). High-grade tumor budding resulted in a relative risk of death of 1.88 (95% CI: 1.2–3.1) in comparison with patients with low-grade tumor budding in this analysis. Although tumor budding was independent of tumor grade (HR (95% CI): 2.24 (1.4–3.6)), pT classification (HR (95% CI): 2.12 (1.3–3.5)), and even the presence of distant metastasis (HR (95% CI: 2.15 (1.3–3.5))

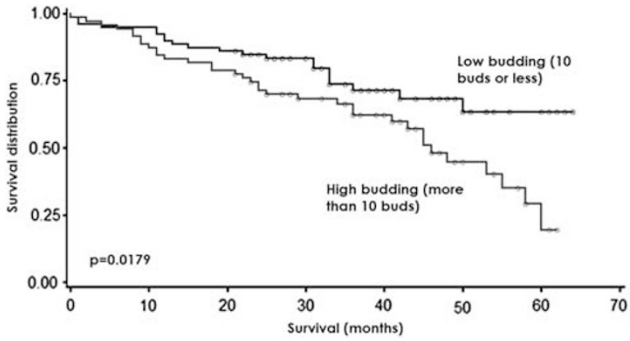

Figure 4 Kaplan–Meier curves for low- and high-grade tumor budding counts and adjusted *P*-values from log-rank test. NS, not significant.

Table 4 Multivariate survival time analysis of tumor budding with TNM stage and adjuvant therapy

Parameter	*HR (95% CI)*	P-*value*
Tumor budding		
≤10 buds	1.0	0.0124
>10 buds	1.88 (1.2–3.1)	
TNM		
Stage I–II	1.0	<0.0001
Stage III–IV	6.55 (3.5–12.4)	
Adjuvant therapy		
None	1.0	0.001
Treated	0.4 (0.3–0.7)	

Abbreviation: HR, hazard ratio.

in this cohort, it was not independent of vessel invasion (HR (95% CI): 1.54 (0.9–2.5)). Focusing only on patients with stage II disease, high-grade tumor budding had a significant adverse effect on outcome (HR (95% CI): 3.55 (1.4–9.2)) and was more important than adjuvant therapy in multivariate analysis. These results suggest that patients with stage II colorectal cancers with high-grade tumor budding have more than 3.5 times greater risk of death in comparison with patients with low-grade tumor budding. As only six patients with TNM stage II disease were pT4, this finding refers mostly to stage II patients with pT3 tumors.

Discussion

Based on the results of this study, we propose that 10 HPFs be used for the assessment of tumor budding after staining with a pan-cytokeratin antibody. Using robust statistical analysis, our results suggest that an average of 10 buds be used as a cutoff value for low ($\leq$10 buds) and high ($>$10 buds) grade tumor budding.

In a first step, we show that the assessment of 10 HPFs leads not only to a strong correlation between the observers' scores but also to an excellent inter-observer agreement. Previous studies using different scoring systems such as those proposed by Hase, Nakamura, Ueno and Wang[7–17] have demonstrated that tumor budding is a strong independent prognostic parameter; however, the implementation of these results in clinical practice is hampered by the lack of a standardized scoring system. In a previous study by our group, after comparison of the several existing scoring methods, the 1 HPF and 10 HPFs approaches came out as the most reliable and reproducible methods of assessing tumor budding.[18] Most importantly, scoring 10 HPFs accounts for heterogeneity and shows a higher inter-observer agreement than 1 HPF because of the improbability of different pathologists selecting the identical 'hot-spots'. In addition, a scoring system based on the evaluation of 10 HPFs has many advantages, as it is already applied in other tumor types such as the mitoses count in breast cancer, soft tissue tumors and gastrointestinal stromal tumors, and therefore, pathologists are familiar with this type of scoring. Further, it will allow the future categorization of colorectal cancer cohorts into prognostic subgroups using a two-tier (low- and high-grade tumor budding) scoring system.

In order to increase the accuracy of tumor budding counts, we and others have shown that the assessment of tumor budding after staining with a pan-cytokeratin antibody greatly improves the visualization of buds.[13,14,19–21]

Although the number of tumor buds as well as the costs are likely to be higher using this approach, it is of significant benefit to differentiate them from activated fibroblasts in the surrounding stroma. Hence, the assessment of budding in this study was undertaken after staining with a pan-cytokeratin antibody of the most representative tumor block.

One issue hindering the widespread use of tumor budding in addition to the scoring method is the determination of a valid cutoff score for declaring a case as low or high grade. Here we used robust statistical analysis on a subsample (30%) of our patient cohort, which allowed us to determine the cutoff value to then be applied on the remaining 70% of the patient data in an unbiased manner. This approach identified a threshold value of 10 buds as the optimal cutoff for discriminating better and worse prognostic subgroups. With this cutoff score, we identified 48% of patients as high-grade budding cases, which is in agreement with the frequency described in the studies by Nakamura and Wang.[11,14,15] Moreover, tumor budding was again found to be associated with factors of poor prognosis, such as higher tumor grade, advanced pT classification, lymph node metastasis, more advanced TNM stage, angioinvasion and infiltrating tumor growth pattern. Our previous work restricted to stage II colorectal cancer found no associations with tumor grade or venous invasion.[18] This discrepancy is mostly due to the unselected nature of the cohort of patients from all stages in this study including those with stage III and IV disease.

