

ORIGINAL PAPER

ASSOCIATIONS BETWEEN ROUTINE HISTOPATHOLOGICAL PARAMETERS AND SITE-SPECIFIC SYNCHRONOUS DISTANT METASTASIS IN COLORECTAL ADENOCARCINOMA

ÖZGEÇAN HAMDEMİR¹, SEMİN AYHAN²¹Department of Pathology, Faculty of Medicine, Kafkas University, Kars, Turkey²Department of Pathology, Faculty of Medicine, Manisa Celal Bayar University, Manisa, Turkey

Synchronous distant metastasis is a major determinant of prognosis in colorectal adenocarcinoma, but its associations with routine histopathological parameters at individual metastatic sites remain incompletely understood.

This retrospective study included 382 patients who underwent surgical resection for colorectal adenocarcinoma between 2006 and 2018. Clinicopathological parameters were evaluated in relation to synchronous distant metastasis overall and to liver, peritoneal, lung, and bone metastases using univariable analyses, exact tests where appropriate, and multivariable logistic regression. Synchronous distant metastasis was present in 83 patients (21.7%).

In two separate multivariable models, lymph node metastasis, positive proximal/distal resection margin, low peritumoral lymphocytic infiltration, and perineural invasion were associated with higher odds of synchronous distant metastasis, whereas deficient mismatch repair (dMMR) status was associated with lower odds. Liver metastasis was associated with lymph node metastasis and positive proximal/distal resection margin, whereas dMMR status was associated with lower odds. In restricted exploratory models, tumor deposits and non-conventional histological subtype were associated with peritoneal metastasis, and tumor perforation was associated with lung metastasis. Bone metastasis was too infrequent for multivariable analysis.

These organ-specific findings should be considered exploratory and require validation in larger independent cohorts.

Key words: colorectal adenocarcinoma, synchronous metastasis, site-specific metastasis, histopathology, mismatch repair.

Introduction

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality worldwide [1, 2]. Approximately 20–25% of patients present with synchronous distant metastasis, and nearly half develop metastatic disease during their illness [3, 4]. Despite therapeutic advances, metastatic CRC continues to have poor outcomes [4, 5], emphasizing the importance of identifying pathological features associated with dissemination.

The liver, lung, and peritoneum are the principal metastatic sites in CRC. Differences in metastatic distribution may reflect anatomical factors, tumor biology, and interactions with organ-specific microenvironments [6–9]. Routinely assessed features – including lymphovascular, venous, and perineural invasion; tumor budding; poorly differentiated clusters (PDCs); tumor deposits; histological subtype; resection margin status; mismatch repair (MMR) status; and host immune response – have been associated with aggressive behavior and adverse outcomes [6, 10–20].

However, studies evaluating both overall synchronous distant metastasis and site-specific associations within the same cohort remain limited [6, 7, 15, 21]. We therefore investigated the associations of routine histopathological parameters with synchronous distant metastasis overall and with metastases at individual anatomical sites.

Material and methods

Study design and patient selection

This retrospective, single-center study included 382 consecutive patients with histopathologically confirmed colorectal adenocarcinoma who underwent surgical resection between 2006 and 2018. Clinical and histopathological data were retrieved from the institutional pathology archives and electronic medical records. The study design is summarized in Figure 1.

Patients with insufficient histopathological material, incomplete clinical and/or radiological information regarding metastatic status, or prior neoadjuvant chemoradiotherapy were excluded because treatment-related changes could influence histopathological evaluation.

Synchronous distant metastasis was defined as distant metastasis detected during baseline staging before or at the time of primary tumor surgery. Metastatic lesions identified only during postoperative follow-up were not classified as synchronous unless they represented radiological confirmation of abnormalities already suspected at baseline. This definition was applied consistently across all metastatic sites.

Metastatic status was established using baseline radio-logical and/or intraoperative findings, with histopathological confirmation when available. Liver and lung metastases were diagnosed primarily by radiological imaging, peritoneal metastases by radiological and intraoperative findings, and bone metastases by radiological imaging performed during baseline staging. Rare metastatic sites, including the adrenal gland, ovary, and pancreas, were identified using baseline radiological, intraoperative, and/or histopathological findings when available. Clinical follow-up was used solely to confirm lesions suspected at baseline and was not used to classify newly detected metastases as synchronous.

All patient data were anonymized before analysis. The requirement for informed consent was waived because of the retrospective study design.

Histopathological evaluation

All hematoxylin and eosin (HE)-stained slides were independently reviewed by two pathologists, including one gastrointestinal pathologist, who were blinded to metastatic status. Cases with discrepant assessments were reviewed jointly until consensus was reached.

The following parameters were evaluated: histological subtype, tumor grade, tumor budding, PDCs, lymphovascular invasion, venous invasion, perineural invasion, tumor deposits, intratumoral and peritumoral lymphocytic infiltration, Crohn-like lymphoid reaction, tumor perforation, resection margin status, tumor location, tumor size, and MMR status. Histological subtype was categorized as conventional adeno-

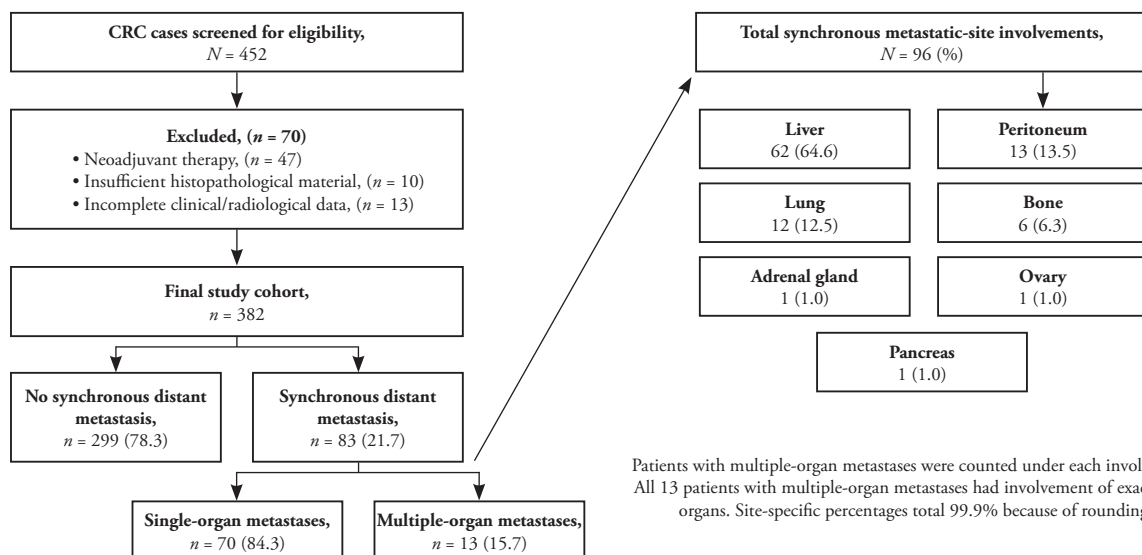


Figure 1. Study flowchart and distribution of synchronous metastatic-site involvements

CRC – colorectal cancer

Among 382 eligible patients, 83 had synchronous distant metastasis. Seventy patients had single-organ metastasis, whereas 13 had multiple-organ metastases; all patients in the latter group had involvement of two organs. These patients accounted for 96 synchronous metastatic-site involvements. Patients with multiple-organ metastases were counted under each involved site. Metastatic-site percentages were calculated using the total number of metastatic-site involvements ($n = 96$) as the denominator and total 99.9% because of rounding.

carcinoma, mucinous adenocarcinoma, or signet-ring cell carcinoma.

Tumor budding was assessed according to the recommendations of the International Tumor Budding Consensus Conference (ITBCC) 2016 [22]. Tumor buds were counted in a hotspot measuring 0.785 mm² at the invasive front and classified as Bd1 (low, 0–4 buds), Bd2 (intermediate, 5–9 buds), or Bd3 (high, ≥ 10 buds).

Poorly differentiated clusters were evaluated at the invasive front and counted in a hotspot measuring 0.785 mm². Poorly differentiated cluster grade was classified as PDC-G1 (< 5 clusters), PDC-G2 (5–9 clusters), or PDC-G3 (≥ 10 clusters) [14]. For statistical analyses, PDC-G1 and PDC-G2 were combined as the low/intermediate group because of the limited number of PDC-G1 cases, whereas PDC-G3 constituted the high-grade group.

Tumor deposits were defined as discrete tumor nodules within the pericolic or perirectal adipose tissue without residual lymph node architecture.

