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REVIEW

Does evaluation of tumour budding in diagnostic biopsies have a clinical relevance? A systematic review

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Does evaluation of tumour budding in diagnostic biopsies have a clinical relevance? A systematic review

Abstract: Tumour budding has emerged as a promising prognostic marker in many cancers. We systematically reviewed all studies that evaluated tumour budding in diagnostic biopsies. We conducted a systematic review of PubMed, MEDLINE, Scopus, Web of Science and Cochrane library for all articles that have assessed tumour budding in diagnostic (i.e. pretreatment or pre-operative) biopsies of any tumour type. Two independent researchers screened the retrieved studies, removed duplicates, excluded irrelevant studies and extracted data from the eligible studies. A total of 13 reports comprising 11 cohorts were found to have studied tumour budding in diagnostic biopsies. All these reports showed that evaluation of tumour budding in diagnostic biopsies was easily applicable. A strong association was observed between tumour budding score in diagnostic biopsies and corresponding surgical samples. Evaluation of tumour budding in diagnostic biopsies had a significant prognostic value for lymph node metastasis and patient survival. In all studies, tumour budding was a valuable marker of tumour aggressiveness and can be evaluated in technically satisfactory diagnostic biopsies. Thus, the assessment of tumour budding seems to identify the behaviour of cancer, and therefore to facilitate treatment planning.

Keywords: biopsy, marker, prognosis, treatment planning, tumour budding

Introduction

Pre-operative/pretreatment biopsies are widely used as diagnostic tools of different epithelial tumours, and they are also used to determine the histological subtype and degree of differentiation. The possibility to use diagnostic biopsies to identify tumours with aggressive behaviour is crucial for proper treatment planning. Such aggressive behaviour is associated with unfavourable histology. Unfortunately, the small amount of tumour tissue in the biopsy (compared with postoperative samples) can impede identification of some histopathological markers (e.g. perineural invasion). Moreover, a superficial diagnostic biopsy may not

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include histopathological features in the deep regions of tumours. Therefore, it is of clinical interest to identify reliable and simple prognostic marker(s) that can be evaluated in diagnostic biopsies, particularly histopathological parameters that can be identified in routine haematoxylin and eosin (H&E) slides.

Tumour budding, or sprouting (Figure 1), is a histopathological phenomenon that refers to the presence of single cancer cell(s) or small cluster(s) of up to four cancer cells that are separated from the main part of the tumour. It was speculated that tumour budding is the result of interactions between cancer cells and tumour microenvironment.[1] Tumour budding represents active invasion, dissociation and epithelial–mesenchymal transition.[1,2] A relationship to cancer stem cells[3] has also been reported. Recent and rapidly accumulating data indicate that tumour budding may open new perspectives for prognostication and treatment planning of many cancers.[2,4–7] The prognostic value of tumour budding in diagnostic biopsies has been examined in recent studies.[8–10] In addition, some of these studies reported the concordance of the score of tumour budding in pre-operative diagnostic biopsy samples and postoperative surgical specimens.[11,12] However, the implementation of tumour budding for pathology reports and treatment planning still requires further studies.[2,13]

We conducted a systematic review of studies that evaluated tumour budding in diagnostic specimens to summarise the current understanding of this topic and to guide pathologists in reporting this histopathological feature in daily practice.

Methods

SEARCH PROTOCOL

We systematically retrieved all studies that evaluated tumour budding in pretreatment diagnostic biopsies. The systematic search included databases of PubMed, OvidMedline, Scopus, Web of Science and Cochrane library from their inception until March 2018. The search strategy was developed by combining the search terms: 'Tumour budding' AND 'biopsy'. Additional search using ('Tumour budding') AND ('diagnostic biopsy' OR 'pretreatment biopsy' OR 'preoperative sample') was also conducted. References of the eligible studies were searched manually to enhance the inclusion of all relevant studies. The Preferred Reporting Items for

