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The prognostic role of tumor budding and tumor-stroma ratio in pulmonary metastasis of colorectal carcinoma

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ABSTRACT

Objective: To evaluate the prognostic value of tumor budding and tumor-stroma ratio (TSR) in resected pulmonary metastases of colorectal carcinoma (CRC).
Methods: In total, 106 pulmonary metastasectomies were performed to 74 patients in two study hospitals during 2000–2020. All relevant clinical data were retrospectively collected. Tumor budding based on the International Tumor Budding Consensus Conference recommendations and TSR in the first resected pulmonary metastases and primary tumors were evaluated from diagnostic hematoxylin-eosin-stained histopathological slides.
Results: 60 patients (85.7%) had low tumor budding (≤5 buds/field) and 10 patients (14.3%) had high tumor budding (>5 buds/field) in their first pulmonary metastases of CRC. 5-year overall survival rates of pulmonary metastasectomy in low and high total tumor budding were 28.3% and 37.3% ($p = 0.387$), respectively. 19 patients (27.1%) had low TSR and 51 patients (72.9%) had high TSR. The 5-year overall survival rates were 32.9% in low and 28.6% in high TSR of first pulmonary metastases ($p = 0.746$). Tumor budding and TSR did not provide prognostic value in Cox multivariate analysis. Tumor budding and TSR in resected pulmonary metastases were not associated with those of the primary tumor.
Conclusion: Tumor budding and TSR in the resected pulmonary metastases of CRC showed no statistically significant prognostic value, however, additional well-powered confirmatory studies are needed.

1. Introduction

Colorectal cancer (CRC) is one of the most common malignancies globally and it is the third leading cause of cancer death worldwide [1]. In Finland, the CRC incidence rate in 2020 was 65.5 cases per 100 000 and the 5-year age-adjusted overall survival rate of all stages was 68% [2]. About 10% of patients have synchronous pulmonary metastases and around 5% of patients have disease

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recurrence with pulmonary metastases within 5 years after treatment of primary CRC [3]. While the 5-year overall survival of CRC in all stages is over 60%, patients with a stage IV CRC at the time of diagnosis have a 5-year survival of only 14% [4]. For decades, cancer staging has relied on anatomy based TNM staging. A need for additional classification systems is recognized as patient survival within TNM-stages varies significantly [5].

Tumor budding is defined as single tumor cell, or 2–4 cell clusters separate from the main tumor bulk. It is proposed to represent epithelial-mesenchymal transition (EMT) in cancer progression and is associated with a strong invasive capacity and poor survival in CRC [6–8]. Tumor budding is included as an additional prognostic factor in the AJCC/UICC cancer staging guidelines [9,10]. In metastatic CRC, tumor budding has been proven a negative prognostic marker in liver metastases in univariate models [11,12], while it has not yet been evaluated in pulmonary metastases.

The tumor microenvironment plays an important part in cancer progression [13]. Stroma is an essential component of the tumor microenvironment and is largely regulated by cancer-associated fibroblasts [14], which have also been linked to tumor budding [15]. Tumor-stroma ratio (TSR) is defined as the percentage of tumor stromal area relative to total tumor area (tumor cells and stroma) and it has been proven as a significant prognostic marker in solid tumors [16]. In CRC, TSR is a robust prognostic marker in the primary tumor [17]. TSR has not yet been evaluated in the pulmonary metastases of CRC.

The objective of this study was to evaluate the prognostic effect of tumor budding and TSR in pulmonary metastases of CRC, including a comparison between primary tumor and metastases.

2. Material and methods

2.1. Study design

All patients with histologically confirmed pulmonary metastases from CRC operated in Oulu University Hospital and Central Finland Central Hospital during 2000–2020 were included in the study. This was a population-based retrospective study. The study hospitals are the only hospitals offering thoracic surgery in the districts. A total of 106 pulmonary metastasectomies from CRC were performed on 74 patients during the study period in the study hospitals. Patients were considered for pulmonary metastasectomy if surgical resection was evaluated to offer curative treatment.

Patients were identified from the archives using surgical registries and pathology reports. All relevant clinical data were retrospectively collected from electronic patient record systems used in the study hospitals. Tumor classification was updated to the American Joint Committee on Cancer (AJCC) 8th edition of tumor-node-metastasis (TNM) classification [18]. Survival data until December 31, 2021 was received from Statistics Finland. The follow-up data was 100% complete.

Prospectively collected diagnostic hematoxylin-eosin-stained histopathological slides of the primary CRC tumor and pulmonary metastases were retrieved from pathology archives and reviewed by a histopathologist (V-P.M). The most representative slide was selected for further analysis in pulmonary metastases. In the primary tumors, the sample with the deepest invasion depth was selected for further analysis. The slides were digitalized using an Aperio digital scanner AT2 Console (Leica Biosystems Imaging Inc., Wetzlar, Germany).

