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Original Article

Biliary Duct Invasion in Colorectal Liver Metastases: Prevalence and Clinicopathologic Correlation in a Retrospective Cohort Study

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Abstract

Background and objectives: Biliary duct invasion (BDI) is an underrecognized growth pattern of colorectal cancer (CRC) liver metastases that can mimic intrahepatic cholangiocarcinoma or intraductal papillary neoplasm of the bile duct. Its prevalence and clinicopathologic associations remain unclear. This study aimed to determine the prevalence of BDI and its clinicopathologic associations in surgically resected CRC liver metastases.

Methods: We retrospectively analyzed 133 consecutive patients who underwent hepatic resection for CRC liver metastases. Clinicopathologic variables, including age, sex, resection type, tumor differentiation, lymphovascular invasion (LVI), tumor budding, surgical margin status, and primary tumor characteristics, were compared between BDI-positive and BDI-negative cases. Categorical variables were analyzed using the chi-square, Fisher exact, or Fisher-Freeman-Halton exact test, as appropriate; continuous variables were analyzed using the Mann-Whitney *U* test.

Results: BDI was identified in 19 of 133 cases (14.3%). Male sex showed the strongest numerical trend toward BDI (84.2% vs. 61.4%, $P = 0.096$), and LVI showed a nonsignificant numerical trend toward higher BDI rates (26.3% vs. 12.3%, $P = 0.149$). No clinicopathologic variable in the primary cohort analysis reached statistical significance after Bonferroni correction ($\alpha = 0.0050$).

Conclusions: BDI occurs in approximately 14% of surgically resected CRC liver metastases in this cohort. Male sex and LVI show nonsignificant numerical trends toward higher BDI rates, but these findings should be interpreted cautiously given the limited sample size. Accurate histopathologic recognition of BDI and its distinction from intrahepatic cholangiocarcinoma remain important for correct pathologic diagnosis. Further prospective studies are warranted to clarify the clinicopathologic and prognostic significance of BDI.

Introduction

Colorectal cancer (CRC) is among the most common malignancies worldwide, and the liver is the most frequent site of distant metastasis, with hepatic involvement occurring in approximately 25% of patients during the course of the disease.[1] Liver metastases may be present synchronously at initial diagnosis or develop metachronously after treatment of the primary tumor, and their presence substantially influences prognosis, treatment strategy, and overall survival. Surgical resection remains the only potentially curative treatment, with 5-year survival rates of 25–58% reported after resection.[2]

The mechanisms by which CRC cells colonize the liver are

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predominantly vascular: tumor cells disseminate through the portal venous system or hepatic arteries and form parenchymal metastatic deposits.[3] However, an alternative and much less common metastatic pattern, intrabiliary or endobiliary growth, has been recognized, in which tumor cells invade, replace, and proliferate along the bile duct epithelium without necessarily forming a discrete parenchymal mass.[4] Histologically, this pattern is characterized by lateral spread of tumor cells along intact basement membranes and, in some cases, the formation of intraluminal polypoid plugs.[5,6]

Biliary duct invasion (BDI) presents substantial diagnostic challenges. Clinically, patients with BDI may be difficult to distinguish from those with primary biliary tumors, particularly intrahepatic cholangiocarcinoma (iCCA), based on findings such as cholestatic or obstructive jaundice and imaging features. The two entities may also appear morphologically similar on histopathologic examination, especially when intrabiliary growth is extensive or a concomitant parenchymal component is absent.[5,6] Misdiagnosis may have important clinical consequences. CRC liver metastases misclassified as iCCA may be inappropriately deemed unresectable, potentially precluding curative surgical intervention and leading to administration of systemic therapies directed at primary biliary malignancies rather than colorectal carcinoma.[7,8]

Reported prevalence estimates of BDI in CRC liver metastases range from approximately 3.6% in Western surgical series to more than 40% in several Japanese cohorts, a discrepancy that may reflect differences in diagnostic awareness, histopathologic sampling protocols, and tumor biology.[9-12] Despite increasing recognition of BDI as a distinct metastatic growth pattern, its clinicopathologic characteristics and prognostic significance remain poorly defined, and comprehensive clinicopathologic studies are limited.