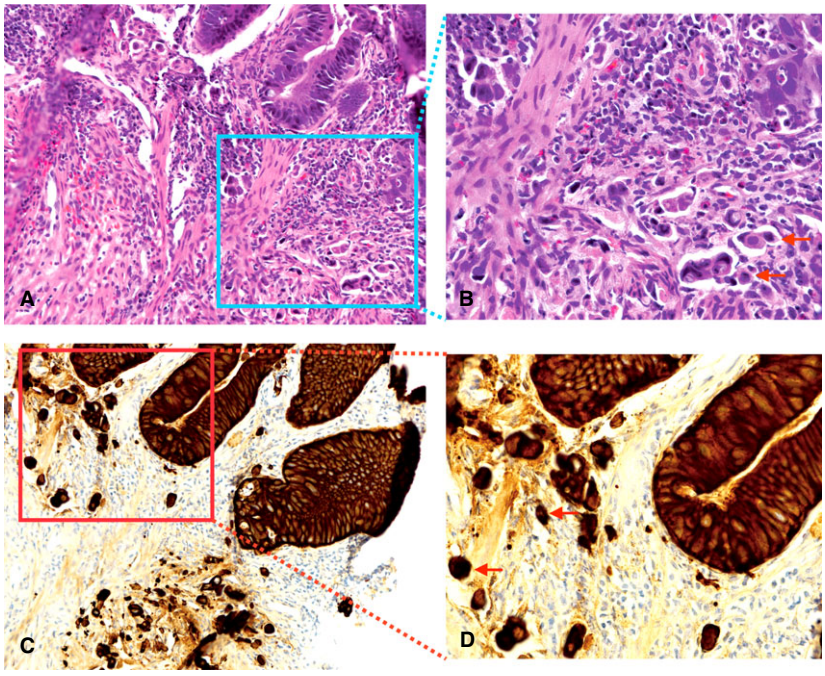

Figure 1. Tumour budding (arrows) in a pre-operative diagnostic biopsy of colorectal cancer. **A**, Tumour budding (haematoxylin and eosin-stained section) in the area inside the insert, which is magnified in **B**. **C**, Tumour budding (pancytokeratin-stained section) in the area inside the insert, which is magnified in **D**.

Systematic Review and Meta-Analysis (PRISMA)[14] guidelines were followed.

INCLUSION AND EXCLUSION CRITERIA

All original reports that used pretreatment biopsies in assessment of tumour budding were included. Eligible studies must have evaluated tumour budding in diagnostic biopsies of a cohort. Review articles, case reports, case series, conference abstracts, editorials, letters to the editor and commentaries were excluded. Our search was limited to articles in the English language.

SCREENING

Two independent researchers (A.A., O.Y.) performed the screening of retrieved studies at all stages to identify the eligible studies. Any disagreements between the two researchers were resolved by discussion to reach a consensus on which studies should be included or excluded.

DATA EXTRACTION

We retrieved the basic information (name of the first author, publication year, country, number of cases, stage, type of tumour and main treatment) from all eligible studies. Data regarding assessment of tumour budding included the definition of buds, cut-off point, microscopic magnification, staining and main findings.

QUALITY ASSESSMENT

As tumour budding is a prognostic marker, we used the guidelines of reporting recommendations for tumour marker prognostic studies (REMARK)[15] to assess the quality of the studies. The main criteria of REMARK guidelines are selected and summarised in Table 1.