2.2. Histopathological examination

The tumor budding and TSR evaluation were performed by 2 independent researchers (T.K and N.K) blinded to the clinical data. Tumor budding was quantified using the hotspot method recommended in the consensus article [8]. A bud was defined as a 1–4 tumor cell cluster surrounded by stroma and detached from the tumor bulk (Fig. 1). The buds were identified with an x20 magnification and counted in a field of view of 0.785 mm^2 using QuPath [19]. Tumor budding was counted separately for peritumoral budding (PTB) in the invasive margin and for intratumoral budding (ITB) in the tumor center of the tumor. Total tumor budding (TTB) was calculated so that the greater of PTB or ITB counts was picked for scoring. The mean of the budding counts between observers was used for scoring.

TSR was analyzed from the same hematoxylin-eosin-stained slides that were used for tumor budding analysis. TSR evaluation was blinded to the tumor budding results. TSR was evaluated according to the recommendation article [17]. A 3.8 mm^2 area with a maximum amount of stroma in relation to tumor cells was selected for evaluation so that tumor cells were present in the corners of the selected field as illustrated in Fig. 1. The amount of stroma tissue was estimated per 10% increment per image field using QuPath. Necrotic tissue, smooth muscle tissue, large blood vessels were avoided in the field selection and if unavoidable it was ignored in scoring. The mean of the TSR values between observers was used for scoring.

Mismatch repair (MMR) and *BRAF* mutation status was determined by immunohistochemical analysis from the pulmonary metastases as described previously [20]. Kristen rat sarcoma virus (*KRAS*) and neuroblastoma rat sarcoma virus (*NRAS*) status was obtained from clinical data. Tumor regression grading was evaluated using the modified Dworak system [21] by a histopathologist (V-M.P).

2.3. Scoring

The median count of PTB, ITB and TTB was 1, 1.5, and 2 in all metastases and 3.25, 1.25 and 4.25 in primary tumor, respectively. A two-tiered scoring (low <5 buds vs. high budding ≥5 buds) was performed based on International Tumor Budding Consensus Conference (ITBCC) recommendations [8], however the intermediate (5–9 buds) and high (over 10 buds) budding groups were combined due to small group sizes in both metastases and primary tumors. Additionally, as the ITBCC recommendations are primarily conducted for primary CRC tumors, an additional two-tiered tumor budding scoring in pulmonary metastases was performed with a cut-off selected using the receiver operating characteristics (ROC) curve, where the cut-offs were 1.25, 1.25 and 1.75 for PTB, ITB and TTB, respectively (Fig. S1).

The median TSR in metastases and primary tumors was 70%. In metastases, a two-tiered scoring was performed using a predefined cut-off of 50% and a median cut-off. In primary tumors, a 50% cut-off was used for two-tiered scoring of TSR.

The Cohen's kappa was calculated for reproducibility assessment in different cut-offs which are presented in the Supplementary Table S1. In pulmonary metastases, the kappa values for TTB with cut-offs of 5, median and ROC-point value, were 0.612, 0.253 and 0.210, respectively. In primary CRC tumor budding, Cohen's kappa for TTB for a cut-off of 5 was 0.762. In TSR assessment, a kappa-value for 50% cut-off was 0.755 in metastases and 0.741 in primary tumors. With a cut-off of 70%, the kappa-value was 0.425.

2.4. Outcomes and definitions

Charlson Comorbidity Index (CCI) was used for comorbidity classification [22]. The cancer under treatment was included as one comorbidity. Disease free interval (DFI) was defined as an interval