In this study, we aimed to determine the prevalence of BDI and its associated clinicopathologic parameters in a consecutive cohort of 133 patients who underwent hepatic resection for CRC liver metastases, describe the clinicomorphologic features used to distinguish BDI from iCCA, and discuss the surgical implications of BDI.

Materials and methods

Patients and study design

For this retrospective clinicopathologic cohort study, cases of histopathologically confirmed colorectal liver metastases were retrospectively identified from the institutional pathology archives at our institution between 2002 and 2018. Eligible patients were required to have available formalin-fixed, paraffin-embedded tissue blocks and sufficient clinicopathologic documentation.

A total of 167 cases were initially screened, of whom 133 patients met the eligibility criteria and were included in the final analysis. Patients were excluded if only biopsy material was available; if they had undergone local ablative therapy with or without surgical resection; if a complete pathologic response after neoadjuvant therapy precluded histopathologic evaluation of the metastatic tumor; if the specimen had been submitted solely for consultation; or if pathologic slides and/or essential clinicopathologic data were unavailable for review. The patient selection process is summarized in Figure 1.

Demographic, clinical, surgical, and pathologic data were retrieved from the institutional pathology information system and operative records. Liver metastases were classified as synchronous when hepatic resection was performed simultaneously with resection of the primary colorectal tumor or within 6 months after primary surgery and as metachronous when hepatic resection was performed more than 6 months after resection of the primary tumor. This study was reported in accordance with the STROBE guidelines.

Histologic criteria for BDI

BDI was defined as involvement of native intrahepatic bile ducts by metastatic colorectal adenocarcinoma, characterized by partial or complete replacement of the biliary epithelium by tumor cells and/or intraluminal tumor growth within preexisting bile ducts. Direct extension of metastatic glands into the surrounding hepatic parenchyma or secondary involvement of small bile ductules by external infiltration, without replacement of native biliary epithelium or intraluminal growth, was not considered BDI. The diagnosis was established on routine hematoxylin and eosin-stained sections according to previously published histopathologic descriptions of intrabiliary growth in colorectal liver metastases.[4,9-11]

Differential diagnosis of intrahepatic cholangiocarcinoma

Distinguishing primary iCCA from CRC liver metastases was facilitated by well-defined morphologic criteria. Features favoring CRC metastasis over iCCA included a predominantly cribriform or tubulovillous glandular architecture, elongated nuclei, intraluminal necrotic debris, and absence of desmoplastic stroma. Marked nuclear pleomorphism, dysplastic *in situ* changes in the adjacent biliary epithelium, abundant desmoplastic stroma, and perineural invasion favored primary iCCA. Immunohistochemistry was not performed routinely in all cases but was reserved for diagnostically challenging lesions. Immunohistochemical studies were performed in 53 of 133 cases, particularly when the primary tumor had been diagnosed at another institution, in poorly differentiated metastatic tumors, or when other primary tumors were included in the differential diagnosis. A subset of immunohistochemical markers, including CK7, CK20, CDX2, and SATB2, was selectively applied on a case-by-case basis to support a diagnosis of metastatic colorectal adenocarcinoma. None of these cases required immunohistochemistry specifically because iCCA was included in the differential diagnosis. When present, a CK20+/CDX2+/SATB2+/CK7− profile supported colorectal origin (Figs. 2 and 3).

