

Keywords: colorectal cancer; tumour budding; prognosis; 5-year survival; lymph node positivity

Systematic review and meta-analysis of the impact of tumour budding in colorectal cancer

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Background: Tumour budding is a histological finding in epithelial cancers indicating an unfavourable phenotype. Previous studies have demonstrated that it is a negative prognostic indicator in colorectal cancer (CRC), and has been proposed as an additional factor to incorporate into staging protocols.

Methods: A systematic review of papers until March 2016 published on Embase, Medline, PubMed, PubMed Central and Cochrane databases pertaining to tumour budding in CRC was performed. Study end points were the presence of lymph node metastases, recurrence (local and distal) and 5-year cancer-related death.

Results: A total of 7821 patients from 34 papers were included, with a mean rate of tumour budding of $36.8 \pm 16.5\%$. Pooled analysis suggested that specimens exhibiting tumour budding were significantly associated with lymph node positivity (OR 4.94, 95% CI 3.96–6.17, $P < 0.00001$), more likely to develop disease recurrence over the time period (OR 5.50, 95% CI 3.64–8.29, $P < 0.00001$) and more likely to lead to cancer-related death at 5 years (OR 4.51, 95% CI 2.55–7.99, $P < 0.00001$).

Conclusions: Tumour budding in CRC is strongly predictive of lymph node metastases, recurrence and cancer-related death at 5 years, and its incorporation into the CRC staging algorithm will contribute to more effective risk stratification.

The TNM staging system for colorectal cancer (CRC) stratifies patients into stages, which influences their treatment pathway; this is especially true for rectal cancer where treatment ranges from local polyp resection to neoadjuvant chemoradiotherapy, surgery and adjuvant chemotherapy. However, there are many flaws with this staging system, including the prognostic heterogeneity of patients with cancers at the same stage (Merkel *et al*, 2001; Bori *et al*, 2009), as well as under- and overtreatment of patient subsets (Merkel *et al*, 2001; Nagtegaal *et al*, 2007; Schiffmann *et al*, 2013; Lea *et al*, 2014; Li *et al*, 2014a), and no superior method of staging and prognostication is currently validated (Gao *et al*, 2012). The future of CRC care will rely on accurate staging and prognostic algorithms, potentially with the addition of demographic, biochemical, morphological, molecular and treatment-related parameters to improve accuracy. One such parameter for inclusion is

tumour budding, a histological phenomenon in epithelial cancers when tumour cells or cell clusters migrate into the surrounding stroma by detaching from the invasive tumour front (Morodomi *et al*, 1989; Hase *et al*, 1993; Ueno *et al*, 2002b). It represents de-differentiation of epithelial cells into more invasive phenotypes in a process known as epithelial–mesenchymal transition (EMT; Kalluri and Weinberg, 2009).

Tumour budding in CRC has been shown significantly associated with lymphatic invasion (Morodomi *et al*, 1989; Okuyama *et al*, 2003a,b; Park *et al*, 2005; Losi *et al*, 2006; Choi *et al*, 2007; Ogawa *et al*, 2009; Wang *et al*, 2009), and accordingly has a well-established association with lymph node metastases, and is a negative prognostic indicator in terms of recurrence and overall survival (Ueno *et al*, 2002a, 2004; Lugli *et al*, 2009; Wang *et al*, 2009; Kevans *et al*, 2011; Petrelli *et al*, 2015). An association

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with distant metastases has also been documented (Nakamura *et al*, 2005; Suzuki *et al*, 2009).

Despite this, tumour budding has not been incorporated into the TNM staging of CRC, partly due to the disagreements in its definition and identification, with 'concerns over reproducibility of assessment, and the wide ranges of percentage of colorectal tumours reported to show budding in different studies' (Loughrey *et al*, 2014).

One other obstacle when trying to introduce budding in clinical practice is the fact that some methods for reporting are conceived for early stages (I–II) and some are for advanced disease (III); therefore, it is difficult to choose a method suitable for all stages.

The aim of the current study was to perform a systematic review and meta-analysis of the implications of tumour budding in operable CRC resection specimens on lymph node metastases, recurrence and cancer-related death.

MATERIALS AND METHODS

Search protocol. Original studies were searched for those that documented patients with surgically resected primary colorectal adenocarcinoma, where the specimens were assessed for the presence of tumour budding. The outcome measures chosen were lymph node metastases at resection, rates of recurrence and 5-year cancer-specific survival rates. Embase, Medline, PubMed, PubMed Central and Cochrane databases were searched using the following Boolean search term: budding AND (cancer OR carcinoma OR adenocarcinoma OR tumor OR tumour) for articles published up to March 2016. All search results were combined in a reference manager database (Endnote) and duplicates removed. Reference lists of included studies were also searched. The inclusion and exclusion criteria are shown in Figure 1.

Data extraction. Two independent reviewers applied the inclusion criteria to retrieved study abstracts and selected full papers for data analysis. These same two reviewers extracted data from full text papers and applied exclusion criteria in order to identify the final included studies; discrepancies were verified by consensus. If multiple publications reported results in the same population, the most recent report or most comprehensive data were chosen. For each study, data on baseline characteristics (author institution,

country, study period, total number of patients, sex, site of cancer, stage, histology, budding definition and methodology) were extracted. The number of patients exhibiting budding, those with co-existing lymph node metastases, merged rates of local or distant recurrence and 5-year cancer-related death rates were obtained where available. We described outcomes as odds ratios with 95% confidence intervals. Where these were not presented in a paper, we followed methods described by Parmar to extract them from Kaplan–Meier curves, or percentage survival. We contacted authors if data were not presented in a useable form.