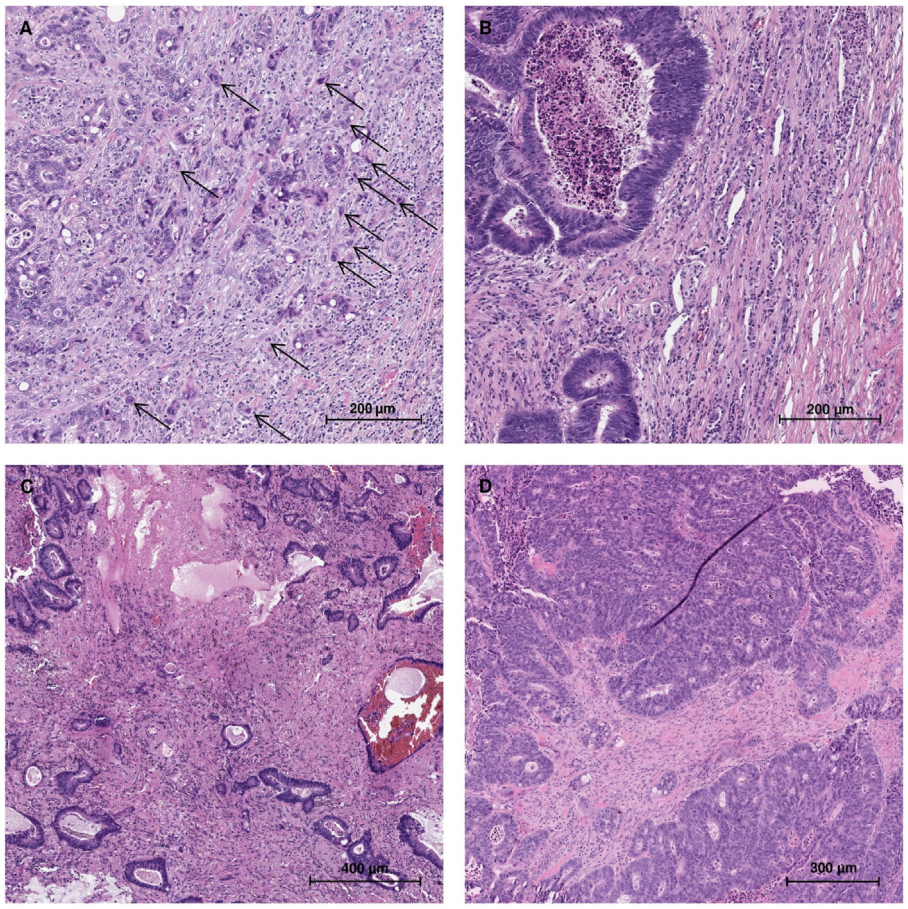

Fig. 1. Tumor budding and TSR of CRC pulmonary metastases. **A.** High tumor budding in the invasive margin of pulmonary metastases. **B.** Low tumor budding in the invasive margin of pulmonary metastases. **C.** Low TSR of pulmonary metastases. **D.** High TSR of pulmonary metastases. Arrows indicate examples of individual buds.

from the surgery of CRC tumor to the date of the detection of the first pulmonary metastasis. Pulmonary metastases that were detected less than 6 months after primary cancer treatment were deemed as synchronous and those after 6 months metachronous.

The primary outcome of the study was 5-year overall survival from the date of pulmonary metastasectomy to death due to any cause before the end of follow-up. Only 1 patient died of other cause than cancer, therefore cancer-specific survival was not analyzed.

2.5. Statistical analysis

Chi-square test was used for group comparison of categorical variables. One-way ANOVA and Kruskal-Wallis tests were used for continuous variable group comparison. Kaplan-Meier survival curves were constructed from the first metastasectomy to death or the end of follow-up to visualize survival up to 5 years after pulmonary metastasectomy. Log rank test was used to compare survivals. The estimates for hazard ratios (HR) with 95% confidence intervals (CI) were calculated using Cox regression. For multivariate analysis, the Cox regression model was adjusted for sex (male/female), age (continuous variable), CCI (1/2/≥3), neoadjuvant therapy (no/yes), number of pulmonary metastases at diagnosis (1/≥2), former liver metastasectomies (no/yes) and synchronicity of pulmonary metastases (synchronous/metachronous). 4 patients had a R1 resection of the first pulmonary metastases and were therefore excluded from the survival analysis. In TSR survival analysis, a sensitivity analysis was performed were only patients who did not receive neoadjuvant treatment were included. In tumor budding survival analysis, a sensitivity analysis was performed based on the location of the primary tumor. Statistical analysis was performed using IBM SPSS Version 28 (IBM corp., Armonk, NY, USA).

3. Results

3.1. Patient characteristics

A total of 106 pulmonary metastasectomies were performed to 74 CRC patients during the study period. Of the metastasectomies, 36 cases were re-metastasectomies and were performed to 21 patients. Adequate samples for tumor budding and TSR analysis were available in 105 pulmonary metastasis and 66 CRC tumors.

The final cohort of first pulmonary metastases and their corresponding primary CRCs consisted of 70 pulmonary metastasis samples and 66 primary CRC samples. At the time of primary CRC treatment, 5 patients (7.1%) had stage I CRC, 18 patients (25.7%) had stage II CRC, 27 patients (38.6%) had stage III CRC and 20 patients (28.6%) had stage IV CRC. The median DFI after primary CRC surgery was 337 (IQR 0–783) days. 12 patients (17.1%) had bilateral pulmonary metastases and 35.7% of patients had more than 1 pulmonary metastasis. 4 patients (5.7%) had a R1 resection of pulmonary metastases. Earlier liver metastases of CRC were curatively operated in 45.7% of patients. Of all patients, 50.0% of CRC tumors were of rectal origin. The median follow-up time was 40.2 months (IQR 20.9–56.3) ranging from 4.9 months to 233 months. The overall 5-year survival rate was 28.4%.

All patients were MMR proficient and 4.5% of all patients had a mutated *BRAF* status. The *KRAS* status was available in 24.3% of patients, of which 64.7% had a mutated status. Only 10% had an available *NRAS* status, of which 14.3% had a mutated status. The mutation status of *BRAF, KRAS* or *NRAS* did not affect survival in our data in univariate models.