Peritumoral and intratumoral lymphocytic reactions were evaluated semiquantitatively using criteria adapted from Ogino *et al.* [19]. Peritumoral lymphocytic reaction was defined as discrete lymphoid aggregates surrounding the tumor, whereas intratumoral lymphocytic reaction was defined as periglandular lymphocytic infiltration within the tumor stroma. Both reactions were scored as 0 (absent), 1+ (mild), 2+ (moderate), or 3+ (marked). Because this was a semiquantitative morphological assessment, no predefined numerical density or percentage thresholds were used to distinguish mild, moderate, and marked reactions. All available HE-stained tumor sections were reviewed, and the highest score observed for each lymphocytic reaction component was recorded. For statistical analyses, peritumoral lymphocytic reaction was dichotomized as low (0–1+) or high (2+ to 3+), whereas intratumoral lymphocytic reaction was categorized as absent (0), low (1+), or high (2+ to 3+).

Crohn-like lymphoid reaction was defined as the presence of at least one transmural lymphoid aggregate located beyond the advancing tumor edge within the muscularis propria or pericolic/perirectal tissue and was categorized as present or absent [19]. No minimum aggregate-size threshold was applied.

Lymphovascular invasion was defined as the presence of tumor cells within endothelial-lined lymphatic or small vascular spaces. Venous invasion was defined as tumor invasion into veins with an identifiable smooth muscle wall. Perineural invasion was defined as the presence of tumor cells within any layer of the nerve sheath or surrounding at least one-third of the nerve circumference.

Tumor grade was categorized as low grade, comprising well- and moderately differentiated adenocar-

cinomas, or high grade, comprising poorly differentiated adenocarcinomas.

Proximal and distal resection margins were categorized as positive or negative according to the presence or absence of tumor involvement at the resection line. Circumferential (radial) resection margin status was evaluated separately and was available for 118 patients.

Tumors were classified as right-sided or left-sided, with rectal tumors included in the left-sided group.

Mismatch repair assessment

Mismatch repair protein expression was assessed immunohistochemically using antibodies against MLH1, PMS2, MSH2, and MSH6. Cases showing loss of nuclear expression of one or more MMR proteins in tumor cells, with intact internal positive controls, were classified as having deficient mismatch repair (dMMR), whereas cases with preserved expression of all four proteins were classified as having proficient mismatch repair.

Assessment of metastatic patterns

A three-stage analytical approach was used. First, associations between routinely assessed clinicopathological parameters and synchronous distant metastasis overall were evaluated. Second, liver, peritoneal, lung, and bone metastases were analyzed separately as site-specific outcomes. For each metastatic site, patients with metastasis at the corresponding site were compared with all patients without involvement of that site. Patients with multiple-organ metastases were included in the analysis of each affected site.

Third, additional analyses were restricted to the 83 patients with synchronous metastatic disease to explore differences among metastatic sites more directly. Patients with liver metastasis were compared with metastatic patients without liver involvement, and patients with peritoneal metastasis were compared with metastatic patients without peritoneal involvement. Patients with multiple-organ metastases were included in the target-site group whenever the corresponding organ was involved.

Accordingly, the analyses were designed to evaluate site-specific metastatic associations and histopathological profiles associated with individual metastatic sites rather than to establish biological organ tropism.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were compared using Pearson's χ^2 test when expected cell-count assumptions were satisfied and the two-sided Fisher's exact test for sparse 2 × 2 tables. The Fisher-Freeman-Halton exact test, with Monte Carlo estimation where indicated, was used for sparse contingency tables with more than

two categories. Because of the very small numbers of lung and bone metastatic events, Fisher's exact test was used for all 2×2 comparisons involving these outcomes. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

For 2×2 comparisons, unadjusted odds ratios (OR) and 95% CI were calculated from the cell frequencies using the standard logarithmic method. For variables with more than two categories, category-specific unadjusted OR and 95% CI were calculated relative to the designated reference category, whereas the reported p -value represented the overall comparison obtained using the Fisher-Freeman-Halton exact test. Odds ratios were not estimated when a zero cell prevented conventional estimation.

For the analysis of synchronous distant metastasis overall, variables with $p < 0.10$ in the univariable analyses, together with selected clinically relevant variables, were considered for multivariable binary logistic regression. To reduce overfitting and minimize collinearity among biologically related variables, two separate models were constructed. Model 1 included lymph node metastasis, proximal/distal resection margin status, MMR status, and peritumoral lymphocytic infiltration. Model 2 included perineural invasion, lymphovascular invasion, tumor deposits, and PDC grade.

The multivariable liver metastasis model included MMR status, lymph node metastasis, proximal/distal resection margin status, perineural invasion, venous invasion, and PDC grade.

Because only 13 patients had synchronous peritoneal metastasis, the peritoneal metastasis model was deliberately restricted to two binary variables selected before model fitting based on their established clinicopathological relevance: histological subtype, categorized as conventional vs. non-conventional (mucinous or signet-ring cell carcinoma), and tumor deposits. Because only 12 patients had synchronous lung metastasis, a restricted exploratory model including tumor perforation and peritumoral lymphocytic infiltration was constructed. All variables included in the multivariable models were entered simultaneously. Because only six patients had synchronous bone metastasis, multivariable analysis was not performed; bone metastasis was evaluated using descriptive and exact univariable analyses only.

Multicollinearity was assessed using the variance inflation factor. Adjusted OR with 95% CI were calculated for the multivariable models. Model fit and discrimination were assessed using the Omnibus test, Hosmer-Lemeshow goodness-of-fit test, Cox and Snell R^2 , Nagelkerke R^2 , and the area under the receiver operating characteristic curve (AUC), as available.

No formal adjustment for multiple testing was applied because the organ-specific analyses were exploratory. Accordingly, these findings should be considered hypothesis-generating and require validation in independent cohorts. No missing data were iden-

tified for the variables included in the multivariable models. Radial resection margin status was excluded from these models because it was available for only 118 patients.

Results

A total of 382 patients with colorectal adenocarcinoma were included. The mean age was 62.1 ± 11.8 years, 226 patients (59.2%) were male, and the mean tumor size was 4.6 ± 1.9 cm. Most tumors were left-sided (70.4%) and pT3–4 (93.7%).

Synchronous distant metastasis was detected in 83 patients (21.7%). Of these, 70 (84.3%) had single-organ metastasis and 13 (15.7%) had multiple-organ metastases, all involving two organs, yielding 96 metastatic-site involvements. The liver was the most frequent site (62/96, 64.6%), followed by the peritoneum (13/96, 13.5%), lung (12/96, 12.5%), bone (6/96, 6.3%), adrenal gland (1/96, 1.0%), ovary (1/96, 1.0%), and pancreas (1/96, 1.0%). Other cohort characteristics are presented in Table I.

Synchronous distant metastasis

Univariable analysis showed significant associations with lymph node metastasis, tumor deposits, proximal/distal and radial resection margin status, MMR status, histological subtype, PDC grade, peritumoral lymphocytic infiltration, lymphovascular invasion, venous invasion, and perineural invasion (all $p < 0.05$) (Table II).

In Model 1, lymph node metastasis (OR = 4.46, 95% CI: 2.41–8.23; $p < 0.001$), positive proximal/distal resection margin (OR = 15.37, 95% CI: 1.42–165.93; $p = 0.024$), and low peritumoral lymphocytic infiltration (OR = 1.81, 95% CI: 1.07–3.07; $p = 0.028$) were independently associated with higher odds of synchronous distant metastasis, whereas dMMR was associated with lower odds (OR = 0.24, 95% CI: 0.08–0.69; $p = 0.008$) (Table III). The area under the receiver operating characteristic curve was 0.739.

In Model 2, perineural invasion was independently associated with higher odds of synchronous distant metastasis (OR = 2.05, 95% CI: 1.20–3.49; $p = 0.008$). Tumor deposits (OR = 2.06, 95% CI: 0.97–4.38; $p = 0.059$), lymphovascular invasion (OR = 1.83, 95% CI: 0.92–3.63; $p = 0.084$), and high-grade PDCs (OR = 1.30, 95% CI: 0.75–2.22; $p = 0.350$) did not reach statistical significance (Table IV). The area under the receiver operating characteristic curve was 0.652.