Budding definition. There remains to be a consensus on the most accurate method of assessing tumour budding; currently there are variations in the cut-off for presence or absence of tumour budding – some groups use ≥ 1 budding focus to define the presence of tumour budding, whereas other groups use > 4 or > 9 foci. For this reason, the defined term was catalogued from each included paper and displayed in the results. In papers where two methods of identifying tumour budding were evaluated, the results of the standard method were used for analysis. For the analysis, budding was determined to be either present or absent. Where budding was graded into groups (e.g., mild/moderate/severe), the results for the mild/moderate groups were combined and compared with the severe or highest grade group.

Quality analysis. This meta-analysis was conducted in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines. We measured the quality of the studies on the basis of the Newcastle–Ottawa Scale that assesses the methodological quality of non-randomised cohort studies for meta-analysis. The studies were judged by two independent assessors using a nine-point scale comprising analysis on the selection of the study group, the comparability of cohorts and the ascertainment of outcome. Scores above 6 points were taken to denote studies of high methodological quality.

Statistical analysis. All analyses were conducted with the RevMan statistical package (Review Manager (RevMan) Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Heterogeneity between studies for each of the outcome measures was tested with Cochran's Q test. The I^2 statistic was calculated for an objective measure of heterogeneity. A fixed-effects meta-analysis was performed in all cases, and where there was appreciable heterogeneity ($I^2 > 50\%$ or chi-squared P -values < 0.10) a random-effects model was also used for meta-analysis. Corresponding funnel plots of Ln standard error as a function of effect size were used to examine the effect of publication bias visually, and were statistically tested using Eggers test. P -values > 0.05 were indicative of no publication bias. For meta-analysis, Mantel–Haenszel odds ratios for lymph node metastasis, recurrence and 5-year cancer-related death were extracted and described with 95% confidence intervals. Sensitivity analysis was performed to identify if any methodological features were indicative of heterogeneity among studies. Studies were excluded if they had poor methodological quality (Newcastle–Ottawa scores < 7), where the definitions of tumour budding were unclear or not predetermined in the Methods section, or where the rate of tumour budding in the study was outside of the mean rate of tumour budding among all studies ± 1 s.d. from the mean. Subgroup analysis was also carried out to compare whether tumour location (colon or rectum), T stage (some studies only included T1 and T2 tumours) and lymph node status affected results.

RESULTS

Search results. The literature search revealed 2728 publications (Figure 2). Of the 143 included for full text review, 34 were

Inclusion criteria
Original publication (reviews, opinions, letters, protocols and conference proceedings excluded)
Case–control or cohort studies with > 50 patients
Patients with primary colorectal adenocarcinoma who underwent primary surgical resection without prior neoadjuvant therapy or endoscopic resection
Reported histopathological lymph node metastasis or outcome measures of recurrence or survival
Exclusion criteria
Papers in languages other than English
Papers where data were unavailable or uninterpretable and authors uncontactable

Figure 1. Study inclusion and exclusion criteria.

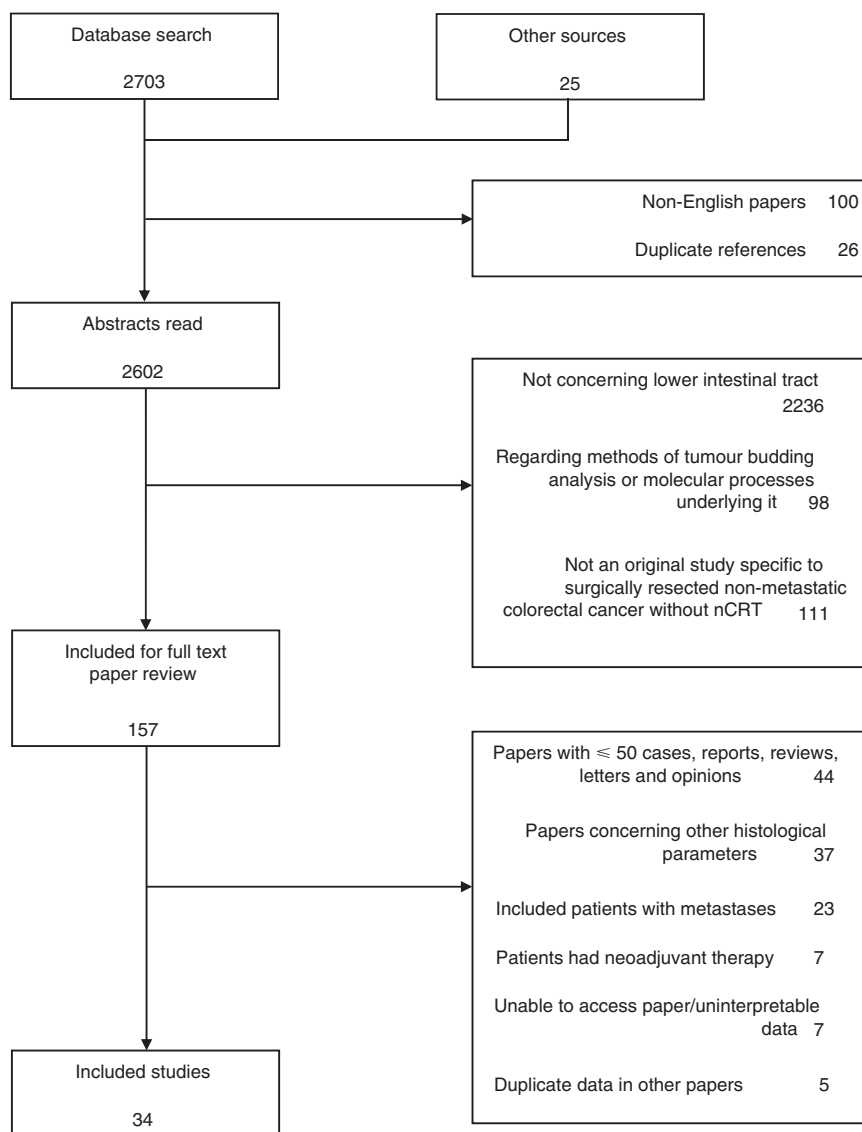


Figure 2. Flow diagram of the systematic review and meta-analysis process.