Results

Our search retrieved 13 studies (11 cohorts) that evaluated tumour budding in pretreatment diagnostic biopsies (Figure 2). The findings of these studies (Table 2) indicated that tumour budding is an evaluable histopathological parameter in biopsy specimens and could be used as a reliable prognosticator for patient survival. The studies evaluated tumour budding in pretreatment samples in the following cancers: five studies (four cohorts) on rectal cancer,[8,16–19] two on colorectal cancer[9,11] and three (two cohorts) on oral cancer.[10,12,20] There was one study on breast cancer,[13] one on epidermoid anal cancer[21] and one on cancer of the external auditory canal.[22]

In colorectal cancer, the studies (of rectal and colorectal cohorts) showed that the pre-operative score of tumour budding was associated with lymph node metastasis,[8,9,11,16,17,19] distant metastasis[9,11,19] and patient survival.[8,19] The relationship between pre-operative tumour budding and the presence of extranodal tumour deposits,[17] lymphovascular invasion,[9] tumour grade[11] and stage[9] was also reported in colorectal cancer. In oral cancer, pre-operative tumour budding had a significant prognostic value for lymph node metastasis, overall survival and relapse-free survival.[10,20] Pre-operative tumour budding in oral cancer also had strong correlations with tumour grade, tumour depth and blood vessel invasion.[10,20] In breast cancer, tumour budding is associated with venous invasion.[13] A study of epidermoid anal cancer revealed that pretreatment tumour budding was a significant predictor of overall survival.[21] In cancer of the external auditory canal, a single study showed that pretreatment tumour budding is associated with expression of laminin 5-γ2 and predicts disease-specific survival.[22] Of note, a significant correlation between tumour budding in pre-operative biopsies and postoperative samples was reported in colorectal cancer,,[11] breast cancer[13] and oral cancer.[12,20]

The quality of the published studies was assessed as satisfactory to good. Some studies did not follow the REMARK guidelines correctly (Table 2), as they reported the prognostic value of tumour budding without multivariate analysis or did not analyse the relationship between tumour budding and classic prognostic factors (e.g. stage, grade, depth of invasion). Some of the published studies suffered from the limitations posed by a low number of cases.[11,17,19,22]

Discussion

Tumour budding is a hallmark of cancer invasion and has been recently validated as a promising prognostic marker in colon cancer,[23] oesophageal cancer,[24] pancreatic cancer,[25] lung cancer[7] and oral cancer.[26] Interestingly, the meta-analyses conducted on the published studies confirm the prognostic value of tumour budding in oesophageal, colorectal and oral cancers.[2,4,5] Moreover, tumour budding is currently considered as an additional prognosticator by

Table 1. Items adapted from REMARK that were used to assess the quality of studies on tumour budding in preoperative biopsies

Item	Criteria
Introduction	• The hypothesis about tumour budding and objectives of the study were explained
Study design	• Retrospective or prospective cohort with a well-defined study population • Medical treatment of the cases was explained
Material	• Patient data such as age, gender, clinical stage and WHO grade were explained
Method of evaluation	• Well-described method including the microscopic field/s and the cutoff point • Routine HE-staining and/or immunohistochemistry (e.g. pan-cytokeratin (AE1/AE3))
Data analysis	• The survival endpoint was well defined • Estimated effect (e.g. hazard ratio, with confidence intervals) of preoperative tumour budding was reported • Univariate estimate: reported the effect of tumour budding in pretreatment samples on outcome • Multivariate estimate: adjusted for the conventional prognostic factors • Inter-observer variability was evaluated • The relationship between the pretreatment score of tumour budding and conventional prognosticators was reported • The prognostic value of the classical prognostic factors (e.g. stage and grade) were reported
Discussion	• The results about tumour budding were discussed in the context of the relevant studies • The limitations of the study were explained • Recommendation for further evaluation of tumour budding was suggested based on published guidelines

the Union for International Cancer Control's tumour–necrosis–metastasis (TNM) classification.[1,27] The assessment of tumour budding in pretreatment diagnostic biopsies has been recently investigated by many researchers. Here, for the first time to our knowledge, we systematically reviewed the literature to summarise the evidence on pretreatment assessment of tumour budding. The published studies showed that evaluation of tumour budding was applicable to pretreatment diagnostic biopsies of oral, breast, colorectal, epidermoid anal and external auditory canal cancers.