Pathologic assessment

All resection specimens were fixed in 10% neutral-buffered formalin before gross examination. Gross examination was performed according to the standardized institutional protocol used at our tertiary referral center. Metastatic lesions measuring ≤ 3 cm were submitted entirely for histologic examination. For lesions > 3 cm, the largest cross-sectional slice was completely mapped and submitted, and at least one additional tissue block was obtained from each remaining slice. Particular attention was paid to sampling the tumor-liver interface, and each metastatic lesion was sampled to include both the interface with the

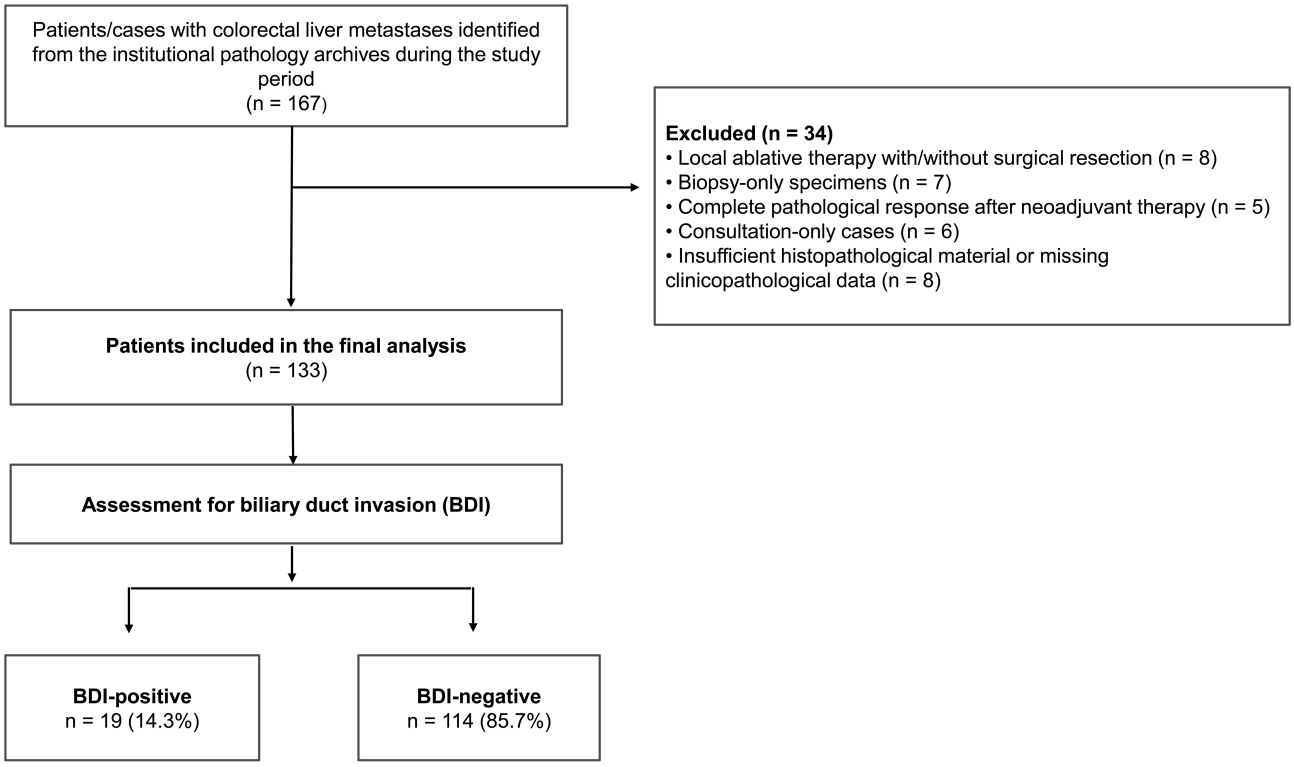


Fig. 1. Patient selection flowchart.

surrounding liver parenchyma and adjacent uninvolved liver tissue. In patients with multiple metastatic lesions, all lesions were sampled according to the same standardized protocol. Tissue was subsequently processed, embedded in paraffin, and sectioned at 5 μm for hematoxylin and eosin staining.