ultimately included in the meta-analysis (Hase *et al*, 1993; Ueno *et al*, 2002a; Okuyama *et al*, 2003a,b; Ueno *et al*, 2004; Prall *et al*, 2005; Wang *et al*, 2005; Yasuda *et al*, 2007; Kanazawa *et al*, 2008; Nakamura *et al*, 2008; Yamauchi *et al*, 2008; Lugli *et al*, 2009; Ogawa *et al*, 2009; Wang *et al*, 2009; Homma *et al*, 2010; Kajiwara *et al*, 2010; Komori *et al*, 2010; Tateishi *et al*, 2010; Akishima-Fukasawa *et al*, 2011; Reggiani Bonetti *et al*, 2011; Betge *et al*, 2012; Nakadoi *et al*, 2012; Sert Bektas *et al*, 2012; Zlobec *et al*, 2012; Wada *et al*, 2013; Huh *et al*, 2014; Lai *et al*, 2014; Nishida *et al*, 2014; Ryu *et al*, 2014; Gilardoni *et al*, 2015; Macias-Garcia *et al*, 2015; Miyachi *et al*, 2015; Tristante *et al*, 2015; Barresi *et al*, 2016). There were a total of 7821 patients included for analysis, with study groups ranging from 58 to 1114 patients. All studies were conducted in Europe or Asia. The main characteristics of eligible studies are characterised in Table 1. Most studies were retrospective and included patients with colon and rectal cancers from a mixture of stages. One study included patients with metastases at diagnosis (Kanazawa *et al*, 2008); these patients were excluded for analysis of disease recurrence and so the non-metastatic data are included here. There were 25 studies that correlated tumour budding status with lymph node metastases in the specimen, 12 that examined recurrence rates, and 9 that

documented 5-year cancer-related death rates. The mean rate of tumour budding was $36.8 \pm 16.5\%$.

Quality of studies. The Newcastle–Ottawa scaling method assessed the methodological quality of the studies and scores are outlined in Table 1, with an overview of the scoring system method in Supplementary Material 1 and raw scores in Supplementary Material 2. The majority (81.6%) of studies had quality scores ≥ 7 , indicating good methodological quality of included studies, and four studies (15.6%; Homma *et al*, 2010; Reggiani Bonetti *et al*, 2011; Sert Bektas *et al*, 2012; Lai *et al*, 2014) had acceptable scores of 6.

Definitions of tumour budding. There were considerable differences and an overt lack of standardisation of tumour budding between studies (Table 2). Twenty-eight studies used standard haematoxylin and eosin (H&E) staining, four studies used immunohistochemistry (IHC) and cytokeratin staining (Prall *et al*, 2005; Lugli *et al*, 2009; Ogawa *et al*, 2009; Zlobec *et al*, 2012), and two did not specify the technique (Gilardoni *et al*, 2015; Macias-Garcia *et al*, 2015), although one could assume standard H&E was used. A ‘budding focus’ or its equivalent was defined as an isolated cancer cell or cluster of less than five cells in 24 studies.

Table 1. Overview of included studies

Author	Year	Country	Study period	Site of cancer	No. of patients	AJCC stage				Newcastle–Ottawa Score
						I	II	III	IV	
Hase	1993	Japan	1970–1985	CR	663	✓	✓	✓		7
Ueno	2002	Japan	1981–1994	R	437			✓		7
Okuyama	2003	Japan	1985–1997	C	196		✓	✓		8
Okuyama	2003	Japan	1985–1997	R	83		✓	✓		7
Ueno	2004	Japan	1960–1980	R	1114	✓	✓	✓		9
Prall	2005	Germany	1994–1999	CR	182	✓	✓			8
Wang	2005	China	1969–2002	CR	159	✓		✓		8
Yasuda	2007	Japan	NA	CR	86	✓		✓		7
Kanazawa	2008	Japan	1996–2001	CR	133		✓	✓	✓	7
Nakamura	2008	Japan	1986–1998	C	200		✓			7
Yamauchi	2008	Japan	1991–2001	CR	164	✓		✓		8
Lugli	2009	Switzerland	1987–1996	CR	273	✓	✓	✓		7
Ogawa	2009	Japan	1995–2003	CR	83	✓		✓		8
Wang	2009	Ireland	1990–2004	CR	128		✓			9
Homma	2010	Japan	2000–2007	R	65	✓		✓		6
Kajiwara	2010	Japan	1985–2005	CR	244	✓		✓		8
Komori	2010	Japan	1990–2004	CR	111	✓		✓		8
Tateishi	2010	Japan	1992–2005	CR	322	✓		✓		8
Akishima-Fukasawa	2011	Japan	1989–2009	CR	111	✓		✓		7
Reggiani Bonetti	2011	Italy	1989–2004	CR	95	✓		✓		6
Betge	2012	Austria	1984–2005	CR	110		✓			8
Nakadoi	2012	Japan	1981–2008	CR	499	✓		✓		8
Sert Bektas	2012	Turkey	2003–2007	CR	73	✓	✓	✓		6
Wada	2013	Japan	1995–2005	CR	120	✓		✓		8
Zlobec	2012	Switzerland	1987–1996	CR	127	✓	✓	✓		8
Nishida	2014	Japan	2000–2011	CR	265	✓		✓		8
Huh	2014	Korea	2007–2009	CR	543	✓		✓		8
Lai	2014	China	1999–2007	C	135		✓			6
Ryu	2014	Korea	2003–2012	CR	179	✓		✓		8
Gilardoni	2015	Italy	2006–2009	C	196	✓	✓			8
Macias-Garcia	2015	Spain	2000–2011	CR	97	✓				8
Tristante	2015	Spain	2011–2014	CR	58		✓	✓		4
Barresi	2016	Italy	NA	CR	82		✓			7
Miyachi	2016	Japan	2001–2014	CR	653	✓				7