The first study that evaluated tumour budding in pre-operative/pretreatment biopsies was published in 1989 by Morodomi and colleagues on a cohort of rectal cancer patients.[16] Notably, most of the studies that evaluated tumour budding in pre-operative biopsies were on colorectal cancer (Table 2), where a strong correlation between tumour budding and lymph node metastasis has been observed.[9,28] The ability of tumour budding to prognosticate nodal metastasis has also been reported in other cancers.[2,29] Moreover, the prognostic impact of tumour budding for nodal metastasis, patient survival or both was prominent in the early stages of other cancers.[30–32] These findings indicate that tumour budding is an important step in the development of metastasis.

The international tumour budding consensus conference 2016 (ITBCC 2016) introduced guidelines to standardise the scoring system of this prognostic marker in colorectal cancer.[33] Interestingly, a recent study on pancreatic cancer[25] used the aforementioned ITBCC evaluation method of ITBCC 2016, and found that this method represents a simple and standardised scoring system that facilitates inclusion of tumour budding in pathology reports. The recommendations included 11 statements,[33] starting with a definition of tumour budding in colorectal cancer as: 'a single tumour cell or a cell cluster consisting of four tumour cells or less' and ending with a statement indicating that: 'Tumour budding and tumour grade are not the same'. There were recommendations specific for prognostic significance of tumour budding in colorectal cancer indicating that: 'Tumour budding is an independent predictor of lymph node metastasis in pT1