3.2. Tumor budding

The tumor budding count was lower in metastases compared to the primary tumors: the median of TTB was 1.5 in the first pulmonary metastases and 4.25 in primary tumors (<0.001). Tumor budding in metastases was not associated with tumor budding in primary tumors (Table 1). 60 patients had low tumor budding (<5) and 10 patients high tumor budding (≥5) in the first pulmonary metastases of CRC. Patient characteristics according to TTB is presented in Table 1. High tumor budding appeared significantly correlated with longer DFI (p = 0.033; Table 1). Neoadjuvant treatment and tumor regression grade did not differ between tumor budding grades. According to the K-M curves, tumor budding in TTB or PTB had no statistically significant effect on 5-year survival (low TTB 28.3% vs. high TTB 37.3%, p = 0.387; low PTB 29.2% vs high PTB 35.7%, p = 0.477; Fig. 2). In multivariate analysis, high TTB had no statistically significant effect of 5-year overall survival (adjusted HR 0.43, 95% CI 0.13–1.42, p = 0.166; Table 2).

In primary CRC tumors, 35 patients had low tumor budding (≤5) and 31 patients high tumor budding (>5). Patient characteristics according to tumor budding of the primary tumor are presented in Table S2. Higher tumor budding was correlated with shorter DFI. In our data, tumor budding in primary tumors had no effect on 10-year survival in K-M survival analysis (p = 0.886; Fig. S2).

3.3. Tumor-stroma ratio

The patient characteristics according to TSR stratified by a predefined cut-off of 50% is presented in Table 3. High TSR was associated with lower proportion of former liver metastasectomy and longer DFI. TSR of the metastases did not correlate with that of the primary tumors. TSR of metastases was not associated with tumor budding. Neoadjuvant treatment or tumor regression grade did not differ between TSR groups. In the K-M survival analysis, TSR of metastases had no effect on 5-year survival (low TSR 32.9% vs. high TSR 28.6%, p = 0.746; Fig. 3). In multivariate analysis, high TSR had no statistically significant effect on 5-year overall survival (adjusted HR 0.86, 95% CI 0.35–2.07, p = 0.727; Table 2). In the primary tumor, TSR did not have any prognostic value according to our data (p = 0.826; Fig. S3).

In a sensitivity analysis including only patients not receiving neoadjuvant treatment, TSR, with a cut-off of 50%, did not have a statistically significant prognostic effect in metastases (low 53.6% vs. high 24.7%, p = 0.279) or primary tumors (low 28.6% vs. high 26.1%, p = 0.447).

3.4. Post-hoc analysis

Since there are very few previous studies on tumor budding or TSR in the pulmonary metastases of CRC, a post-hoc analysis on different cut-offs was performed. For tumor budding, ROC-curve based cut-offs were used, which are explained in the methods section. 36 patients (51.4%) had low PTB (<1.5) and 34 (48.6%) high PTB (≥1.5); whereas in TTB, 36 patients (51.4%) had low TTB (<2) and 34 (48.6%) high TTB (≥2). Using ROC-picked cut-offs, TTB or PTB had no effect on 5-year survival (low TTB 31.8% vs. high TTB 26.3%; $p = 0.794$; Fig. S4B). In the sensitivity analysis of tumor budding based on the location of the primary tumor, tumor budding (<5 vs. ≥5 buds) did not have a significant effect on 5-year overall survival in pulmonary metastases of rectal cancers (low TTB 38.6% vs. high TTB 100%, $p = 0.151$) or colon cancers (low TTB 18.9% vs. high TTB 21.4%, $p = 0.697$). In the sensitivity analysis, only 2 patients in colon cancer and 7 patients in rectal cancer had high tumor budding.

A median cut-off of 70% was used in post-hoc classification of TSR in metastases. 33 patients had low TSR (<70%), and 37 patients had high TSR (≥70%). TSR with median cut-offs had no statistically significant effect on 5-year survival in all patients (low 44.0% vs. high 17.4%; p = 0.084; Fig. S5) or in only patients not receiving neoadjuvant treatment (low 53.6% vs. high 17.8%, p = 0.153).

4. Discussion

We performed a study on the prognostic effect of tumor budding and TSR in the resected pulmonary metastases of CRC. The main finding of this study indicated that tumor budding and TSR determined from the first resected CRC pulmonary metastases has no prognostic value.