Abbreviations: C = colon only; CR = colon and rectum; NA = not available; R = rectum only.

The remaining studies were either unclear about the definition of a budding focus (Yasuda *et al*, 2007; Kanazawa *et al*, 2008; Nakamura *et al*, 2008) or defined it as an isolated cancer cell or cluster of less than six cells (Okuyama *et al*, 2003a; Lugli *et al*, 2009; Reggiani Bonetti *et al*, 2011; Nakadoi *et al*, 2012; Zlobec *et al*, 2012), or a cluster of greater than four cancer cells (Okuyama *et al*, 2003b; Wang *et al*, 2005). Tumour budding was then defined according to the number of budding foci in the majority of studies; there was some heterogeneity with the classifications between studies. Ten studies identified tumour budding positivity if there were >4 budding foci present in the specimen. Other studies used >9 budding foci (eight studies), ≥1 focus (five studies), >5 foci (three studies), >10, >16 or >24 foci (one study each). Three studies did not quantify the number of budding foci, but graded tumour budding as mild, moderate or severe, depending on

subjective opinion (Hase *et al*, 1993) or the proportion of the tumour margin involved by budding (Kanazawa *et al*, 2008; Nakamura *et al*, 2008). For these studies, the severe group data were compared with mild and moderate (Kanazawa *et al*, 2008; Nakamura *et al*, 2008). One study was unclear as to the methodology used to determine tumour budding (Homma *et al*, 2010).

Meta-analysis results

Lymph node metastasis. There were 25 included studies that compared the rate of tumour budding to regional lymph node metastases in the resected specimens, involving 6724 patients. The pooled random-effects analysis suggested that specimens exhibiting tumour budding were significantly associated with lymph node positivity (OR 4.94, 95% CI 3.96–6.17,

Table 2. Methods of defining tumour budding among included studies

Author	Year	Staining method	Definition of budding focus	Tumour budding method
Hase	1993	H&E	Cluster of <5 cancer cells	Graded into two groups: none/mild or moderate/severe at invasive front
Ueno	2002	H&E	Cluster of <5 cancer cells	> 5 Budding foci
Okuyama	2002	H&E	Cluster of <6 cancer cells	≥ 1 Budding focus (Morodomi)
Okuyama	2003	H&E	Cluster of >4 cancer cells	≥ 1 Budding focus
Ueno	2004	H&E	Cluster of <5 cancer cells	> 9 Budding foci
Prall	2005	Cytokeratin/IHC	Cluster of <5 cancer cells	> 24 Budding foci
Wang	2005	H&E	Cluster of > 4 cancer cells	≥ 1 Budding focus (Morodomi)
Yasuda	2007	H&E	Cluster of an unspecified number of cancer cells	≥ 1 Budding focus (Hase)
Kanazawa	2008	H&E	Cluster of an unspecified number of cancer cells	Mild: < 1/3 invasive margin Moderate: 1/3–2/3 invasive margin Severe: > 2/3 invasive margin
Nakamura	2008	H&E	Cluster of an unspecified number of cancer cells	As per Kanazawa above
Yamauchi	2008	H&E	Cluster of <5 cancer cells	> 4 Budding foci (Ueno modified)
Lugli	2009	Cytokeratin/IHC	Cluster of <6 cancer cells	> 16 Buds per hpf
Ogawa	2009	Cytokeratin/IHC	Cluster of <5 cancer cells	> 9 Budding foci
Wang	2009	H&E	Cluster of <5 cancer cells	≥ 1 Budding focus
Homma	2010	H&E	Cluster of <5 cancer cells	Graded none, mild, mod, severe but did not delineate what comprised each
Kajiwara	2010	H&E	Cluster of <5 cancer cells	> 9 Budding foci
Komori	2010	H&E	Cluster of <5 cancer cells	> 4 Budding foci (Ueno modified)
Tateishi	2010	H&E	Cluster of <5 cancer cells	≥ 1 Budding focus (Ueno)
Akishima-Fukasawa	2011	H&E	Cluster of <5 cancer cells	> 4 Budding foci (Ueno)
Reggiani Bonetti	2011	H&E	Cluster of <6 cancer cells	> 4 Budding foci (Ueno)
Betge	2012	H&E	Cluster of <5 cancer cells	> 9 Budding foci (Ueno modified)
Nakadoi	2012	H&E	Cluster of <6 cancer cells	> 4 Budding foci (Ueno modified)
Sert Bektas	2012	H&E	Cluster of <5 cancer cells	> 9 Budding foci (Ueno modified)
Wada	2013	H&E	Cluster of <5 cancer cells	> 4 Budding foci (Ueno)
Zlobec	2012	Cytokeratin/IHC	Cluster of <6 cancer cells	> 5 Budding foci
Nishida	2014	H&E	Cluster of <5 cancer cells	> 4 Budding foci (Ueno)
Huh	2014	H&E	Cluster of <5 cancer cells	> 5 Budding foci
Lai	2014	H&E	Cluster of <5 cancer cells	> 9 Budding foci (Ueno modified)
Ryu	2014	H&E	Cluster of <5 cancer cells	> 4 Budding foci (Ueno)
Gilardoni	2014	NS	Cluster of <5 cancer cells	> 4 Budding foci (Ueno)
Macias-Garcia	2014	NS	Cluster of <5 cancer cells	> 10 Budding foci (Ueno modified)
Tristante	2014	NS	NS	NS
Barresi	2016	H&E	Cluster of <5 cancer cells	> 4 Budding foci (Ueno)
Miyachi	2015	H&E	Cluster of <5 cancer cells	> 4 Budding foci (Ueno)