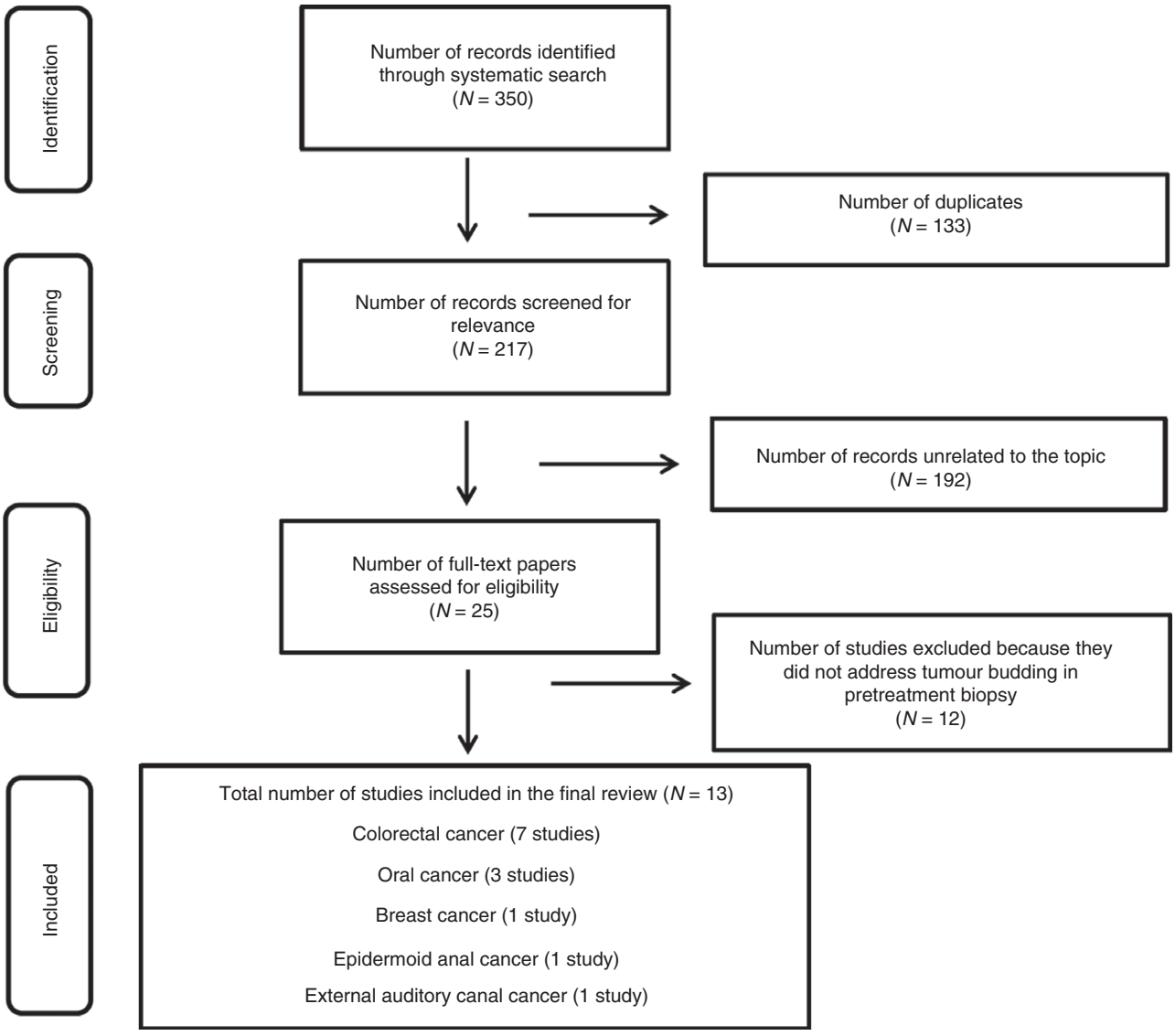

Figure 2. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart. Number of articles included and excluded along the steps of systematic searching for studies that examined tumour budding in pretreatment diagnostic biopsies.

colorectal cancer, and for survival in stage II colorectal cancer'. We noted that the majority of the relevant studies were in accordance with the main recommendations especially in colorectal cancer studies (Table 2). It is important to take into consideration that the studies included in our systematic review were not limited to colorectal cancer. In addition, the authors of the recommendations[33] stated that the ITBCC is not an end-point, but rather a step towards further research.[33] Therefore, it is necessary for future research to follow the introduced guidelines and update them if needed for each specific cancer type.

Tumour budding evaluated on H&E-stained slides has shown a reliable predictive value.[34–36] This simple method has the potential to make the evaluation of tumour budding more applicable to daily practice than the use of biomarkers that require immunohistochemical staining. It has been stated in the recent recommendation (ITBCC 2016)[33] that: 'Tumour budding is counted on HE' because a majority of the published data were based on H&E assessment. Also, the low cost of H&E staining is a factor that allows a worldwide evaluation of tumour budding. The ITBCC group admitted that this can change if future data on immunohistochemical assessment indicate it to be superior to H&E staining.[33] Of note, Kai and colleagues[37] compared the usefulness of cytokeratin staining compared with H&E staining (in postoperative samples of colorectal cancer) and found

Table 2. Summary of the studies that examined tumour budding in pretreatment diagnostic biopsies