In the multivariate analysis, the effect on survival remained independent of the TNM stage and administration of adjuvant therapy. This was confirmed in subgroup analysis of stage II patients only. This is particularly critical as a proportion of stage II patients are likely to be undertreated. In fact, the identification of biomarkers capable of stratifying stage II colorectal cancer into better prognostic subgroups has been the objective of several studies over the past few years. However, most of these results are not currently implemented because of lack of reproducibility, validation or standardization. In the present study, we followed the REMARK guidelines, proposed in 2005 in order to improve the quality and reproducibility of biomarker studies by suggesting a uniform study design.[22] Within this context, additional prognostic information regarding tumor budding would help to identify a subgroup of stage II patients that would be suited for adjuvant therapy.

In conclusion, our results suggest that the evaluation of 10 HPFs for the presence of buds and a cutoff score of 10 buds on average after staining with a pan-cytokeratin antibody is not only reproducible for the assessment of tumor budding in colorectal cancer but could be a defining factor for refining criteria to identify patients with stage II disease who may benefit from postoperative therapy.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Prall F. Tumour budding in colorectal carcinoma. Histopathology 2007;50:151–162.
2 Brabletz T, Jung A, Spaderna S, *et al.* Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. Nat Rev Cancer 2005;5: 744–749.
3 Zlobec I, Lugli A, Baker C, *et al.* Role of APAF-1, E-cadherin and peritumoural lymphocytic infiltration in tumour budding in colorectal cancer. J Pathol 2007; 212:260–268.
4 Kalluri R. EMT: when epithelial cells decide to become mesenchymal-like cells. J Clin Invest 2009;119: 1417–1419.
5 Compton C. Prognostic Factors in Cancer, 3rd edn. John Wiley & Sons, Inc.: New York, 2006.
6 Compton C. Pathology and prognostic determinants of colorectal cancer, In: Tanabe K, Savarese D(eds) UpToDate. Wolters Kluwer Health: Waltham, MA, 2012.
7 Hase K, Shatney C, Johnson D, *et al.* Prognostic value of tumor 'budding' in patients with colorectal cancer. Dis Colon Rectum 1993;36:627–635.
8 Ueno H, Murphy J, Jass JR, *et al.* Tumour 'budding' as an index to estimate the potential of aggressiveness in rectal cancer. Histopathology 2002;40:127–132.
9 Ishikawa Y, Akishima-Fukasawa Y, Ito K, *et al.* Histopathologic determinants of regional lymph node metastasis in early colorectal cancer. Cancer 2008;112: 924–933.
10 Kazama S, Watanabe T, Ajioka Y, *et al.* 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;94:293–298.
11 Nakamura T, Mitomi H, Kikuchi S, *et al.* Evaluation of the usefulness of tumor budding on the prediction of metastasis to the lung and liver after curative excision of colorectal cancer. Hepatogastroenterology 2005;52: 1432–1435.
12 Ueno H, Mochizuki H, Hashiguchi Y, *et al.* Predictors of extrahepatic recurrence after resection of colorectal liver metastases. Br J Surg 2004;91:327–333.
13 Ueno H, Mochizuki H, Hashiguchi Y, *et al.* Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology 2004;127:385–394.
14 Wang LM, Kevans D, Mulkahy H, *et al.* Tumor budding is a strong and reproducible prognostic marker in T3N0 colorectal cancer. Am J Surg Pathol 2009;1:134–141.
15 Nakamura T, Mitomi H, Kanazawa H, *et al.* Tumor budding as an index to identify high-risk patients with stage II colon cancer. Dis Colon Rectum 2008;51: 568–572.
16 Ueno H, Mochizuki H, Shinto E, *et al.* Histologic indices in biopsy specimens for estimating the probability of extended local spread in patients with rectal carcinoma. Cancer 2002;94:2882–2891.
17 Ueno H, Price AB, Wilkinson KH, *et al.* A new prognostic staging system for rectal cancer. Ann Surg 2004;240:832–839.
18 Koelzer V, Horcic M, Karamitopoulou E, *et al.* Tumour budding score based on 10 high-power fields (HPFs) is a reliable basis for a standardized scoring system in colorectal cancer (Abstract). Virchows Arch 2011; 459:S156.
19 Park KJ, Choi HJ, Roh MS, *et al.* Intensity of tumor budding and its prognostic implications in invasive colon carcinoma. Dis Colon Rectum 2005;48: 1597–1602.
20 Prall F, Nizze H, Barten M. Tumour budding as prognostic factor in stage I/II colorectal carcinoma. Histopathology 2005;47:2005.
21 Zlobec I, Molinari F, Martin V, *et al.* Tumor budding predicts response to anti-EGFR therapies in metastatic colorectal cancer patients. World J Gastroenterol 2010;16:4823–4831.
22 McShane LM, Altman DG, Sauerbrei W, *et al.* Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005;97: 1180–1184.