Abbreviations: H&E = haematoxylin and eosin; IHC = Immunohistochemistry; NS, not significant.

$P < 0.00001$; Figure 3). There was moderate heterogeneity between the studies ($I^2 = 53\%$) and thus a random-effects model is shown. When sensitivity analysis was performed, omission of those studies with Newcastle–Ottawa quality scores < 7 , those with an unclear definition of tumour budding or those which had overall rates of tumour budding outside of 1 s.d. of the mean tumour budding rate did not alter the overall effect of tumour budding on lymph node metastasis or affect heterogeneity (OR 4.90, CI 3.90–6.16, $I^2 = 55\%$, OR 5.13, CI 4.02–6.53, $I^2 = 52\%$ and OR 3.98, CI 2.96–5.35, $I^2 = 54\%$, respectively). Subgroup analysis of studies by tumour location improved homogeneity; those that included rectal cancer only had an I^2 of 0%, with a fixed-effects model OR remaining significant at 5.36, CI 4.25–6.75. Only one study in this group included colon cancer only. The remaining studies did not differentiate

between colon and rectal cancers and had an OR for lymph node metastasis similar to the overall study group (OR 4.83, CI 3.61–6.45, $I^2 = 59\%$). Fourteen studies only examined lymph node metastases in T1 and T2 tumours, this group was also heterogeneous ($I^2 = 52\%$) with an OR of 5.67, CI 3.92–8.21. Conversely, the 11 studies that did not differentiate between T stage were also heterogeneous with $I^2 = 58\%$ and OR 4.52, CI 3.39–6.02.

Local or distant recurrence. Twelve studies compared the rate of tumour budding with local or distant recurrence, involving 2773 patients. The pooled random-effects analysis suggested that specimens exhibiting tumour budding were significantly more likely to develop disease recurrence over the time period (OR 5.50, 95% CI 3.64–8.29, $P < 0.00001$; Figure 4). There was moderate

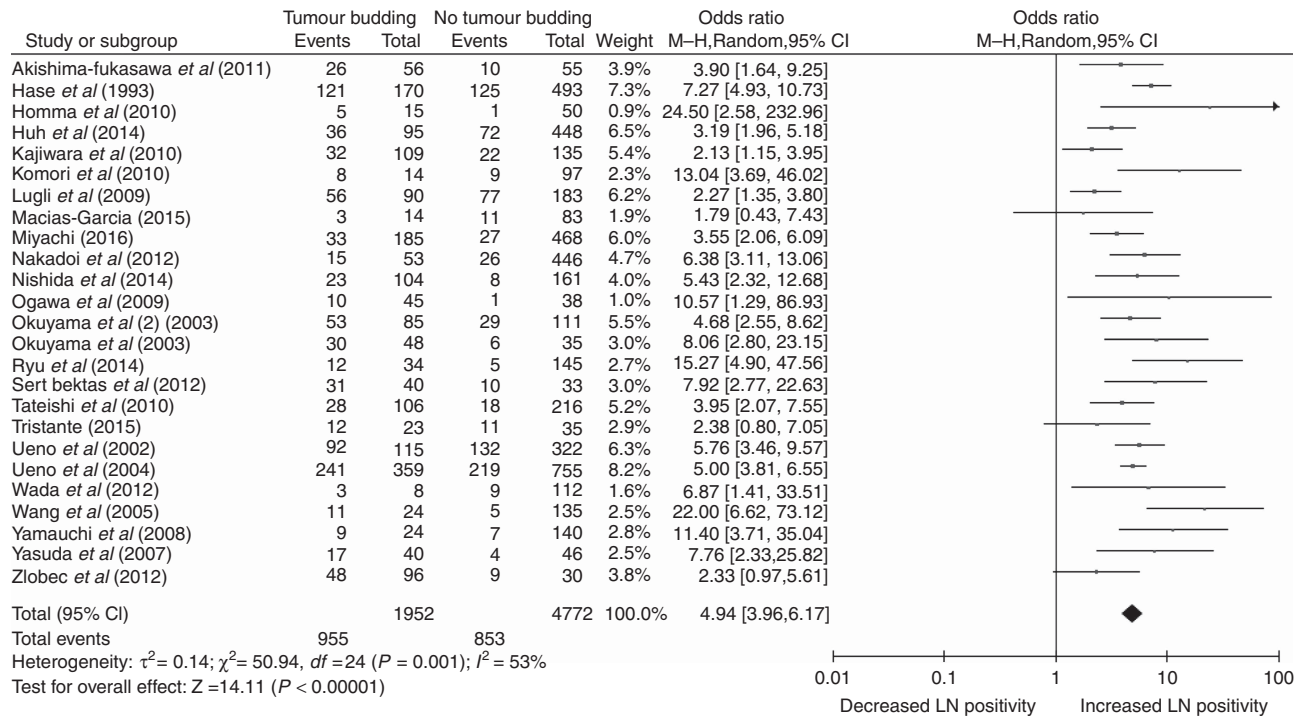


Figure 3. The association of tumour budding with lymph node metastasis in resected colorectal cancer.