Macroscopic assessment included tumor location, resection type, number of metastatic foci, and maximum lesion diameter. Microscopic evaluation included metastatic tumor differentiation (low grade [well- or moderately differentiated adenocarcinoma], high grade [poorly differentiated adenocarcinoma], or mucinous, according to current pathologic practice), presence or absence of lymphovascular invasion (LVI), BDI status, tumor budding, and parenchymal resection margin status. For primary tumors, cases in which differentiation could not be reliably assessed following neoadjuvant therapy were classified as "not assessable after neoadjuvant therapy." LVI was evaluated on routine hematoxylin and eosin-stained sections and was defined as unequivocal tumor cell clusters within endothelial-lined vascular or lymphatic spaces. When retraction artifact could not be confidently distinguished from true LVI, CD34 immunohistochemistry was performed to assist with evaluation. For metastatic lesions, the invasive front was defined as the tumor-liver interface. Tumor budding was assessed at the hotspot within this interface according to the morphologic principles of the International Tumor Budding Consensus Conference; a tumor bud was defined as a single tumor cell or a cluster of up to four tumor cells.[13] Because no internationally accepted grading system currently exists for colorectal liver metastases, tumor budding was recorded as present or absent rather than categorized as low, intermediate, or high grade.[14] Surgical margin status was assessed histologically, and a positive margin was defined as the presence of tumor at the inked resection margin. Margin status reported in this study refers to the parenchymal resection margin. All clinicopathologic variables were evaluated and recorded at the patient level. In patients with multiple metastatic lesions, a patient was classified as BDI-positive if BDI was identified in any examined metastatic lesion. In patients with multiple simultaneous metastases, the largest lesion was used for microscopic grading because pathologic features were concordant across lesions when multiple samples were examined. Primary CRC data, including differentiation grade, lymph node metastasis status, and tumor diameter, were recorded when primary tumor resection specimens were available in the institutional archive.

Statistical analysis

Statistical analyses were performed using Python (SciPy v1.11). Categorical variables were compared using the chi-square test or exact tests, as appropriate. For 2×2 contingency tables, the chi-square test with Yates' continuity correction (scipy.stats.chi2_contingency) was used when all expected cell counts were ≥5; Fisher's exact test (scipy.stats.fisher_exact) was used when any expected cell count was <5. For contingency tables with more than two categories in either dimension, the Fisher-Freeman-Halton exact test — a generalization of Fisher's exact test to r×c tables, not natively available in SciPy v1.11 and computed here using a custom exact-enumeration implementation validated against