Tumor budding has been suggested to represent EMT of malignant tumor cells and the invasive metastatic capacity of the tumor. In the primary CRC tumors, tumor budding has been linked to the lowered expression of cell adhesion molecules, such as E-cadherin [23,24], supporting the linkage between tumor budding and EMT. The prognostic value of tumor budding in primary CRC tumors has been proven in early to metastatic stages of the disease [7,25]. As cancer cells migrate to the peripheral tissues and form metastatic nodules, they are proposed to regain their epithelial characteristics via mesenchymal-epithelial transition (MET) [26,27]. Cancer cells in the metastases express higher levels of cell adhesion molecules compared to the primary tumors [28], however there is also contradictory reports [29]. There is limited research on the prognostic effect of tumor budding in CRC metastases. Several studies have suggested tumor budding in CRC liver metastases having prognostic value in univariate models [11,12,30]. In our study, tumor budding in pulmonary metastases of CRC did not show a survival effect. With the predefined cut-off of 5 buds in a two-tiered classification, TTB and PTB showed no prognostic effect on 5-year overall survival. However, in this analysis, only up to 10 patients had high tumor. In the ROC curve analysis, the areas under curves in TTB, PTB and ITB were 0.647, 0.623 and 0.561, respectively. Using the cut-offs picked from the ROC-curve, TTB and PTB did not provide prognostic value. Also, the reproducibility weakened as the kappa-values with the ROC cut-off values were only 0.21 and 0.362 in TTB and PTB, respectively. Whereas the kappa-values with a cut-off of 5 were 0.696 and 0.697 in TTB and PTB, respectively. The use of median cut-offs also provided no additional prognostic value in the pulmonary metastases.

There are discordant reports of the tumor budding comparison between metastases and primary CRC tumors. Yonemura et al. [12] reported a significant association between tumor budding in liver metastases and primary tumor. Contrarily, Blank et al. [31] reported primary CRC and liver metastases tumor budding as separate phenomena with no association with each other. In our study, there was no association of tumor budding between pulmonary metastases and primary tumor in group comparison using different cut-offs. In continuous variable correlation analysis, only ITB in primary tumor had a statistically significant correlation with PTB in pulmonary metastases ($r_s = 0.368$; $p = 0.002$). In the baseline characteristics, high TTB in primary tumors was correlated with shorter DFI, whereas in pulmonary metastases, high TTB correlated with longer DFI, pointing out the possible difference of the effect of tumor budding in pulmonary metastases and primary tumor.

The lack of prognostic value of tumor budding in our study could

Table 1
Patient characteristics (n = 70) according to total tumor budding (TTB) of the first pulmonary metastasis of colorectal carcinoma.

	Total tumor budding (TTB)		p-value
	Low (<5)	High (≥5)	
	n (%)	n (%)	
Gender			0.306
Female	32 (53.3%)	3 (30.0%)	
Male	28 (46.7%)	7 (70.0%)	
Age (M; SD)	67.6 (10.5)	65.8 (11.4)	0.622
CCI			0.164
1	33 (55.0%)	9 (90.0%)	
2	27 (25.0%)	1 (10.0%)	
≥3	12 (20.0%	0 (0.0%)	
Neoadjuvant therapy			>0.999
No	35 (58.3%)	6 (60.0%)	
Yes	25 (41.7%)	4 (40.0%)	
CRC stage			0.432
1-2	19 (31.7%)	4 (40.0%)	
3	22 (36.7%)	5 (50.0%)	
4	19 (31.7%)	1 (10.0%)	
CRC location			0.306
Colon	32 (53.3%)	3 (30.0%)	
Rectum	28 (46.7%)	7 (70.0%)	
DFI (days; MD; IQR)	286 (0–749)	939 (326–1253)	**0.033***
Size of largest PM (cm; MD; IQR)	2 (1.1–3.5)	2.3 (1.5–2.6)	0.748
Synchronicity			0.674
Synchronous	13 (21.7%)	1 (10.0%)	
Metachronous	47 (78.3%)	9 (90.0%)	
CEA (MD; IQR)	3.15 (1.80–5.28)	2.3 (0.70–4.10)	0.228
Former liver metastasectomy			0.326
No	31 (51.7%)	7 (70.0%)	
Yes	29 (48.3%)	3 (30.0%)	
Number of metastases			>0.999
1	38 (63.3%)	7 (70.0%)	
2	17 (28.3%)	3 (30.0%)	
≥3	5 (8.3%)	0 (0.0%)	
Laterality of metastases			0.359
Unilateral	51 (85.0%)	7 (70.0%)	
Bilateral	9 (15.0%)	3 (30.0%)	
Tumor regression grade			0.618
Minimal regression	55 (91.7%)	9 (90.0%)	
Moderate regression	3 (5.0%)	1 (10.0%)	
Subtotal regression	2 (3.3%)	0 (0.0%)	
met PTB			**<0.001***
Low	60 (100.0%)	3 (30.0%)	
High	0 (0.0%)	7 (70.0%)	
met ITB			**<0.001***
Low	60 (100.0%)	3 (30.0%)	
High	0 (0.0%)	7 (70.0%)	
prim PTB			>0.999
Low	32 (57.1%)	6 (60.0%)	
High	24 (42.9%)	4 (40.0%)	
prim ITB			0.372
Low	47 (83.9%)	7 (70.0%)	
High	9 (16.1%)	3 (30.0%)	
prim TTB			>0.999
Low	30 (53.6%)	5 (50.0%)	
High	26 (46.4%)	5 (50.0%)	