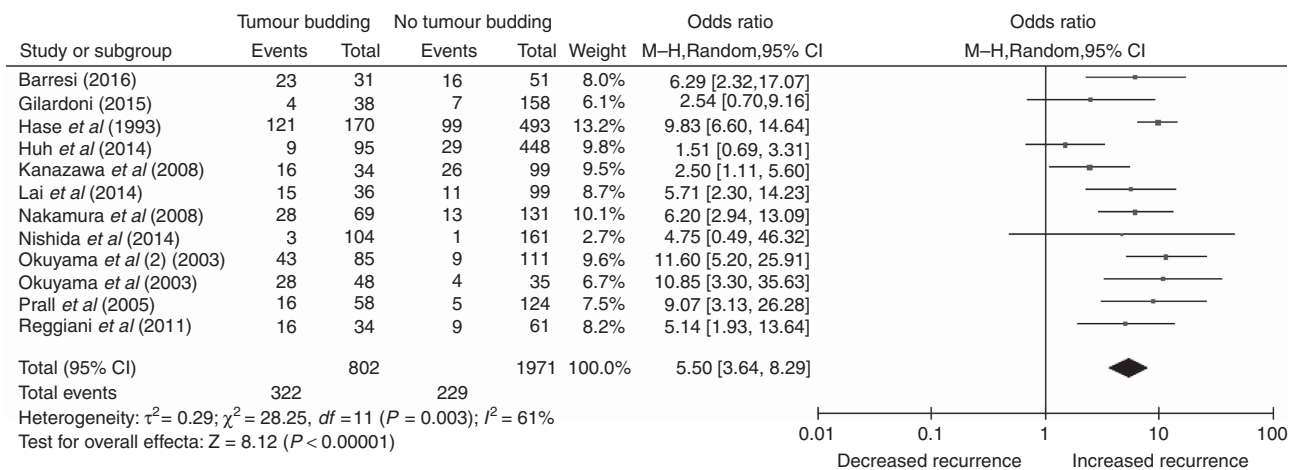


Figure 4. The association of tumour budding with local or distal recurrence in resected colorectal cancer.

heterogeneity between the studies ($I^2 = 61\%$). Sensitivity analysis with omission of studies of poor methodological quality or those with unclear definitions of tumour budding did not change the effect of tumour budding on disease recurrence or affect heterogeneity results (OR 5.48, CI 3.35–8.97, $I^2 = 68\%$ and OR 5.01, CI 2.88–8.73, $I^2 = 65\%$). Exclusion of studies with outlying rates of overall tumour budding did not improve heterogeneity with an I^2 of 56%, and an OR of 4.54, CI 2.75–7.49. Subgroup analysis by tumour location could not be performed, as only one included study focussed on rectal cancer in relation to recurrence. In the group that examined recurrence in T1/T2 tumours only, three studies had a heterogeneous I^2 of 50%, OR 2.87, CI 1.12–7.35. The remaining nine studies did not stratify by T stage and displayed improved homogeneity ($I^2 = 42\%$) and a fixed effects OR of 7.41, CI 5.77–9.50. In the four studies that only examined recurrence in node negative resections, the fixed-effects model gave an OR of 6.57, CI 4.18–10.32, $I^2 = 0\%$. The remaining seven studies

included both node negative and node positive disease, and were heterogeneous ($I^2 = 75\%$), OR 4.91, CI 2.63–9.17.

Five-year cancer-related death rates. The rate of tumour budding with 5-year cancer-related death was documented in nine studies, involving 2234 patients. When analysed using a pooled random-effects model, the results demonstrate that CRCs with tumour budding were significantly more likely to lead to cancer-related death at 5 years (OR 4.51, 95% CI 2.55–7.99, $P < 0.00001$; Figure 5). There was significant heterogeneity between the studies ($I^2 = 78\%$) and thus a random-effects model is shown. Sensitivity analysis excluding studies with Newcastle–Ottawa quality scores < 7 and those with unclear criteria for tumour budding did not change the overall effect of tumour budding on 5-year cancer-related death rates or on heterogeneity between studies (OR 4.39, CI 2.32–8.30, $I^2 = 80\%$ and OR 3.58, CI 1.61–7.99, $I^2 = 85\%$). Including only those studies with rates of tumour

budding within 1 s.d. of the overall mean rate improved I^2 to 0%, with a fixed-effects model OR of 7.43, CI 5.84–9.45. Subgroup analysis by tumour location included two studies examining tumour budding with relation to 5-year cancer-related death in rectal cancer, OR 6.97, CI 5.29–9.19, $I^2=0\%$. Three studies included only colon cancers for analysis, OR 7.71, CI 4.46–13.33, $I^2=6\%$. The remaining four studies did not differentiate between colon and rectal cancers and were heterogeneous, $I^2=82\%$, OR 2.59, CI 0.85–7.92. Only one study examined 5-year cancer-related death in those with early (T1/T2) tumours, but when this was excluded, the remaining studies were homogenous ($I^2=0\%$) with the fixed-effects model giving a significant OR of 6.50, CI 5.19–8.14. Where node negative tumours only were included, the four studies were homogenous ($I^2=0\%$), with an OR of 5.43, CI 3.31–8.91, on the fixed-effects model. The remaining studies included node negative and positive disease and were heterogeneous ($I^2=89\%$), OR 3.86, CI 1.43–10.41.