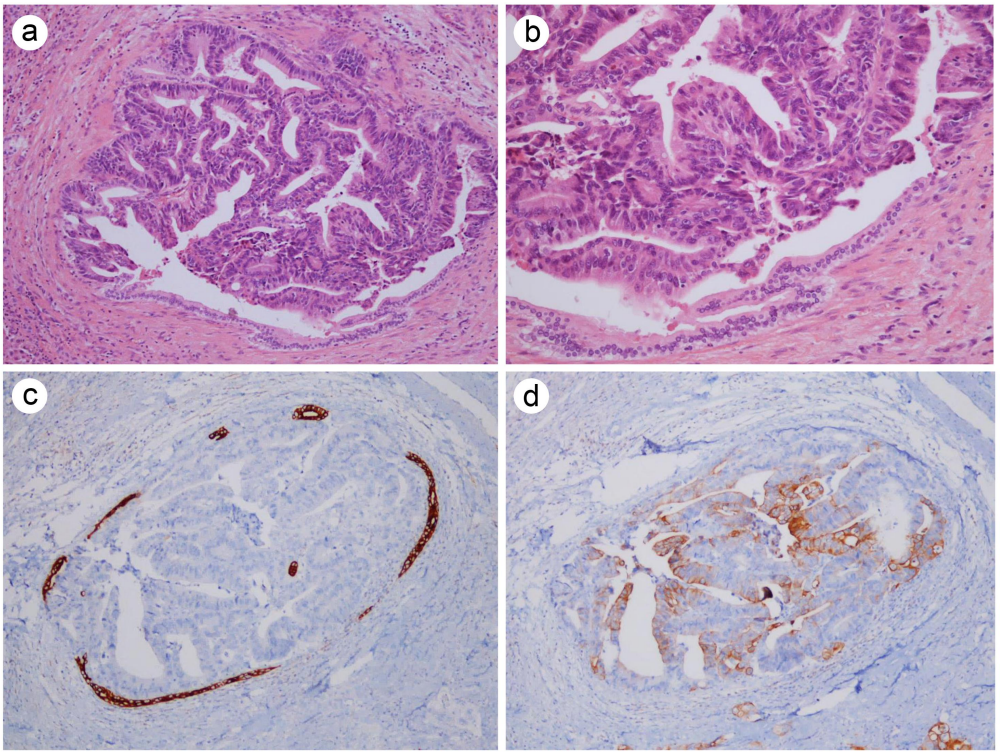


Fig. 2. Histopathologic and immunohistochemical features of biliary duct invasion in colorectal liver metastasis. (a) H&E, ×10: colorectal adenocarcinoma cells forming cribriform glandular structures within the bile duct lumen, with partial replacement of the native biliary epithelium; residual nonneoplastic biliary epithelial cells are visible at the periphery. (b) H&E, ×20: higher magnification showing nuclear elongation, intraluminal necrotic debris, and absence of a desmoplastic stromal reaction. (c) CK7 immunohistochemistry, ×10: selective immunoreactivity in residual nonneoplastic biliary epithelial cells; tumor cells are CK7 negative. (d) CK20 immunohistochemistry, ×10: strong cytoplasmic CK20 positivity in tumor cells, supporting colorectal origin. CK7, cytokeratin 7; CK20, cytokeratin 20; H&E, hematoxylin and eosin.

scipy.stats.fisher_exact on 2×2 tables — was applied under the same expected-cell-count criterion. To account for multiple comparisons among the 10 prespecified clinicopathologic comparisons assessed in the primary cohort analysis (Table 1), a Bonferroni-corrected significance threshold of α=0.0050 (0.05/10) was applied. The primary tumor subgroup analysis (Table 2) was considered exploratory because of the smaller sample size and missing data, and p values were reported without correction for multiple comparisons. Continuous variables were analyzed using the Mann-Whitney U test because their distributions were nonnormal based on histogram inspection and Shapiro-Wilk testing. Continuous data are presented as median [interquartile range (IQR)].

Results

Cohort characteristics

The study cohort comprised 133 patients: 86 men (64.7%) and 47 women (35.3%), with a median age of 60 years (IQR, 52–67; range, 22–82 years). Surgical procedures included 91 segmentectomies (68.4%) and 42 metastasectomies (31.6%). Ninety patients (67.7%) had a single metastatic focus, and 43 (32.3%) had multiple foci. The median diameter of the largest metastatic lesion was 3.5 cm (IQR, 1.8–5.0 cm; range, 0.6–16.0 cm).

Histopathologic analysis of metastatic foci identified 110 low-grade adenocarcinomas (82.7%), 11 high-grade adenocarcinomas (8.3%), and 12 mucinous adenocarcinomas (9.0%). LVI was present in 19 cases (14.3%), tumor budding in 30 cases (22.6%), and positive surgical margins in 61 cases (45.9%). Perineural invasion was absent in all cases.

Primary tumor specimens were available for 100 patients (75.2%). Among these, 59 patients (59.0%) had synchronous metastases and 41 (41.0%) had metachronous metastases. Primary tumors were classified as 80 low-grade adenocarcinomas (80.0%), 9 high-grade adenocarcinomas (9.0%), and 7 mucinous adenocarcinomas (7.0%). Primary tumor differentiation was not assessable after neoadjuvant therapy in four patients. Primary tumor lymph node metastasis was present in 66 of 100 patients (66.0%).