Site-specific metastatic associations

Synchronous liver metastasis

Univariable analysis showed significant associations of liver metastasis with lymph node metastasis, proximal/distal resection margin status, MMR status,

Table I. Clinicopathological characteristics of the study cohort and distribution of synchronous metastatic sites

PATIENT AND TUMOR CHARACTERISTICS	VALUE	PATIENT AND TUMOR CHARACTERISTICS	VALUE
Age [years], mean $\pm$ SD	62.1 $\pm$ 11.8	Intratumoral lymphocytic infiltration	
Sex, <i>n</i> (%)		Absent	97 (25.4)
Male	226 (59.2)	Low	252 (66.0)
Female	156 (40.8)	High	33 (8.6)
Tumor size [cm], mean $\pm$ SD	4.6 $\pm$ 1.9	Peritumoral lymphocytic infiltration	
PARAMETERS	<i>N</i> (%)	High	200 (52.4)
Tumor location		Low	182 (47.6)
Right-sided	113 (29.6)	Crohn-like lymphoid reaction	
Left-sided*	269 (70.4)	Present	242 (63.4)
Invasion depth		Absent	140 (36.6)
pT3–4	358 (93.7)	Lymphovascular invasion	
pT1–2	24 (6.3)	Present	264 (69.1)
Tumor perforation		Absent	118 (30.9)
Present	28 (7.3)	Venous invasion	
Absent	354 (92.7)	Present	36 (9.4)
Lymph node metastasis		Absent	346 (90.6)
Present	215 (56.3)	Perineural invasion	
Absent	167 (43.7)	Present	160 (41.9)
Tumor deposits		Absent	222 (58.1)
Present	38 (9.9)	Metastatic status	
Absent	344 (90.1)	Synchronous distant metastasis	
Proximal/distal resection margin		Present	83 (21.7)
Positive	5 (1.3)	Absent	299 (78.3)
Negative	377 (98.7)	Metastatic pattern among patients with synchronous metastasis**	
Radial resection margin***		Single-organ metastasis	70 (84.3)
Positive	22 (18.6)	Multiple-organ metastases	13 (15.7)
Negative	96 (81.4)	Distribution of synchronous metastatic-site involvements****	
MMR status		Liver metastasis – present	62 (64.6)
dMMR	59 (15.4)	Peritoneal metastasis – present	13 (13.5)
pMMR	323 (84.6)	Lung metastasis – present	12 (12.5)
Histological subtype		Bone metastasis – present	6 (6.3)
Mucinous adenocarcinoma	31 (8.1)	Adrenal gland metastasis – present	1 (1.0)
Conventional adenocarcinoma	347 (90.8)	Ovarian metastasis – present	1 (1.0)
Signet-ring cell carcinoma	4 (1.0)	Pancreatic metastasis – present	1 (1.0)
Tumor grade			
High grade (poorly differentiated)	52 (13.6)		
Low grade (well/moderately differentiated)	330 (86.4)		
PDC			
High	174 (45.5)		
Low/intermediate	208 (54.5)		
Tumor budding			
High (Bd3)	182 (47.6)		
Intermediate (Bd2)	81 (21.2)		
Low (Bd1)	119 (31.2)		

Bd – tumor budding grade, dMMR – deficient mismatch repair, MMR – mismatch repair, PDCs – poorly differentiated clusters, pMMR – proficient mismatch repair, SD – standard deviation

Data are presented as *n* (%) unless otherwise stated. Age and tumor size are presented as mean $\pm$ SD.

* Left-sided tumors include rectal tumors.

** Percentages for single- and multiple-organ metastases were calculated among patients with synchronous distant metastasis (*n* = 83). All 13 patients with multiple-organ metastases had involvement of two metastatic sites.

*** Radial resection margin status was available for 118 patients; percentages were calculated using the available cases as the denominator.

**** Metastatic-site percentages were calculated using the total number of synchronous metastatic-site involvements (*n* = 96) as the denominator. Patients with multiple-organ metastases were counted under each involved site.

Individual percentages total 99.9% because of rounding to one decimal place.

Table II. Univariable analysis of associations between clinicopathological parameters and synchronous distant metastasis

PARAMETERS	SYNCHRONOUS METASTASIS PRESENT (N = 83)	ABSENT (N = 299)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Age [years]					
> 55	58 (69.9)	225 (75.3)	0.76 (0.45–1.31)	Pearson χ^2	0.323
≤ 55	25 (30.1)	74 (24.7)	Reference		
Sex					
Male	46 (55.4)	180 (60.2)	0.82 (0.50–1.34)	Pearson χ^2	0.433
Female	37 (44.6)	119 (39.8)	Reference		
Tumor size [cm]					
> 5.5	24 (28.9)	77 (25.8)	1.17 (0.68–2.01)	Pearson χ^2	0.563
≤ 5.5	59 (71.1)	222 (74.2)	Reference		
Tumor location					
Right-sided	26 (31.3)	87 (29.1)	1.11 (0.66–1.88)	Pearson χ^2	0.694
Left-sided	57 (68.7)	212 (70.9)	Reference		
Invasion depth (pT stage)					
pT3–4	80 (96.4)	278 (93.0)	2.01 (0.59–6.93)	Pearson χ^2	0.257
pT1–2	3 (3.6)	21 (7.0)	Reference		
Tumor perforation					
Present	8 (9.6)	20 (6.7)	1.49 (0.63–3.51)	Pearson χ^2	0.362
Absent	75 (90.4)	279 (93.3)	Reference		
Lymph node metastasis					
Present	67 (80.7)	148 (49.5)	4.27 (2.37–7.71)	Pearson χ^2	< 0.001
Absent	16 (19.3)	151 (50.5)	Reference		
Tumor deposits					
Present	14 (16.9)	24 (8.0)	2.32 (1.14–4.73)	Pearson χ^2	0.017
Absent	69 (83.1)	275 (92.0)	Reference		
Proximal/distal resection margin					
Positive	4 (4.8)	1 (0.3)	15.09 (1.66–136.90)	Fisher's exact	0.009
Negative	79 (95.2)	298 (99.7)	Reference		
Radial resection margin*					
Positive	8 (38.1)	14 (14.4)	3.65 (1.28–10.40)	Fisher's exact	0.026
Negative	13 (61.9)	83 (85.6)	Reference		
MMR status					
dMMR	5 (6.0)	54 (18.1)	0.29 (0.11–0.75)	Pearson χ^2	0.007
pMMR	78 (94.0)	245 (81.9)	Reference		
Histological subtype					
Mucinous adenocarcinoma	10 (12.0)	21 (7.0)	1.88 (0.85–4.18)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.014
Conventional adenocarcinoma	70 (84.3)	277 (92.6)	Reference		
Signet-ring cell carcinoma	3 (3.6)	1 (0.3)	11.87 (1.22–115.87)		
Tumor grade					
High grade (poorly differentiated)	15 (18.1)	37 (12.4)	1.56 (0.81–3.01)	Pearson χ^2	0.181
Low grade (well/moderately differentiated)	68 (81.9)	262 (87.6)	Reference		
PDC					
High	48 (57.8)	126 (42.1)	1.88 (1.15–3.08)	Pearson χ^2	0.011
Low/intermediate	35 (42.2)	173 (57.9)	Reference		

Table II. Cont.

PARAMETERS	SYNCHRONOUS METASTASIS PRESENT (N = 83)	ABSENT (N = 299)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Tumor budding					
High	46 (55.4)	136 (45.5)	1.67 (0.93–3.01)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.214
Intermediate	17 (20.5)	64 (21.4)	1.31 (0.64–2.70)		
Low	20 (24.1)	99 (33.1)	Reference		
Intratumoral lymphocytic infiltration					
Absent	28 (33.7)	69 (23.1)	1.51 (0.59–3.87)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.141
Low	48 (57.8)	204 (68.2)	0.87 (0.36–2.13)		
High	7 (8.4)	26 (8.7)	Reference		
Peritumoral lymphocytic infiltration					
High	33 (39.8)	167 (55.9)	Reference	Pearson χ^2	0.009
Low	50 (60.2)	132 (44.1)	1.92 (1.17–3.15)		
Crohn-like lymphoid reaction					
Present	46 (55.4)	196 (65.6)	0.65 (0.40–1.07)	Pearson χ^2	0.090
Absent	37 (44.6)	103 (34.4)	Reference		
Lymphovascular invasion					
Present	69 (83.1)	195 (65.2)	2.63 (1.41–4.90)	Pearson χ^2	0.002
Absent	14 (16.9)	104 (34.8)	Reference		
Venous invasion					
Present	17 (20.5)	19 (6.4)	3.80 (1.87–7.70)	Pearson χ^2	< 0.001
Absent	66 (79.5)	280 (93.6)	Reference		
Perineural invasion					
Present	50 (60.2)	110 (36.8)	2.60 (1.58–4.29)	Pearson χ^2	< 0.001
Absent	33 (39.8)	189 (63.2)	Reference		

dMMR – deficient mismatch repair, MMR – mismatch repair, OR – odds ratio, PDC – poorly differentiated clusters, pMMR – proficient mismatch repair

* Radial resection margin status was available for 118 patients.

Data are presented as n (%). Unadjusted odds ratios (ORs) and 95% CI were calculated for the presence of synchronous distant metastasis. For variables with more than two categories, category-specific ORs were calculated relative to the indicated reference category, whereas the reported p-value represents the overall association across all categories. All p-values are two-sided.