Publication bias. Funnel plots were created for each outcome measure. The shapes of the funnel plots and the statistical tests revealed no evidence of bias in any of the three groups (P -values for lymph node metastasis, recurrence and 5-year cancer-related death were 0.103, 0.402 and 0.363, respectively; Figure 6).

DISCUSSION

The meta-analysis demonstrates that the histopathologic finding of tumour budding in resected CRC specimens is associated with concurrent lymph node metastases, disease recurrence and cancer-related death, despite variations in tumour budding definition between included studies. Tumour budding appears to indicate an aggressive phenotype, independent of staging according to the current TNM guidelines. Personalised patient care is an emerging concept in the future of cancer treatment (Piccart, 2013; Wazir and Mokbel, 2014; Jackson and Chester, 2015), and incorporation of tumour budding into the staging of CRC could assist in tailoring the management of currently contentious subgroups (Koelzer *et al*, 2016). Tumour budding may indicate early colorectal tumours requiring more rigorous treatment approaches, or its absence may aid decision-making in later stage cancers.

At present there is no proven benefit in treating early stage CRC patients with chemotherapy (Quasar Collaborative Group *et al*, 2007; Tournigand *et al*, 2012); however, we know there are subsets of stage I and II CRC who have poor prognostic outcomes. Although the current results suggest that tumour budding identifies high-risk early stage CRCs, it remains to be determined whether its presence indicates the need for further treatment in this

subset. Clinical trials stratifying patients according to tumour budding status may be warranted.

Studies have also looked at tumour budding in other epithelial cancers, demonstrating a negative prognostic impact in oesophageal (Koike *et al*, 2008; Brown *et al*, 2010), breast (Salhia *et al*, 2015), pancreatic (Karamitopoulou *et al*, 2013) and lung (Masuda *et al*, 2012) carcinomas, but as yet, tumour budding remains a non-core component of colorectal adenocarcinoma pathologic staging (Koelzer *et al*, 2016) and is not formally part of staging of other cancers. Most studies advocate its identification using standard pathologic processing methods (H&E, light microscopy), with no extra staining or pathological processing required – thus this is potentially a cost-effective addition to current staging protocols. Cytokeratin staining appears to assist tumour budding detection and may be worthwhile investigating further (Suzuki *et al*, 2009; Puppa *et al*, 2012). Interestingly, although studies vary in their definition of how many cells constitute a tumour bud, and how many buds represent budding, and with considerable inter-observer variability in its reporting, most studies definitively demonstrate that the finding is a strong negative prognostic marker in CRC. A recent systematic review summarises methods of identifying tumour budding and their relationship to prognosticating CRC and surmises that despite differences in methodologies between studies, ‘most methods, if practised with care and some training, will yield relevant prognostic information as high-degree tumour budding places a patient at a significant risk for an adverse outcome’ (van Wyk *et al*, 2015). However, it is clear that the definition needs standardisation, and for this, more prospective trials need to be designed to assess its reproducibility and inter-observer variation.

As the search herein was performed, other studies have also supported a negative prognostic role of tumour budding in CRC (Barresi *et al*, 2014; Graham *et al*, 2015). Petrelli *et al* (2015) recently undertook a systematic review of tumour budding and its relation to survival limited to stage II CRC. Their meta-analysis includes 10 studies and compares favourably with ours in that it also demonstrates a significantly increased OR for death at 5 years (OR 6.25, 95% CI 4.04–9.67, $P<0.00001$).

The results herein strongly demonstrate that tumour budding is a negative prognostic indicator in almost 7000 patients with CRC – these results remain robust even with sensitivity and subgroup analyses. Interestingly, when sensitivity analysis was performed only to include those studies with a percentage finding of tumour budding within 1 s.d. above and below the mean for all patients, the heterogeneity on the studies significantly decreased. This provides further evidence that standardisation of the definition and characterisation is imperative in the future of CRC staging.

Staging of CRC using the TNM system is notoriously inaccurate for prognosticating patient subsets, and thus many other

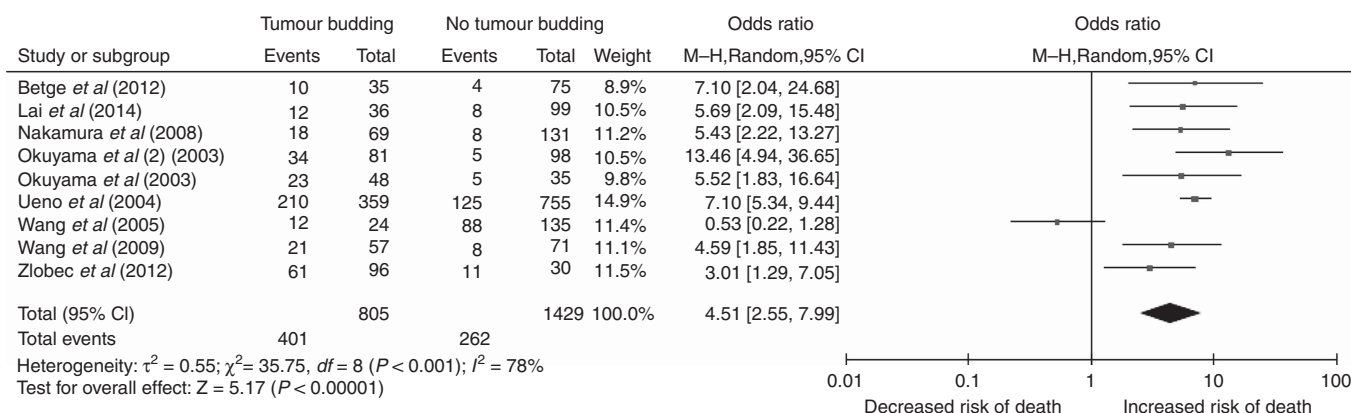


Figure 5. The association of tumour budding with 5-year cancer-related death in resected colorectal cancer.