CCI=Charlson comorbidity index; CEA = premetastasectomy carcinoembryonic antigen; DFI = disease free interval; ITB = intratumoral budding; met = metastases; prim = primary tumor; PTB = peritumoral budding; TTB = total tumor budding.
*statistically significant at the level of <0.05.

be related to several factors. The possible selection bias for pulmonary metastasectomy might have affected our result, as generally only patients with controlled/absent extrathoracic metastases are considered for pulmonary metastasectomy and therefore patients with widespread metastases with presumably a more aggressive disease and higher tumor budding are excluded from the pulmonary metastasectomy cohorts. The finding of lower tumor budding values in the pulmonary metastases compared to the primary tumors support this speculation. Additionally, our data did not include the adjuvant oncological therapy of the primary tumor, which might have influenced tumor budding in the pulmonary metastases. We also speculate that the reduced prognostic value of tumor budding in the pulmonary metastases in our study might also be related to the immune host response as represented in the 'pro/anti-tumor model' [32,33], as the immune infiltration is reported to be higher in the metastases compared to the primary tumors [20,34]. The possible artifacts and "pseudobudding" might also have affected the budding analysis in our article. Pseudobudding is a known to be a challenge especially in adenocarcinomas following neoadjuvant therapy and tumor samples with a dense

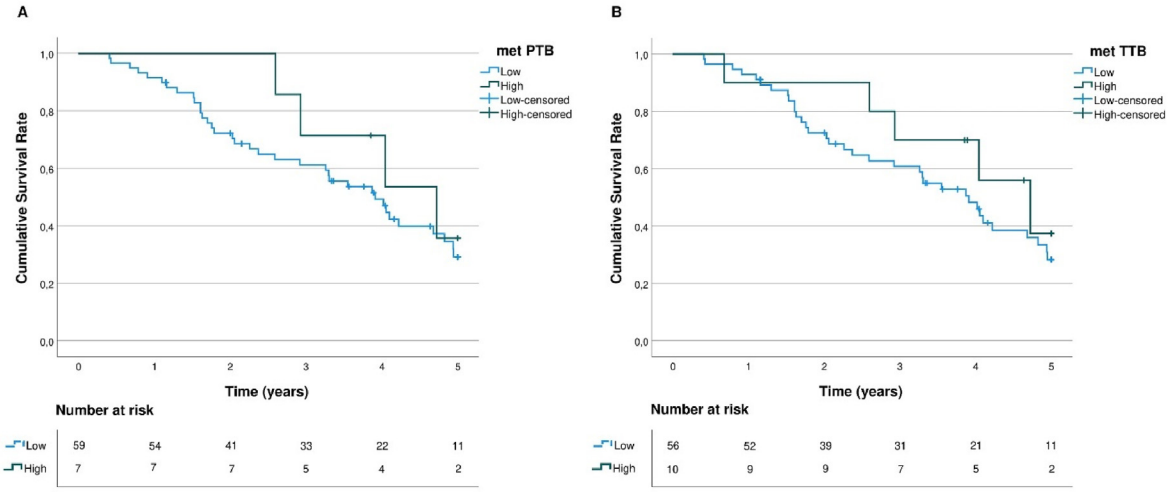

Fig. 2. K-M curves of 5-year overall survival. **A.** Peritumoral budding (PTB) with a cut-off of 5 in the first resected pulmonary metastases of colorectal carcinoma. Log rank p = 0.447. **B.** Total tumor budding (TTB) with a cut-off of 5 in the first resected pulmonary metastases of colorectal carcinoma. Log rank p = 0.387.

Table 2
Hazard ratios (HR) for 5-year all-cause mortality with 95% confidence intervals in first pulmonary metastases and primary colorectal tumor stratified by total tumor budding (low/high) and TSR (low/high).

		Total tumor budding (TTB)		Tumor-stroma ratio (TSR)	
Metastases	N	Low, HR (95%CI)	High, HR (95%CI)	Low, HR (95%CI)	High, HR (95%CI)
Crude	66	1.00 (reference)	0.66 (0.26–1.69; p = 0.390)	1.00 (reference)	1.13 (0.55–2.31; p = 0.746)
Adjusted[a]	66	1.00 (reference)	0.43 (0.13–1.42; p = 0.166)	1.00 (reference)	0.86 (0.35–2.07; p = 0.727)

[a] Adjusted for gender (female/male), age (continuous), CCI (1/2/≥3), neoadjuvant therapy (no/yes), synchronicity of pulmonary metastases (synchronous/metachronous), number of pulmonary metastases at diagnosis (1/≥1) former liver metastasectomy (no/yes).