Table III. Multivariable logistic regression analysis of factors associated with synchronous distant metastasis (Model 1)

PARAMETERS	COMPARISON	OR	95% CI	P-VALUE
Lymph node metastasis	Present vs. absent	4.46	2.41–8.23	< 0.001
Proximal/distal resection margin	Positive vs. negative	15.37	1.42–165.93	0.024
MMR status	dMMR vs. pMMR	0.24	0.08–0.69	0.008
Peritumoral lymphocytic infiltration	Low vs. high	1.81	1.07–3.07	0.028

AUC – area under the receiver operating characteristic curve, dMMR – deficient mismatch repair, MMR – mismatch repair, OR – odds ratio, pMMR – proficient mismatch repair
Model performance: Omnibus test $\chi^2 = 49.674$, $p < 0.001$; Hosmer-Lemeshow test $p = 0.199$; Nagelkerke $R^2 = 0.192$; AUC = 0.739.

Table IV. Multivariable logistic regression analysis of pathological factors associated with synchronous distant metastasis (Model 2)

PARAMETERS	COMPARISON	OR	95% CI	P-VALUE
Perineural invasion	Present vs. absent	2.05	1.20–3.49	0.008
Lymphovascular invasion	Present vs. absent	1.83	0.92–3.63	0.084
Tumor deposits	Present vs. absent	2.06	0.97–4.38	0.059
PDC	High vs. low/intermediate	1.30	0.75–2.22	0.350

AUC – area under the receiver operating characteristic curve, OR – odds ratio, PDC – poorly differentiated clusters

Model performance: Omnibus test $\chi^2 = 23.217$, $P < 0.001$; Hosmer-Lemeshow test $p = 0.574$; Nagelkerke $R^2 = 0.092$; AUC = 0.652.

Crohn-like lymphoid reaction, lymphovascular invasion, venous invasion, and perineural invasion (all $p < 0.05$) (Table V).

In multivariable analysis, lymph node metastasis (OR = 3.23, 95% CI: 1.62–6.43; $p < 0.001$) and positive

proximal/distal resection margin (OR = 23.78, 95% CI: 2.08–271.66; $p = 0.011$) were associated with higher odds of liver metastasis, whereas dMMR was associated with lower odds (OR = 0.27, 95% CI: 0.08–0.90; $p = 0.033$). Venous invasion showed a borderline as-

Table V. Univariable analysis of the associations between clinicopathological characteristics and synchronous liver metastasis

PARAMETERS	LIVER METASTASIS PRESENT ($N = 62$)	ABSENT ($N = 320$)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Age [years]					
> 55	47 (75.8)	236 (73.8)	1.12 (0.59–2.10)	Pearson χ^2	0.735
≤ 55	15 (24.2)	84 (26.3)	Reference		
Sex					
Male	33 (53.2)	193 (60.3)	0.75 (0.43–1.29)	Pearson χ^2	0.299
Female	29 (46.8)	127 (39.7)	Reference		
Tumor size [cm]					
> 5.5	15 (24.2)	86 (26.9)	0.87 (0.46–1.63)	Pearson χ^2	0.661
≤ 5.5	47 (75.8)	234 (73.1)	Reference		
Tumor location					
Right-sided	21 (33.9)	92 (28.8)	1.27 (0.71–2.26)	Pearson χ^2	0.419
Left-sided	41 (66.1)	228 (71.2)	Reference		
Invasion depth (pT stage)					
pT3–4	60 (96.8)	298 (93.1)	2.21 (0.51–9.67)	Fisher's exact	0.396
pT1–2	2 (3.2)	22 (6.9)	Reference		
Tumor perforation					
Present	5 (8.1)	23 (7.2)	1.13 (0.41–3.10)	Fisher's exact	0.791
Absent	57 (91.9)	297 (92.8)	Reference		
Lymph node metastasis					
Present	49 (79.0)	166 (51.9)	3.50 (1.83–6.70)	Pearson χ^2	< 0.001
Absent	13 (21.0)	154 (48.1)	Reference		
Tumor deposits					
Present	10 (16.1)	28 (8.8)	2.01 (0.92–4.37)	Pearson χ^2	0.076
Absent	52 (83.9)	292 (91.2)	Reference		
Proximal/distal resection margin					
Positive	4 (6.5)	1 (0.3)	22.00 (2.42–200.37)	Fisher's exact	0.003
Negative	58 (93.5)	319 (99.7)	Reference		
Radial resection margin*					
Positive	5 (35.7)	17 (16.3)	2.84 (0.85–9.54)	Fisher's exact	0.135
Negative	9 (64.3)	87 (83.7)	Reference		
MMR status					
dMMR	3 (4.8)	56 (17.5)	0.24 (0.07–0.79)	Pearson χ^2	0.012
pMMR	59 (95.2)	264 (82.5)	Reference		
Histological subtype					
Mucinous adenocarcinoma	3 (4.8)	28 (8.8)	0.53 (0.16–1.81)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.403
Conventional adenocarcinoma	58 (93.5)	289 (90.3)	Reference		
Signet-ring cell carcinoma	1 (1.6)	3 (0.9)	1.66 (0.17–16.25)		

Table V. Cont.

PARAMETERS	LIVER METASTASIS PRESENT (N = 62)	ABSENT (N = 320)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Tumor grade					
High grade (poorly differentiated)	9 (14.5)	43 (13.4)	1.09 (0.50–2.38)	Pearson χ^2	0.821
Low grade (well/ moderately differentiated)	53 (85.5)	277 (86.6)	Reference		
PDC					
High	35 (56.5)	139 (43.4)	1.69 (0.98–2.92)	Pearson χ^2	0.060
Low/intermediate	27 (43.5)	181 (56.6)	Reference		
Tumor budding					
High (Bd3)	32 (51.6)	150 (46.9)	1.74 (0.87–3.47)	Fisher-Freeman- Halton exact test (Monte Carlo)	0.119
Intermediate (Bd2)	17 (27.4)	64 (20.0)	2.17 (0.99–4.75)		
Low (Bd1)	13 (21.0)	106 (33.1)	Reference		
Intratumoral lymphocytic infiltration					
Absent	15 (24.2)	82 (25.6)	0.82 (0.29–2.33)	Fisher-Freeman- Halton exact test (Monte Carlo)	0.927
Low	41 (66.1)	211 (65.9)	0.87 (0.34–2.25)		
High	6 (9.7)	27 (8.4)	Reference		
Peritumoral lymphocytic infiltration					
High	26 (41.9)	174 (54.4)	Reference	Pearson χ^2	0.073
Low	36 (58.1)	146 (45.6)	1.65 (0.95–2.86)		
Crohn-like lymphoid reaction					
Present	32 (51.6)	210 (65.6)	0.56 (0.32–0.97)	Pearson χ^2	0.036
Absent	30 (48.4)	110 (34.4)	Reference		
Lymphovascular invasion					
Present	53 (85.5)	211 (65.9)	3.04 (1.45–6.40)	Pearson χ^2	0.002
Absent	9 (14.5)	109 (34.1)	Reference		
Venous invasion					
Present	14 (22.6)	22 (6.9)	3.95 (1.89–8.25)	Pearson χ^2	< 0.001
Absent	48 (77.4)	298 (93.1)	Reference		
Perineural invasion					
Present	36 (58.1)	124 (38.8)	2.19 (1.26–3.80)	Pearson χ^2	0.005
Absent	26 (41.9)	196 (61.2)	Reference		

Bd – tumor budding grade, dMMR – deficient mismatch repair, MMR – mismatch repair, OR – odds ratio, PDC – poorly differentiated clusters, pMMR – proficient mismatch repair

* Radial resection margin status was available for 118 patients.

Data are presented as n (%). Unadjusted odds ratios (ORs) and 95% CI were calculated for the presence of synchronous liver metastasis. For variables with more than two categories, category-specific ORs were calculated relative to the indicated reference category, whereas the reported p-value represents the overall association across all categories. All p-values are two-sided.

sociation (OR = 2.22, 95% CI: 0.96–5.12; $p = 0.062$), while perineural invasion and PDC grade were not significant (Table VI). The area under the receiver operating characteristic curve was 0.741.

Synchronous peritoneal metastasis

Univariable analysis showed significant associations of peritoneal metastasis with younger age, lymph node metastasis, tumor deposits, histological subtype, tumor budding grade, intratumoral lymphocytic infiltration, and perineural invasion (all $p < 0.05$) (Table VII).

Because only 13 events occurred, the restricted exploratory model included two binary variables selected before model fitting based on their clinicopathological relevance. Non-conventional histological subtype (OR = 38.91, 95% CI: 9.33–162.23; $p < 0.001$) and tumor deposits (OR = 13.53, 95% CI: 3.05–60.08; $p < 0.001$) were associated with higher odds of peritoneal metastasis (Table VIII). The area under the receiver operating characteristic curve was 0.879. The wide confidence intervals indicate substantial imprecision; therefore, these findings should be considered exploratory.