Authors, year	Country	NO. of cases	Stage	Type of tumour	Main treatment	Bud definition	Cutoff	Magnification/ field	Stain	Summary of relevant findings on tumour budding	Quality of the study
Morodomi et al,[16] 1989	Japan	112	NA	Rectal cancer	Surgery	<5 cells	5 buds[1]	×400	HE	TB in preoperative biopsies had a strong correlation with LN metastasis ($P < 0.001$; OR 8.0)	Satisfactory
Ueno et al,[17] 2002*	Japan	85	Advanced stage	Rectal cancer	Surgery	<5 cells	5 buds	×200	HE	TB in preoperative biopsy significantly associated with number of LN involved (OR 5.3, 95% CI 1.7–16.9) and with presence of extranodal tumour deposits (OR 5.5, 95% CI 1.8–17.1)	Good
Ueno et al,[18] 2004*	Japan	120	Advanced stage	Rectal cancer	Surgery	<5 cells	5 buds	×200	HE	Preoperative assessment of TB was among parameters of unfavourable histology that associated with distal intramural spread	Satisfactory
Nilsson et al,[21] 2005	Sweden	209	T1-T4	Epidermoid anal cancer	Radiotherapy[2]	<5 cells	NA	NA	Laminin-5 gamma 2-chain	TB in pretreatment diagnostic biopsies had a statistically significant relationship with 5-year OS ($P < 0.05$)	Satisfactory
Guzinska-Ustymowicz,[19] 2005	Poland	34	T1	Rectal cancer	Surgery	<5 cells	1 bud	Field size[3]	HE	The presence of preoperative TB strongly correlated with local recurrence, LN and distant metastases ($P < 0.05$)	Satisfactory
Giger et al,[11] 2012	Switzerland	72	pT1-T4	Colorectal cancer	Surgery	<5 cells	Referred to Nakamura *et al.* method[4]	×2.5 & ×10	HE	Preoperative and postoperative TB were correlated ($P = 0.008$). There was a significant correlation between preoperative TB and LN metastasis, distant metastasis and tumour grade ($P < 0.05$)	Satisfactory
Zlobec et al,[9] 2014	Switzerland	133	pT1-T4	Colorectal cancer	Surgery	<5 cells	Scale	×40	AE1/AE3	Preoperative assessment of TB associated with LN metastasis, distant metastasis, pT stage, lymphatic and venous vessel invasion ($P < 0.05$)	Good
Rogers et al,[8] 2014	Ireland	89	T1-T4	Rectal cancer	Surgery + neoadjuvant chemotherapy	<5 cells	1 bud	×10	HE	The presence of TB in pre-treatment biopsies significantly associated with stage, LN, DFS, and response to neoadjuvant chemoradiotherapy ($P < 0.05$). TB in pretreatment biopsies was a significant predictor of cancer-related deaths (HR 3.51, 95% CI 1.03–11.93, $P = 0.040$)	Good

Table 2. *(Continued)*

Authors, year	Country	NO. of cases	Stage	Type of tumour	Main treatment	Bud definition	Cutoff	Magnification/ field	Stain	Summary of relevant findings on tumour budding	Quality of the study
Salhia et al,[13] 2015	Switzerland	99	T1-T4	Breast cancer	Surgery	<5 cells	10 buds	×400	AE1/AE3	High TB in preoperative core biopsies was significantly ($P = 0.0063$) associated with venous invasion. There was a significant correlation between TB in the preoperative core biopsies and in the surgical resection specimens ($P < 0.0001$)	Satisfactory
Okado et al,[22] 2015	Japan	46	T1-T4	SCC of external auditory canal	Surgery; neoadjuvantchemo-radiotherapy.	<5 cells	10 buds	×20*	Cytokeratin	TB in pretreatment biopsy specimens associated with expression of Ln5-γ2 ($P = 0.04$). Pretreatment TB associated with DSS ($P = 0.0007$)	Satisfactory
Seki et al,[20] 2016**	Japan	91	T1-T4	Tongue and FOM SCC	Surgery; preoperative CT (47 cases)	<5 cells	3 buds	×20	Cytokeratin	Preoperative TB was significantly associated with LN metastasis (OR 31, $P < 0.01$), OS and RFS ($P < 0.05$)	Good
Seki et al,[10] 2017**	Japan	209	cT1-T4	Oral SCC	Surgery; preoperative CT (111 cases)	<5 cells	3 buds; 5 buds	×20*	Cytokeratin	Strong correlations ($P < 0.01$) were observed between preoperative TB and tumour grade, tumour depth, INF and blood vessel invasion. Preoperative TB correlated with LNM (OR 30.05, $P < 0.01$)	Good
Almangush et al,[12] 2018	Finland	100	I-IV	Oral tongue cancer	Surgery	<5 cells	5 buds	×20*	HE	There was a statistically significant relationship ($P < 0.001$) between TB score in preoperative and postoperative samples	Satisfactory

AE1/AE3, Pancytokeratin marker AE1/AE3; CI, Confidence interval; CT, Chemotherapy; DFS, Disease free survival; FOM, floor of the mouth; HR, Hazard ratio; HE, haematoxylin and eosin staining; IHC, Immunohistochemically for cytokeratin (AE1/AE3); INF, Infiltrative pattern; LN, Lymph node; Ln5-γ2, Laminin 5-γ2; NA, Not available; OR, Odds ratio; OS, Overall survival; RFS, relapse-free survival; SCC; squamous cell carcinoma; TB, Tumour budding.