immune infiltration, where the immune reaction is proposed to fragment to cancer cells from the tumor epithelia not representing true tumor budding [35]. In our study, tumor budding did not have prognostic value even when excluding patients receiving neoadjuvant therapy. In comparison to the CRC liver metastases, where tumor budding had prognostic value in univariate models [11,12], a possible explanation of the difference to our results might be related to the difference of the tissue structure between lungs and liver. However, high tumor budding has negative impact on prognosis in primary lung cancer [36] suggesting that the lung tissue itself does not explain our negative results. At the molecular level, the MET hypothesis in metastases [27,37] might explain the lack of prognostic value of tumor budding in our study. On the other hand, there are a few studies indicating that cell adhesion molecule expression in metastases is inversely correlated with the size of the metastases [38,39], as it is also shown to occur in primary tumors [40]. This might indicate that EMT in the metastases might occur as the metastases progress in size. Molecular studies on cell adhesion molecule expression and tumor budding analysis in combination to size of metastases would shed light to tumor budding as a prognostic marker in cancer metastases.

To date, the prognostic value of TSR has been proven in multiple solid cancers [16,41]. It is proposed that the stroma mediated by the cancer-associated fibroblasts supply growth factors, cytokines and stimulate angiogenesis and thus promote cancer progression [42]. In primary CRC, high stromal content has been proven to be associated with worse overall and cancer-specific survival, as well as disease-free survival in all stages [43,44]. To the best of our knowledge, TSR has not yet been evaluated from the metastases of CRC or any other malignancy. According to our study, TSR evaluated from the resected pulmonary metastases of CRC does not have prognostic value in univariate or multivariate analysis. Surgical patient selection might have affected our results as discussed above: more aggressive metastatic tumors possibly end up being excluded from surgical treatment of pulmonary metastases. Also, our study lacks data on adjuvant therapy, which might have affected our results. However, as neoadjuvant treatment induces changes to the cellular morphology and composition of the TME, resulting in stromal formation surrounding to tumor [17], patients receiving neoadjuvant therapy are suggested to be excluded from TSR analysis. In all resected pulmonary metastases of our study, TSR was suggestively associated with neoadjuvant treatment: patients receiving neoadjuvant treatment had a greater proportion of low TSR compared to patients not receiving neoadjuvant therapy ($p = 0.088$). In the sensitivity analysis, excluding neoadjuvant treated patients, TSR did not show a statistically significant effect on survival. However, due to the small sample size in the sensitivity analysis and the relatively large survival difference in survival between high and low TSR, our study requires validation in further studies.

The novelty of this study can be considered as a strength; to the best of our knowledge, tumor budding and TSR in CRC pulmonary metastases is evaluated here for the first time. This is a dual-institutional study, which can be considered as a strength in a clinical point of view. However, the quality of staining in the diagnostic hematoxylin-eosin-stained slides varied considerably between the study hospital laboratories which might have affected the tumor budding evaluation especially in slides with a dense immune-infiltration. As a population-based study, the selection bias is minimal and restricted to surgical patient selection. Nevertheless, there might be some differences in the patient selection for pulmonary metastasectomy between the study hospitals, since in

Table 3
Patient characteristics (n = 70) according to tumor-stroma ratio (TSR) of the first pulmonary metastases of colorectal carcinoma.

	Tumor-stroma ratio (TSR)		*p*-value
	Low (<50%)	High (≥50%)	
	n (%)	n (%)	
Gender			0.788
Female	9 (47.4%)	26 (51.0%)	
Male	10 (52.6%)	25 (49.0%)	
Age (M; SD)	67.6 (10.2)	67.3 (10.8)	0.910
CCI			0.199
1	12 (63.2%)	30 (58.8%)	
2	2 (10.5%)	14 (27.5%)	
≥3	5 (26.3%)	7 (13.7%)	
Neoadjuvant therapy			0.088
No	8 (42.1%)	33 (64.7%)	
Yes	11 (57.9%)	18 (35.3%)	
CRC stage			0.247
1–2	4 (21.1%)	19 (37.3%)	
3	7 (36.8%)	20 (39.2%)	
4	8 (42.1%)	12 (23.5%)	
CRC location			0.420
Colon	11 (57.9%)	24 (47.1%)	
Rectum	8 (42.1%)	27 (52.9%)	
DFI (days; MD; IQR)	67 (0–350)	587 (0–977)	**0.010***
Synchronicity			0.893
Synchronous	4 (21.1%)	10 (19.6%)	
Metachronous	15 (78.9%)	41 (80.4%)	
CEA (MD; IQR)	2.25 (1.02–4.43)	3.30 (1.90–5.00)	0.148
Former liver metastases			**0.004***
No	5 (26.3%)	33 (64.7%)	
Yes	14 (73.7%)	18 (35.3%)	
Number of metastases			0.317
1	14 (73.7%)	31 (60.8%)	
>1	5 (26.3%)	20 (39.2%)	
Laterality			0.370
Unilateral	17 (89.5%)	41 (80.4%)	
Bilateral	2 (10.5%)	10 (19.6%)	
Tumor regression grade			0.332
Minimal regression	18 (94.7%)	46 (90.2%)	
Moderate regression	0 (0.0%)	4 (7.8%)	
Subtotal regression	1 (5.3%)	1 (2.0%)	
met PTB (cutoff 5)			0.089
Low	19 (100.0%)	44 (86.3%)	
High	0 (0.0%)	7 (13.7%)	
met ITB (cutoff 5)			0.420
Low	18 (94.7%)	45 (88.2%)	
High	1 (5.3%)	6 (11.8%)	
met TTB (cutoff 5)			0.188
Low	18 (94.7%)	42 (82.4%)	
High	1 (5.3%)	9 (17.6%)	
TSR of primary tumor			0.705
Low	3 (16.7%)	10 (20.8%)	
High	15 (83.3%)	38 (79.2%)	