Table VI. Multivariable logistic regression analysis of factors associated with synchronous liver metastasis

PARAMETERS	COMPARISON	OR	95% CI	P-VALUE
MMR status	dMMR vs. pMMR	0.27	0.08–0.90	0.033
Lymph node metastasis	Present vs. absent	3.23	1.62–6.43	< 0.001
Proximal/distal resection margin	Positive vs. negative	23.78	2.08–271.66	0.011
Perineural invasion	Present vs. absent	1.47	0.79–2.74	0.225
Venous invasion	Present vs. absent	2.22	0.96–5.12	0.062
PDC	High vs. low/intermediate	1.19	0.64–2.21	0.578

AUC – area under the receiver operating characteristic curve, dMMR – deficient mismatch repair, MMR – mismatch repair, OR – odds ratio, PDC – poorly differentiated clusters, pMMR – proficient mismatch repair

Model performance: Omnibus test $\chi^2 = 42.497$, $p < 0.001$; Hosmer-Lemeshow test $p = 0.839$; $-2 \log \text{likelihood} = 296.314$; Cox and Snell $R^2 = 0.105$;

Nagelkerke $R^2 = 0.179$; AUC = 0.741.

Table VII. Univariable analysis of the associations between clinicopathological characteristics and synchronous peritoneal metastasis

PARAMETERS	PERITONEAL METASTASIS PRESENT (N = 13)	ABSENT (N = 369)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Age [years]					
> 55	6 (46.2)	277 (75.1)	0.28 (0.09–0.87)	Fisher's exact	0.046
≤ 55	7 (53.8)	92 (24.9)	Reference		
Sex					
Male	8 (61.5)	218 (59.1)	1.11 (0.36–3.45)	Pearson χ^2	0.859
Female	5 (38.5)	151 (40.9)	Reference		
Tumor size [cm]					
> 5.5	6 (46.2)	95 (25.7)	2.47 (0.81–7.54)	Fisher's exact	0.114
≤ 5.5	7 (53.8)	274 (74.3)	Reference		
Tumor location					
Right-sided	3 (23.1)	110 (29.8)	0.71 (0.19–2.62)	Fisher's exact	0.763
Left-sided	10 (76.9)	259 (70.2)	Reference		
Invasion depth (pT stage)					
pT3–4	13 (100.0)	345 (93.5)	NE	Fisher's exact	1.000
pT1–2	0 (0.0)	24 (6.5)	Reference		
Tumor perforation					
Present	2 (15.4)	26 (7.0)	2.40 (0.50–11.40)	Fisher's exact	0.245
Absent	11 (84.6)	343 (93.0)	Reference		
Lymph node metastasis					
Present	12 (92.3)	203 (55.0)	9.81 (1.26–76.25)	Pearson χ^2	0.008
Absent	1 (7.7)	166 (45.0)	Reference		
Tumor deposits					
Present	6 (46.2)	32 (8.7)	9.03 (2.86–28.48)	Fisher's exact	< 0.001
Absent	7 (53.8)	337 (91.3)	Reference		
Proximal/distal resection margin					
Positive	1 (7.7)	4 (1.1)	7.60 (0.79–73.28)	Fisher's exact	0.160
Negative	12 (92.3)	365 (98.9)	Reference		
Radial resection margin*					
Positive	0 (0.0)	22 (19.0)	NE	Fisher's exact	1.000
Negative	2 (100.0)	94 (81.0)	Reference		
MMR status					
dMMR	3 (23.1)	56 (15.2)	1.68 (0.45–6.28)	Fisher's exact	0.433
pMMR	10 (76.9)	313 (84.8)	Reference		

Table VII. Cont.

PARAMETERS	PERITONEAL METASTASIS PRESENT (N = 13)	ABSENT (N = 369)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Histological subtype					
Mucinous adenocarcinoma	6 (46.2)	25 (6.8)	20.58 (5.45–77.72)	Fisher-Freeman-Halton exact test (Monte Carlo)	< 0.001
Conventional adenocarcinoma	4 (30.8)	343 (93.0)	Reference		
Signet-ring cell carcinoma	3 (23.1)	1 (0.3)	257.25 (21.79–3036.91)		
Tumor grade					
High grade (poorly differentiated)	4 (30.8)	48 (13.0)	2.97 (0.88–10.03)	Fisher's exact	0.086
Low grade (well/moderately differentiated)	9 (69.2)	321 (87.0)	Reference		
PDC					
High	9 (69.2)	165 (44.7)	2.78 (0.84–9.20)	Pearson χ^2	0.081
Low/intermediate	4 (30.8)	204 (55.3)	Reference		
Tumor budding					
High	11 (84.6)	171 (46.3)	7.59 (0.97–59.59)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.035
Intermediate	1 (7.7)	80 (21.7)	1.48 (0.09–23.93)		
Low	1 (7.7)	118 (32.0)	Reference		
Intratumoral lymphocytic infiltration					
Absent	10 (76.9)	87 (23.6)	9.54 (2.57–35.47)	Fisher-Freeman-Halton exact test (Monte Carlo)	< 0.001
Low	3 (23.1)	249 (67.5)	Reference		
High	0 (0.0)	33 (8.9)	NE		
Peritumoral lymphocytic infiltration					
High	4 (30.8)	196 (53.1)	Reference	Pearson χ^2	0.113
Low	9 (69.2)	173 (46.9)	2.55 (0.77–8.42)		
Crohn-like lymphoid reaction					
Present	10 (76.9)	232 (62.9)	1.97 (0.53–7.28)	Fisher's exact	0.389
Absent	3 (23.1)	137 (37.1)	Reference		
Lymphovascular invasion					
Present	11 (84.6)	253 (68.6)	2.52 (0.55–11.56)	Fisher's exact	0.360
Absent	2 (15.4)	116 (31.4)	Reference		
Venous invasion					
Present	2 (15.4)	34 (9.2)	1.79 (0.38–8.42)	Fisher's exact	0.351
Absent	11 (84.6)	335 (90.8)	Reference		
Perineural invasion					
Present	11 (84.6)	149 (40.4)	8.12 (1.77–37.17)	Pearson χ^2	0.001
Absent	2 (15.4)	220 (59.6)	Reference		

dMMR – deficient mismatch repair, MMR – mismatch repair, NE – not estimable, OR – odds ratio, PDC – poorly differentiated clusters, pMMR – proficient mismatch repair

* Radial resection margin status was available for 118 patients.

Data are presented as n (%). Unadjusted odds ratios (ORs) and 95% CI were calculated for the presence of synchronous peritoneal metastasis. For variables with more than two categories, category-specific ORs were calculated relative to the indicated reference category, whereas the reported p-value represents the overall association across all categories. All p-values are two-sided. Not estimable indicates that OR could not be estimated because of a zero cell.

Table VIII. Restricted exploratory multivariable logistic regression analysis for synchronous peritoneal metastasis

PARAMETERS	COMPARISON	OR	95% CI	P-VALUE
Histological subtype	Non-conventional vs. conventional	38.91	9.33–162.23	< 0.001
Tumor deposits	Present vs. absent	13.53	3.05–60.08	< 0.001

AUC – area under the receiver operating characteristic curve, OR – odds ratio

Model performance: Omnibus test $\chi^2 = 41.913$, $p < 0.001$; $-2 \log \text{likelihood} = 71.532$; Cox and Snell $R^2 = 0.104$; Nagelkerke $R^2 = 0.404$; Hosmer–Lemeshow test $p = 0.770$; AUC = 0.879.

Non-conventional histological subtype comprised mucinous adenocarcinoma and signet-ring cell carcinoma.

Synchronous lung metastasis

In univariable analysis, tumor perforation showed a borderline association with lung metastasis (25.0% vs. 6.8%; Fisher's exact $p = 0.050$). No other association reached statistical significance (Table IX).

The restricted two-variable exploratory model showed an association between tumor perforation and higher odds of lung metastasis (OR = 4.22, 95% CI: 1.06–16.83; $p = 0.042$), whereas low peritumoral lymphocytic infiltration did not reach statistical significance (OR = 3.24, 95% CI: 0.86–12.20; $p = 0.084$) (Table X). The area under the receiver operating characteristic curve was 0.703. Given the limited number of events and wide confidence intervals, these findings should be considered exploratory.

Synchronous bone metastasis

Among six patients with bone metastasis, five (83.3%) had low-grade budding, one (16.7%) had high-grade budding, and none had intermediate-grade budding ($p = 0.036$). No other characteristic was significantly associated with bone metastasis (Table XI). Multivariable analysis was not performed, and this unexpected distribution should be considered descriptive and hypothesis-generating.