Prevalence and clinicopathologic associations of BDI

Microscopic BDI was identified in 19 of 133 surgically resected cases (14.3%). One case showed a predominantly intrabiliary growth pattern with only minimal focal parenchymal invasion. Preoperative imaging showed a hepatic mass with biliary duct dilatation, and the differential diagnosis included both colorectal liver metastasis and a second primary biliary malignancy;

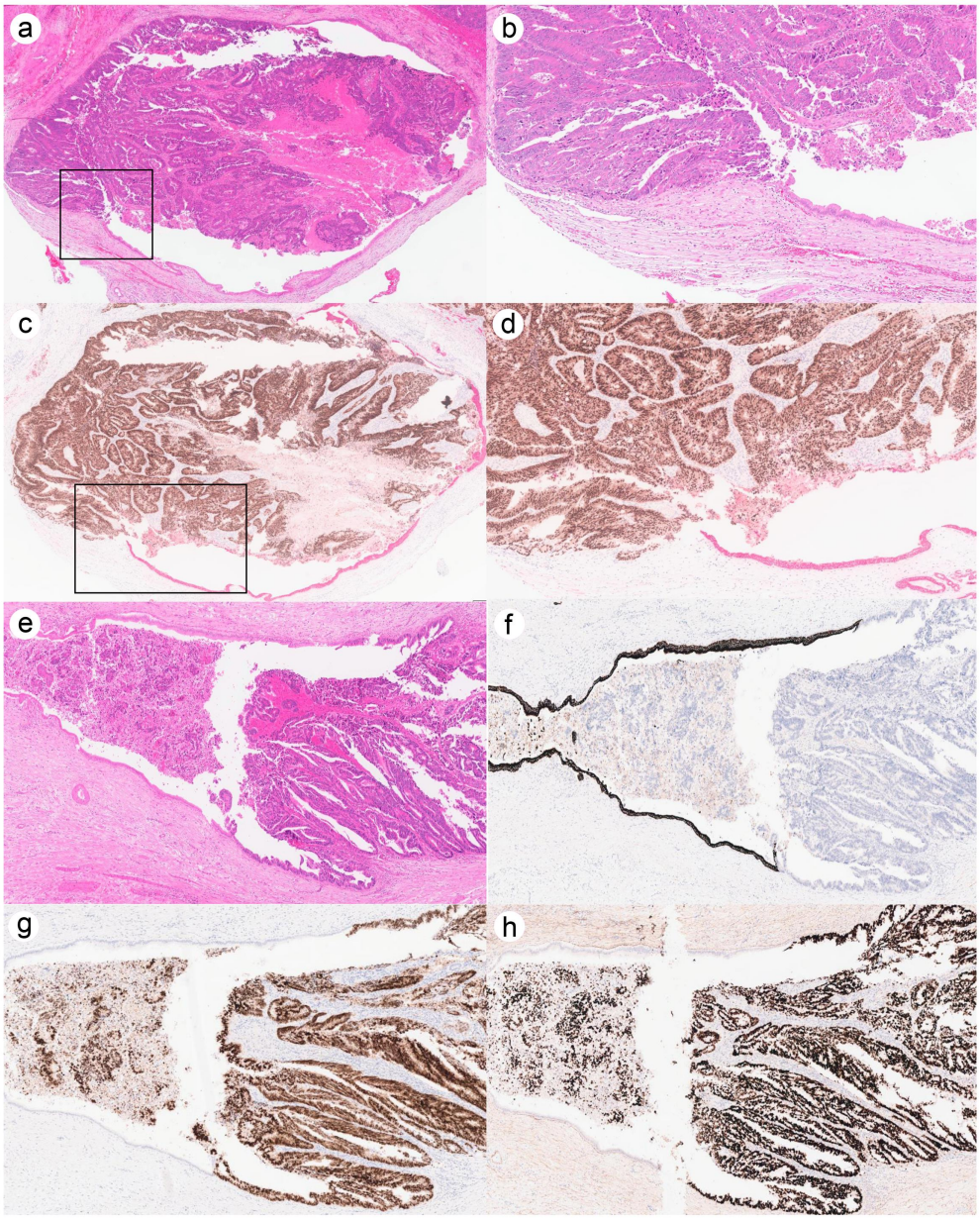


Fig. 3. Histopathologic and immunohistochemical features of biliary duct invasion (BDI) in colorectal liver metastases. (a) Low-power hematoxylin and eosin (H&E) image (×2) showing metastatic colorectal adenocarcinoma colonizing a native intrahepatic bile duct. Residual native biliary epithelium is preserved and is particularly evident along the lower aspect of the duct. (b) Higher-magnification H&E image (×8) of the boxed area in panel a, showing the interface between metastatic colorectal adenocarcinoma and residual native biliary epithelium. (c) Low-power (×2) dual immunohistochemical stain (CK7, red; CDX2, brown) corresponding to the lesion shown in panel a. Native biliary epithelium shows diffuse cytoplasmic CK7 positivity (red), whereas metastatic colorectal adenocarcinoma shows diffuse nuclear CDX2 positivity (brown). The dual stain delineates the transition between native bile duct epithelium and intrabiliary metastatic tumor. (d) Higher-magnification (×8) view of the boxed area in panel c. The sharp transition between CK7-positive native biliary epithelium (red) and CDX2-positive metastatic colorectal adenocarcinoma (brown nuclear staining) supports replacement of biliary epithelium by metastatic colorectal adenocarcinoma. (e) Low-power H&E image (×2) from a different case showing biliary duct invasion by metastatic colorectal adenocarcinoma. (f) CK7 immunohistochemistry showing strong cytoplasmic positivity in native