CCI=Charlson comorbidit y index; CEA = premetastasectomy carcinoembryonic antigen; DFI = disease free interval; ITB = intratumoral budding; met = metastases; prim = primary tumor; PTB = peritumoral budding; TTB = total tumor budding.
*statistically significant at the level of <0.05.

Oulu University Hospital district, the treatment and follow-up of primary CRC in under a third of patients has not occurred in our study hospital where the patient received pulmonary metastasectomy. The relatively small sample size is a limitation in our study. Concerning the TSR analysis, our study included also patients receiving neoadjuvant therapy, wherein the abundance of stroma might also reflect the neoadjuvant response and not the changes in the cellular morphology and tumor microenvironment. This limitation is addressed in the sensitivity analysis including only patients not receiving neoadjuvant therapy. The lack of data on other proven histological markers in primary tumors, such as lymphovascular and perineural invasion, might be considered a limitation, however their role is yet to be determined in the pulmonary

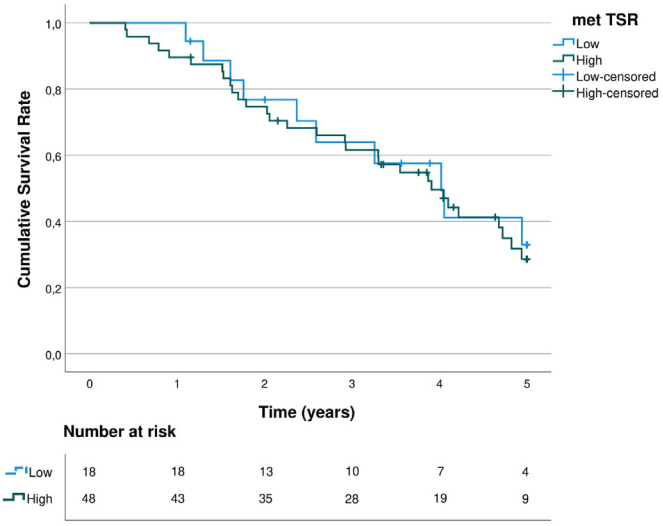

Fig. 3. K-M curves of 5-year overall survival stratified by TSR with a cut-off of 50%. $p = 0.746$.

metastases of CRC. The lack of data on *RAS* mutation status is also a limitation. However, since the clinical data was acquired during a long period of time, and thus the *RAS* mutation status was not used in clinical decision making in all patients, especially regarding chemotherapy regimens, the post hoc determination of *RAS* status was not performed. Predefined cut-off values were used for both tumor budding and TSR to avoid false positive results and data dredging [45]. Additionally, to explore potential cut-off values, ROC curve analyses were performed, all of which resulted with negative result making our negative finding more robust.

5. Conclusion

Our study concludes that tumor budding and TSR evaluated from the resected pulmonary metastases of CRC do not have prognostic value. Tumor budding is significantly decreased in the resected pulmonary metastases compared to the primary tumors. Tumor budding or TSR in the resected pulmonary metastases were not associated with those of the primary tumor.

Ethics approval and consent to participate

The Oulu University Hospital Ethics Committee (EETMK 81/2008) approved the study. The Finnish National Authority of Medicolegal Affairs (VALVIRA) waived the need for informed consent due to the retrospective nature of the study. The study was performed in accordance with the Declaration of Helsinki.

Consent for publication

All data were anonymized before analysis and writing.

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CRediT authorship contribution statement

Topias Karjula: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Niko Kemi:** Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Anne Niskakangas:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Olli Mustonen:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Iiris Puro:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Vesa-Matti Pohjanen:** Validation, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Teijo Kuopio:** Validation, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Hanna Elomaa:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Maarit Ahtiainen:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Jukka-Pekka Mecklin:** Investigation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Toni T. Seppälä:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Erkki-Ville Wirta:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Eero Sihvo:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Juha P. Väyrynen:** Conceptualization, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Fredrik Yannopoulos:** Conceptualization, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Olli Helminen:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

T.T.S. reports consultation fees from Boehringer Ingelheim Finland and Amgen Finland and being a co-owner and CEA of Healthfund Finland.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2023.02.009.

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