Additional comparisons among patients with synchronous metastatic disease

Among the 83 patients with synchronous metastatic disease, liver involvement was associated with age, histological subtype, tumor budding grade, and intratumoral

Table IX. Univariable analysis of the associations between clinicopathological characteristics and synchronous lung metastasis

PARAMETERS	LUNG METASTASIS PRESENT (N = 12)	ABSENT (N = 370)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Age [years]					
> 55	11 (91.7)	272 (73.5)	3.96 (0.51–31.10)	Fisher's exact	0.199
≤ 55	1 (8.3)	98 (26.5)	Reference		
Sex					
Male	8 (66.7)	218 (58.9)	1.39 (0.41–4.71)	Fisher's exact	0.768
Female	4 (33.3)	152 (41.1)	Reference		
Tumor size [cm]					
> 5.5	3 (25.0)	98 (26.5)	0.93 (0.25–3.49)	Fisher's exact	1.000
≤ 5.5	9 (75.0)	272 (73.5)	Reference		
Tumor location					
Right-sided	5 (41.7)	108 (29.2)	1.73 (0.54–5.58)	Fisher's exact	0.349
Left-sided	7 (58.3)	262 (70.8)	Reference		
Invasion depth (pT stage)					
pT3–4	12 (100.0)	346 (93.5)	NE	Fisher's exact	1.000
pT1–2	0 (0.0)	24 (6.5)	Reference		
Tumor perforation					
Present	3 (25.0)	25 (6.8)	4.60 (1.17–18.07)	Fisher's exact	0.050
Absent	9 (75.0)	345 (93.2)	Reference		
Lymph node metastasis					
Present	9 (75.0)	206 (55.7)	2.39 (0.64–8.96)	Fisher's exact	0.243
Absent	3 (25.0)	164 (44.3)	Reference		
Tumor deposits					
Present	3 (25.0)	35 (9.5)	3.19 (0.83–12.33)	Fisher's exact	0.106
Absent	9 (75.0)	335 (90.5)	Reference		
Proximal/distal resection margin					
Positive	0 (0.0)	5 (1.4)	NE	Fisher's exact	1.000
Negative	12 (100.0)	365 (98.6)	Reference		
Radial resection margin*					
Positive	2 (33.3)	20 (17.9)	2.30 (0.39–13.43)	Fisher's exact	0.311
Negative	4 (66.7)	92 (82.1)	Reference		

Table IX. Cont.

PARAMETERS	LUNG METASTASIS PRESENT (N = 12)	ABSENT (N = 370)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
MMR status					
dMMR	0 (0.0)	59 (15.9)	NE	Fisher's exact	0.227
pMMR	12 (100.0)	311 (84.1)	Reference		
Histological subtype					
Mucinous adenocarcinoma	0 (0.0)	31 (8.4)	NE	Fisher-Freeman-Halton exact test (Monte Carlo)	0.082
Conventional adenocarcinoma	11 (91.7)	336 (90.8)	Reference		
Signet-ring cell carcinoma	1 (8.3)	3 (0.8)	10.18 (0.98–105.86)		
Tumor grade					
High grade (poorly differentiated)	2 (16.7)	50 (13.5)	1.28 (0.27–6.01)	Fisher's exact	0.671
Low grade (well/moderately differentiated)	10 (83.3)	320 (86.5)	Reference		
PDC					
High	5 (41.7)	169 (45.7)	0.85 (0.26–2.73)	Fisher's exact	1.000
Low/intermediate	7 (58.3)	201 (54.3)	Reference		
Tumor budding					
High	7 (58.3)	175 (47.3)	0.91 (0.28–2.94)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.168
Intermediate	0 (0.0)	81 (21.9)	NE		
Low	5 (41.7)	114 (30.8)	Reference		
Intratumoral lymphocytic infiltration					
Absent	6 (50.0)	91 (24.6)	2.11 (0.24–18.20)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.100
Low	5 (41.7)	247 (66.8)	0.65 (0.07–5.72)		
High	1 (8.3)	32 (8.6)	Reference		
Peritumoral lymphocytic infiltration					
High	3 (25.0)	197 (53.2)	Reference	Fisher's exact	0.076
Low	9 (75.0)	173 (46.8)	3.42 (0.91–12.82)		
Crohn-like lymphoid reaction					
Present	7 (58.3)	235 (63.5)	0.80 (0.25–2.58)	Fisher's exact	0.765
Absent	5 (41.7)	135 (36.5)	Reference		
Lymphovascular invasion					
Present	11 (91.7)	253 (68.4)	5.09 (0.65–39.87)	Fisher's exact	0.115
Absent	1 (8.3)	117 (31.6)	Reference		
Venous invasion					
Present	1 (8.3)	35 (9.5)	0.87 (0.11–6.94)	Fisher's exact	1.000
Absent	11 (91.7)	335 (90.5)	Reference		
Perineural invasion					
Present	7 (58.3)	153 (41.4)	1.99 (0.62–6.37)	Fisher's exact	0.252
Absent	5 (41.7)	217 (58.6)	Reference		

dMMR – deficient mismatch repair, MMR – mismatch repair, NE – not estimable, OR – odds ratio, PDC – poorly differentiated clusters, pMMR – proficient mismatch repair
 * Radial resection margin status was available for 118 patients.

Data are presented as n (%). Unadjusted odds ratios (ORs) and 95% CI were calculated for the presence of synchronous lung metastasis. For variables with more than two categories, category-specific ORs were calculated relative to the indicated reference category, whereas the reported p-value represents the overall association across all categories. All p-values are two-sided. Not estimable indicates that OR could not be estimated because of a zero cell.

Table X. Restricted exploratory multivariable logistic regression analysis for synchronous lung metastasis

PARAMETERS	COMPARISON	OR	95% CI	P-VALUE
Tumor perforation	Present vs. absent	4.22	1.06–16.83	0.042
Peritumoral lymphocytic infiltration	Low vs. high	3.24	0.86–12.20	0.084

AUC – area under the receiver operating characteristic curve, OR – odds ratio

Model performance: Omnibus test $\chi^2 = 7.175$, $p = 0.028$; $-2 \log \text{likelihood} = 99.497$; Cox and Snell $R^2 = 0.019$; Nagelkerke $R^2 = 0.076$; Hosmer-Lemeshow test $p = 0.770$; AUC = 0.703.

Table XI. Univariable analysis of the associations between clinicopathological characteristics and synchronous bone metastasis

PARAMETERS	BONE METASTASIS PRESENT (N = 6)	ABSENT (N = 376)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Age [years]					
> 55	3 (50.0)	280 (74.5)	0.34 (0.07–1.73)	Fisher's exact	0.183
≤ 55	3 (50.0)	96 (25.5)	Reference		
Sex					
Male	4 (66.7)	222 (59.0)	1.39 (0.25–7.67)	Fisher's exact	1.000
Female	2 (33.3)	154 (41.0)	Reference		
Tumor size [cm]					
> 5.5	1 (16.7)	100 (26.6)	0.55 (0.06–4.78)	Fisher's exact	1.000
≤ 5.5	5 (83.3)	276 (73.4)	Reference		
Tumor location					
Right-sided	1 (16.7)	112 (29.8)	0.47 (0.05–4.08)	Fisher's exact	0.675
Left-sided	5 (83.3)	264 (70.2)	Reference		
Invasion depth (pT stage)					
pT3–4	5 (83.3)	353 (93.9)	0.33 (0.04–2.91)	Fisher's exact	0.324
pT1–2	1 (16.7)	23 (6.1)	Reference		
Tumor perforation					
Present	0 (0.0)	28 (7.4)	NE	Fisher's exact	1.000
Absent	6 (100.0)	348 (92.6)	Reference		
Lymph node metastasis					
Present	5 (83.3)	210 (55.9)	3.95 (0.46–34.16)	Fisher's exact	0.237
Absent	1 (16.7)	166 (44.1)	Reference		
Tumor deposits					
Present	0 (0.0)	38 (10.1)	NE	Fisher's exact	1.000
Absent	6 (100.0)	338 (89.9)	Reference		
Proximal/distal resection margin					
Positive	0 (0.0)	5 (1.3)	NE	Fisher's exact	1.000
Negative	6 (100.0)	371 (98.7)	Reference		
Radial resection margin*					
Positive	2 (40.0)	20 (17.7)	3.10 (0.49–19.78)	Fisher's exact	0.233
Negative	3 (60.0)	93 (82.3)	Reference		
MMR status					
dMMR	0 (0.0)	59 (15.7)	NE	Fisher's exact	0.596
pMMR	6 (100.0)	317 (84.3)	Reference		
Histological subtype					
Mucinous adenocarcinoma	0 (0.0)	31 (8.2)	NE	Fisher-Freeman-Halton exact test (Monte Carlo)	1.000
Conventional adenocarcinoma	6 (100.0)	341 (90.7)	Reference		
Signet-ring cell carcinoma	0 (0.0)	4 (1.1)	NE		
Tumor grade					
High grade (poorly differentiated)	1 (16.7)	51 (13.6)	1.27 (0.15–11.13)	Fisher's exact	0.587
Low grade (well/moderately differentiated)	5 (83.3)	325 (86.4)	Reference		
PDC					
High	2 (33.3)	172 (45.7)	0.59 (0.11–3.28)	Fisher's exact	0.693
Low/intermediate	4 (66.7)	204 (54.3)	Reference		

Table XI. Cont.