Categories for Morodomi *et al.* 1989 were as follows: 0–4 buds was considered negative (−), 5–14 was mildly positive (+), and 15 or more was strongly positive (++). Treatment in Nilsson *et al.* 2005 was radiotherapy with or without concomitant bleomycin. Surgery was used for poor responders. Field size in study of Guzinska-Ustymowicz, 2005 was 500 μm × 2500 μm square at 4 locations in each slide. Nakamura *et al.*[36]

×20 objective lens. 10 buds cutoff point: Low <10 buds/HPF ***vs***. High ≥10 buds/HPF. 5 buds cutoff point: Low (or negative) <5 buds ***vs***. High (or positive) ≥5 buds. 3 buds cutoff point: Low <3 buds ***vs***. High ≥3 buds. 1 bud cutoff point: Budding negative when no bud was observed, or budding positive when at least one bud was found.

*Ueno *et al.* 2002 and Ueno *et al.* 2004 are overlapped.

**Seki *et al.* 2016 and Seki *et al.* 2017 are overlapped.

that cytokeratin staining was useful in the evaluation of tumour budding by unexperienced pathologists, but for expert pathologists the benefit of using cytokeratin was only slight. In oral squamous cell carcinoma, evaluation of tumour budding using cytokeratin produced higher reproducibility than H&E staining, and interobserver variation was higher among less experienced examiners.[38] Indeed, while pan-cytokeratin staining can detect more tumour budding,[39] H&E staining has been used successfully in several studies with good reliability and reproducibility.[11,25,30] Thus, at present routine assessment of tumour budding with H&E staining can be used, while pan-cytokeratin staining may be considered in selected cases. For example, immunostaining could be used if there is a high density of inflammatory infiltrate preventing accurate evaluation of tumour budding.

A satisfactory biopsy is necessary for appropriate evaluation of tumour budding. A histopathological study on pre-operative biopsies in colorectal cancer reported that cases where at least three biopsies had been taken yielded satisfactory results in assessment of poorly differentiated tumour clusters of five cancer cells or more.[40] Such a procedure of multiple biopsies might be necessary for representative samples. However, excessive fragmentation of the specimens, artefacts, effects of tangential biopsies and the presence of extensive necrosis often reduce the quality of pretreatment diagnostic biopsies, and these can prevent a proper assessment of tumour budding. Interestingly, intratumoural budding (i.e. buds within the tumour centre) correlated significantly with peritumoural budding at the invasive front.[41,42] Diagnostic biopsies often do not include the invasive front, and it is also challenging to identify this area from small biopsies. Due to this fact, it seems more reliable to analyse intratumoural budding in these diagnostic specimens.[11]

In conclusion, our systematic review revealed that tumour budding could be successfully evaluated in diagnostic biopsies. The published studies had some limitations; they were mainly retrospective in nature and were commonly based on a single-institution experience. Due to heterogeneity between tumour types in the published studies, we were not able to perform meta-analyses. Therefore, the finding of our systematic review is still preliminary, and requires further validation and multicentre collaborative efforts. Of note, the finding was consistent between all eligible studies, indicating that tumour budding is an evaluable marker in diagnostic biopsy specimens and has a significant prognostic value. Thus, the current evidence summarised in our systematic review can be used as a starting-point for future research. Such research should aim to define distinctive criteria for assessment of tumour budding in diagnostic biopsies, and to be considered in therapeutic decision-making.

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Conflicts of interest

None declared.

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