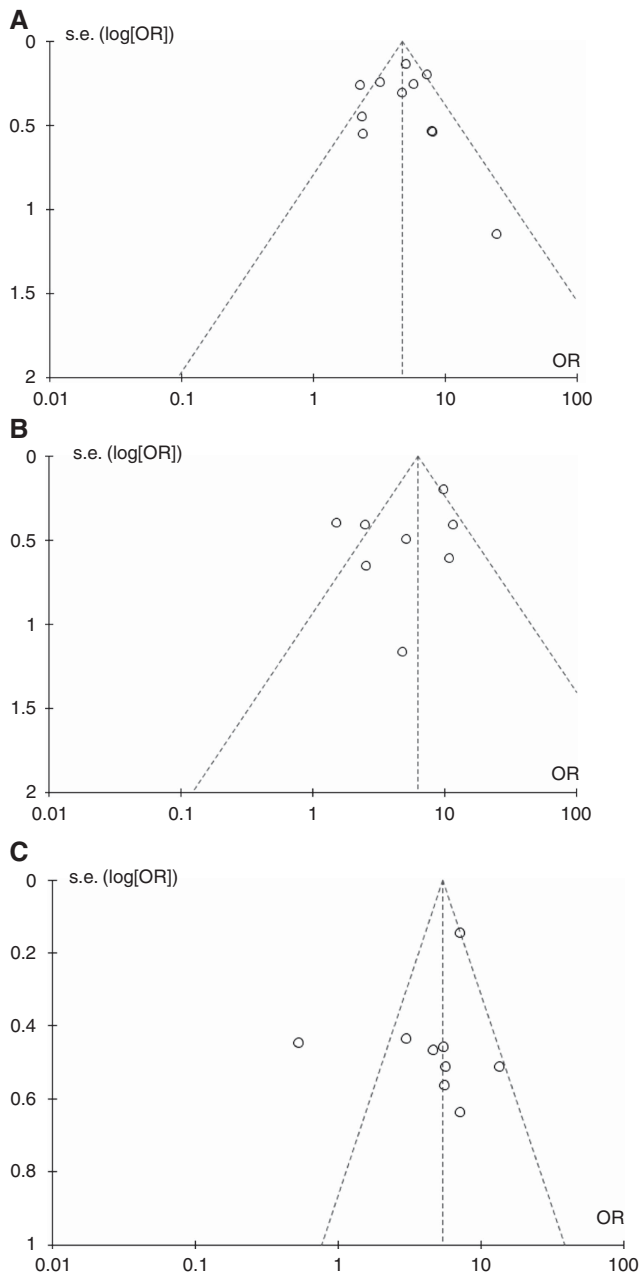


Figure 6. Funnel plots for visual inspection of publication bias. Each point represents a standardised comparison of a separate study, comparing the outcome effect (OR) with the standard error of its logarithm (SE(log[OR])). (A) Lymph node metastases, (B) recurrence, (C) 5-year cancer-related death.

prognostic markers have been studied. These include circulating and tumour markers such as cyclin D1 expression, CXCR4, VEGF, microRNA-21, survivin, CDKN2A hypermethylation, BRAF and K-ras mutation, as well as the findings of microsatellite instability, mucinous tumour phenotype and a high neutrophil–lymphocyte ratio (Guastadisegni *et al*, 2010; Chen *et al*, 2012; Safaee Ardekani *et al*, 2012; Verhulst *et al*, 2012; Huang *et al*, 2013; Xia *et al*, 2013; Xing *et al*, 2013; Lv *et al*, 2014; Wang *et al*, 2014; Li *et al*, 2014b,c; Rui *et al*, 2015). Despite the fact that all are negative prognostic markers for overall survival in CRC, none have pooled hazard or odds ratios > 2 on systematic review. Even more well-established prognostic indicators such as perineural invasion and high lymph node ratio are associated with relative risks for poorer overall survival of < 2.5 (Ceelen *et al*, 2010; Knijn *et al*, 2015). The results

of the current study demonstrate that tumour budding has an effect on survival that is markedly worse than any other previously studied pathological phenotype, with an OR of 4.5 for cancer-related death at 5 years in those exhibiting tumour budding. These findings support its inclusion into CRC pathologic staging as a marker of a more aggressive disease phenotype.

Early evidence suggests that tumours exhibiting budding do not respond to neoadjuvant chemotherapy (Bhangu *et al*, 2014; Rogers *et al*, 2014; Sannier *et al*, 2014). In this study, we excluded rectal cancers undergoing neoadjuvant chemoradiotherapy (nCRT). Although this subset of included rectal tumours are likely to be less advanced, we felt that nCRT inclusion may weaken the data, and a further meta-analysis of this subset is warranted when more results are available. It remains to be determined whether tumour budding should be seen as an indicator for a primary surgical approach in rectal cancer and foregoing nCRT as these patients are less likely to respond; more data are required to elucidate this point. However, those tumours exhibiting budding certainly are worth considering as a subset to target with adjuvant chemotherapy in CRC as a whole.

Tumour budding in CRC is strongly predictive of lymph node metastases, recurrence and cancer-related death at 5 years. Incorporation of this histological finding into the CRC staging algorithm is imminent, but will require standardisation of the pathological description of tumour budding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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