PARAMETERS	BONE METASTASIS PRESENT (N = 6)	ABSENT (N = 376)	UNADJUSTED OR (95% CI)	TEST	P-VALUE
Tumor budding					
High	1 (16.7)	181 (48.1)	0.13 (0.01–1.09)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.036
Intermediate	0 (0.0)	81 (21.5)	NE		
Low	5 (83.3)	114 (30.3)	Reference		
Intratumoral lymphocytic infiltration					
Absent	3 (50.0)	94 (25.0)	2.65 (0.53–13.36)	Fisher-Freeman-Halton exact test (Monte Carlo)	0.403
Low	3 (50.0)	249 (66.2)	Reference		
High	0 (0.0)	33 (8.8)	NE		
Peritumoral lymphocytic infiltration					
High	3 (50.0)	197 (52.4)	Reference	Fisher's exact	1.000
Low	3 (50.0)	179 (47.6)	1.10 (0.22–5.52)		
Crohn-like lymphoid reaction					
Present	5 (83.3)	237 (63.0)	2.93 (0.34–25.36)	Fisher's exact	0.421
Absent	1 (16.7)	139 (37.0)	Reference		
Lymphovascular invasion					
Present	3 (50.0)	261 (69.4)	0.44 (0.09–2.22)	Fisher's exact	0.378
Absent	3 (50.0)	115 (30.6)	Reference		
Venous invasion					
Present	1 (16.7)	35 (9.3)	1.95 (0.22–17.15)	Fisher's exact	0.450
Absent	5 (83.3)	341 (90.7)	Reference		
Perineural invasion					
Present	2 (33.3)	158 (42.0)	0.69 (0.12–3.81)	Fisher's exact	1.000
Absent	4 (66.7)	218 (58.0)	Reference		

dMMR – deficient mismatch repair, MMR – mismatch repair, NE – not estimable, OR – odds ratio, PDC – poorly differentiated clusters, pMMR – proficient mismatch repair

* Radial resection margin status was available for 118 patients.

Data are presented as n (%). Unadjusted odds ratios (ORs) and 95% CI were calculated for the presence of synchronous bone metastasis. For variables with more than two categories, category-specific ORs were calculated relative to the indicated reference category, whereas the reported p-value represents the overall association across all categories. All p-values are two-sided. NE indicates that OR could not be estimated because of a zero cell.

lymphocytic infiltration (all $p < 0.05$) (Supplementary Table S1). Peritoneal involvement was associated with tumor deposits, MMR status, histological subtype, and intratumoral lymphocytic infiltration (all $p < 0.05$) (Supplementary Table S2). Because these analyses involved multiple comparisons within a small subgroup, they should be considered hypothesis-generating and not evidence of preferential organ tropism.

Discussion

In this study, synchronous distant metastasis was associated with multiple routinely assessed features reflecting disease burden, local invasiveness, molecular characteristics, and tumor-host interactions. Different patterns of association were also observed across individual metastatic sites. A strength of the study is the assessment of both overall synchronous metastasis and site-specific associations within the same cohort.

Synchronous distant metastasis

Two multivariable models were constructed to reduce collinearity among biologically related variables. Model 1 demonstrated moderate discrimination (AUC = 0.739), whereas Model 2 showed more limited discrimination (AUC = 0.652). Because the models contained different variables and their AUCs were not formally compared, this difference does not establish the relative importance of the represented biological processes. Rather, it supports the multifactorial nature of metastatic dissemination.

Lymph node metastasis was strongly associated with synchronous distant metastasis and remained associated with liver metastasis in the organ-specific model. Previous studies have linked lymph node involvement and invasive histopathological features to synchronous metastasis and adverse outcomes [21, 23]. Regional nodal involvement may therefore indicate greater metastatic potential or disease burden, although the present

observational findings do not establish a direct pathway between lymphatic and hepatic dissemination.

Deficient mismatch repair was associated with lower odds of both overall synchronous metastasis and liver metastasis. Deficient mismatch repair CRCs are characterized by high mutational and neo-antigen burdens and prominent immune infiltration [17, 18], which have been proposed to contribute to their lower frequency of distant dissemination [24, 25]. Previous studies have similarly reported a reduced likelihood of metastasis and favorable stage-adjusted outcomes in selected patients with dMMR/microsatellite instability-high CRC [25–27]. Nevertheless, molecular data beyond MMR status were unavailable, and these associations should not be interpreted as evidence of a causal protective effect.

Low peritumoral lymphocytic infiltration was associated with higher odds of synchronous distant metastasis, with high infiltration as the reference category. This finding, together with the association observed for dMMR, is consistent with a potential relationship between host immune response and metastatic dissemination [18–20]. However, whether these variables represent related or independent biological processes could not be determined.

Perineural invasion remained independently associated with synchronous distant metastasis in Model 2. This finding is consistent with previous evidence linking perineural invasion to advanced clinicopathological features, lymph node involvement, and adverse outcomes [12, 23]. Conversely, lymphovascular invasion and tumor deposits showed borderline adjusted associations, while PDC grade was not independently associated. These findings may reflect correlations among adverse pathological features, limited precision, or residual confounding. Lymphovascular invasion and tumor deposits have previously been associated with metastatic disease and adverse outcomes [10, 15, 23, 28], and PDCs with adverse prognosis [14, 29, 30]; therefore, the present nonsignificant adjusted findings should not be interpreted as evidence of no association.

Histological subtype showed a strong association with peritoneal metastasis: non-conventional subtypes, comprising mucinous and signet-ring cell carcinomas, were associated with higher odds than conventional adenocarcinoma. This is consistent with previous reports of more frequent peritoneal dissemination in these subtypes [6, 7]. However, the estimate was based on only 13 peritoneal metastatic events and had a wide confidence interval, indicating substantial imprecision and possible sparse-data bias. It should therefore be considered exploratory rather than a precise measure of association.

Site-specific metastatic associations

Histopathological associations differed across metastatic sites. Although metastatic distribution may

be influenced by tumor biology and organ-specific microenvironments [4, 6, 8, 9], the present results demonstrate statistical associations rather than mechanisms of biological organ tropism.

Synchronous liver metastasis

The liver was the most frequent metastatic site, consistent with previous reports [3, 6]. Its predominance reflects portal venous drainage, although hepatic colonization may also involve tumor-microenvironment interactions described by the “seed and soil” hypothesis [8, 9].

Lymph node metastasis and positive proximal/distal resection margin were associated with higher odds of liver metastasis, whereas dMMR was associated with lower odds. The latter finding may relate to immune characteristics of dMMR CRC [18, 24, 25], but the underlying mechanism remains uncertain. The high OR for positive margin status was based on only five positive cases and had a wide confidence interval. Margin positivity may therefore represent locally advanced or technically difficult disease rather than a causal relationship with liver metastasis.

Synchronous peritoneal metastasis

Tumor deposits and non-conventional histological subtype were associated with higher odds of peritoneal metastasis. Peritoneal dissemination is thought to involve tumor-cell exfoliation and intraperitoneal implantation [8, 9]. The association with mucinous and signet-ring cell carcinomas is consistent with their reported tendency toward peritoneal involvement [6, 7], while tumor deposits have been linked to metastatic disease and adverse outcomes [15, 28, 31]. However, with only 13 events, the large estimates and wide confidence intervals indicate imprecision and possible sparse-data bias; thus, the restricted model remains exploratory.

Synchronous lung metastasis

Tumor perforation was associated with higher odds of lung metastasis in the restricted model. Perforation reflects advanced local disease and has been associated with recurrence and adverse outcomes [32, 33], although its specific relationship with lung metastasis is poorly characterized. The univariable association was borderline ($p = 0.050$), and the adjusted estimate (OR = 4.22, 95% CI: 1.06–16.83; $p = 0.042$) was based on only 12 events. Low peritumoral lymphocytic infiltration was not statistically significant. Accordingly, no firm conclusions can be drawn from this model.

Synchronous bone metastasis

Only six patients had bone metastasis, precluding multivariable analysis. The unexpected predominance

of low-grade budding ($p = 0.036$) conflicts with the established adverse significance of tumor budding [13, 22] and may represent a chance finding. The bone metastasis results should therefore be considered descriptive and hypothesis-generating.

Additional metastatic-only comparisons identified differences in age, histological subtype, tumor budding, and intratumoral lymphocytic infiltration according to liver involvement, and in tumor deposits, MMR status, histological subtype, and intratumoral lymphocytic infiltration according to peritoneal involvement. These analyses further illustrate differing histopathological profiles but remain exploratory because of small comparison groups, multiple testing, and inclusion of multiple-organ cases.

Overall, the site-specific findings require validation in larger independent cohorts and should not be interpreted as evidence of preferential organ tropism.

Limitations

A major strength of this study is the evaluation of overall and site-specific synchronous metastatic associations within the same cohort. All slides were independently reviewed by two pathologists, including a gastrointestinal pathologist, who were blinded to metastatic status. Tumor budding was assessed according to ITBCC recommendations, MMR status was evaluated immunohistochemically, and all examined parameters are routinely available in pathological practice.

The retrospective, single-center design and inclusion of only surgically treated patients may have introduced selection bias and limited generalizability, particularly to unresectable disease. Radial margin status was available for only 118 patients and was excluded from multivariable analyses. The small numbers of peritoneal, lung, and bone metastases increased the risks of sparse-data bias, overfitting, and imprecise estimates. Thus, the peritoneal and lung models remain exploratory despite restriction to two variables, whereas the bone analysis is descriptive.

In the primary site-specific analyses, each target-site group was compared with all patients without involvement of that site, including both nonmetastatic patients and patients with metastases elsewhere. Therefore, these analyses do not demonstrate preferential organ tropism. The metastatic-only comparisons were also limited by small sample sizes and inclusion of multiple-organ cases.

No adjustment for multiple comparisons was applied because the organ-specific analyses were exploratory, increasing the risk of false-positive findings. Additional limitations include temporal heterogeneity during the 2006–2018 inclusion period, molecular characterization limited to MMR status, lack of external validation, and the inability of the observational design to support causal conclusions.

Conclusions

Synchronous distant metastasis was associated with features reflecting disease burden, local invasiveness, tumor-host interactions, and MMR status, with different association patterns across metastatic sites. These findings do not establish preferential organ tropism. The peritoneal and lung results should be considered exploratory and the bone findings descriptive and hypothesis-generating. External validation is required before these observations can inform individual risk prediction or clinical management.

Disclosures

1. Institutional review board statement: This study was approved by the Ethics Committee of Kafkas University Faculty of Medicine (approval decision no.: KAÜ-TFEK 2025/07/15, dated: 24.09.2025).
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024; 74: 229-263.
2. Douaier J, Ravipati A, Grams B, Chowdhury S, Alatis O, Are C. Colorectal cancer-global burden, trends, and geographical variations. *J Surg Oncol* 2017; 115: 619-630.
3. VanderGeest LG, Lam-Boer J, Koopman M, Verhoef C, Elferink MA, de Wilt JH. Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. *Clin Exp Metastasis* 2015; 32: 457-465.
4. Shin AE, Giaccotti FG, Rustgi AK. Metastatic colorectal cancer: mechanisms and emerging therapeutics. *Trends Pharmacol Sci* 2023; 44: 222-236.
5. Kopetz S, Chang GJ, Overman MJ, Eng C, Sargent DJ, Larson DW, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. *J Clin Oncol* 2009; 27: 3677-3683.
6. Hugen N, van de Velde CJH, de Wilt JHW, Nagtegaal ID. Metastatic pattern in colorectal cancer is strongly influenced by histological subtype. *Ann Oncol* 2014; 25: 651-657.
7. Lian L, Xu XF, Shen XM, Huang TA, Li XM, Han SG, et al. Pattern of distant metastases and predictive nomograms in colorectal mucinous adenocarcinoma: a SEER analysis. *J Gastrointest Oncol* 2021; 12: 2906-2918.
8. Langley RR, Fidler IJ. The seed and soil hypothesis revisited: the role of tumor-stroma interactions in metastasis to different organs. *Int J Cancer* 2011; 128: 2527-2535.
9. Fidler IJ. The pathogenesis of cancer metastasis: the "seed and soil" hypothesis revisited. *Nat Rev Cancer* 2003; 3: 453-458.
10. Lim SB, Yu CS, Jang SJ, Kim TW, Kim JH, Kim JC. Prognostic significance of lymphovascular invasion in sporadic colorectal cancer. *Dis Colon Rectum* 2010; 53: 377-384.
11. Chand M, Siddiqui MR, Swift I, Brown G. Systematic review of prognostic importance of extramural venous invasion in rectal cancer. *World J Gastroenterol* 2016; 22: 1721-1726.
12. Kniijn N, Mogk SC, Teerenstra S, Simmer F, Nagtegaal ID. Perineural invasion is a strong prognostic factor in colorectal cancer: a systematic review. *Am J Surg Pathol* 2016; 40: 103-112.

13. Ryan É, Khaw YL, Creavin B, Geraghty R, Ryan EJ, Gibbons D, et al. Tumor budding and PDC grade are stage-independent predictors of clinical outcome in mismatch repair-deficient colorectal cancer. *Am J Surg Pathol* 2018; 42: 60-68.
14. Ueno H, Kajiura Y, Shimazaki H, Shinto E, Hashiguchi Y, Nakanishi K, et al. New criteria for histologic grading of colorectal cancer. *Am J Surg Pathol* 2012; 36: 193-201.
15. Emile SH, Emile MH, Garoufalia Z, Dourado J, Wexner SD. Association between tumor deposits and liver and lung metastases at diagnosis of colorectal cancer: a SEER-based analysis. *Surgery* 2025; 178: 108904.
16. Nagtegaal ID, Quirke P. What is the role for the circumferential margin in the modern treatment of rectal cancer? *J Clin Oncol* 2008; 26: 303-312.
17. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; 138: 2073-87.e3.
18. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 2015; 5: 43-51.
19. Ogino S, Noshio K, Irahara N, Meyerhardt JA, Baba Y, Shima K, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res* 2009; 15: 6412-6420.
20. He R, Huang S, Lu J, Su L, Gao X, Chi H. Unveiling the immune symphony: decoding colorectal cancer metastasis through immune interactions. *Front Immunol* 2024; 15: 1362709.
21. Li Q, Wang G, Luo J, Li B, Chen W. Clinicopathological factors associated with synchronous distant metastasis and prognosis of stage T1 colorectal cancer patients. *Sci Rep* 2021; 11: 8722.
22. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. *Mod Pathol* 2017; 30: 1299-1311.
23. Al-Sukhni E, Attwood K, Gabriel EM, LeVea CM, Kanehira K, Nurkin SJ. Lymphovascular and perineural invasion are associated with poor prognostic features and outcomes in colorectal cancer: a retrospective cohort study. *Int J Surg* 2017; 37: 42-49.
24. Buckowitz A, Knaebel HP, Benner A, Bläker H, Gebert J, Kienle P, et al. Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. *Br J Cancer* 2005; 92: 1746-1753.
25. Malesci A, Laghi L, Bianchi P, Delconte G, Randolph A, Torri V, et al. Reduced likelihood of metastases in patients with microsatellite-unstable colorectal cancer. *Clin Cancer Res* 2007; 13: 3831-3839.
26. Benatti P, Gafà R, Barana D, Marino M, Scarselli A, Pedroni M, et al. Microsatellite instability and colorectal cancer prognosis. *Clin Cancer Res* 2005; 11: 8332-8340.
27. Kang S, Na Y, Joung SY, Lee SI, Oh SC, Min BW. The significance of microsatellite instability in colorectal cancer after controlling for clinicopathological factors. *Medicine (Baltimore)* 2018; 97: e0019.
28. Bendari A, Ucar E, Bendari A, Hammoud H, Kiran FNU, Al-Refai R, et al. A high number of tumor deposits (≥ 4) is an isolated predictor of local recurrence and distant metastasis in non-metastatic colorectal cancer: a single center study. *Surg Exp Pathol* 2024; 7: 30.
29. Reggiani Bonetti L, Barresi V, Bettelli S, Domati F, Palmiere C. Poorly differentiated clusters (PDC) in colorectal cancer: what is and ought to be known. *Diagn Pathol* 2016; 11: 31.
30. Konishi T, Shimada Y, Lee LH, Cavalcanti MS, Hsu M, Smith JJ, et al. Poorly differentiated clusters predict colon cancer recurrence: an in-depth comparative analysis of invasive-front prognostic markers. *Am J Surg Pathol* 2018; 42: 705-714.
31. Lin Q, Wei Y, Ren L, Zhong Y, Qin C, Zheng P, et al. Tumor deposit is a poor prognostic indicator in patients who underwent simultaneous resection for synchronous colorectal liver metastases. *Onco Targets Ther* 2015; 8: 233-240.
32. Zamaray B, van Velzen RA, Snaebjornsson P, Consten ECJ, Tanis PJ, van Westreenen HL. Outcomes of patients with perforated colon cancer: a systematic review. *Eur J Surg Oncol* 2023; 49: 1-8.
33. Takagi M, Kim S, Suzuki M, Takayama T, Asano H. Factors associated with postoperative recurrence in perforated colorectal cancer: unraveling the high recurrence rate of perforated colorectal cancer. *World J Surg Oncol* 2025; 23: 139.

Address for correspondence

Özgecan Hamdemir

Department of Pathology, Faculty of Medicine
Kafkas University
Kafkas University Campus, 36100 Kars, Turkey
e-mail: ozgecandemir_92@hotmail.com