biliary epithelium, whereas metastatic tumor is negative (×2). (g) CDX2 immunohistochemistry showing diffuse nuclear positivity in metastatic colorectal adenocarcinoma, while native biliary epithelium is negative (×2). (h) SATB2 immunohistochemistry showing diffuse nuclear positivity in metastatic colorectal adenocarcinoma, with no staining in native biliary epithelium (×2). BDI, biliary duct invasion; CDX2, caudal type homeobox 2; CK7, cytokeratin 7; H&E, hematoxylin and eosin; SATB2, special AT-rich

sequence-binding protein 2.

Table 1. Clinicopathologic features by biliary duct invasion status (*n* = 133)

Variable	BDI (+), *n* = 19 (14.3%)	BDI (–), *n* = 114 (85.7%)	*P* value
Sex			
Female	3 (15.8%)	44 (38.6%)	0.096
Male	16 (84.2%)	70 (61.4%)	
Age			
Median [IQR], years	64 [54–70]	60 [52–67]	0.369[a]
< 50 years	4 (21.1%)	21 (18.4%)	0.756[b]
≥ 50 years	15 (78.9%)	93 (81.6%)	
Hepatic resection type			
Segmentectomy	14 (73.7%)	77 (67.5%)	0.790
Metastasectomy	5 (26.3%)	37 (32.5%)	
Number of metastatic foci			
Single	11 (57.9%)	79 (69.3%)	0.472
Multiple	8 (42.1%)	35 (30.7%)	
Largest metastasis diameter			
Median [IQR], cm	3.0 [1.9–4.8]	3.5 [1.8–5.0]	0.728[a]
Metastasis differentiation			
Low grade	17 (89.5%)	93 (81.6%)	1.000[c]
High grade	1 (5.3%)	10 (8.8%)	
Mucinous	1 (5.3%)	11 (9.6%)	
Surgical margin status			
Negative	12 (63.2%)	60 (52.6%)	0.546
Positive	7 (36.8%)	54 (47.4%)	
Lymphovascular invasion (LVI)			
Present	5 (26.3%)	14 (12.3%)	0.149[b]
Absent	14 (73.7%)	100 (87.7%)	
Tumor budding			
Present	6 (31.6%)	24 (21.1%)	0.374[b]
Absent	13 (68.4%)	90 (78.9%)	

[a]Mann-Whitney *U* test. [b]Fisher's exact test (2 × 2 tables with an expected cell count < 5). [c]Fisher-Freeman-Halton exact test (*r* × *c* tables with an expected cell count < 5). All other *P* values: chi-square test. Bonferroni-corrected significance threshold for the 10 clinicopathological comparisons in this table: α=0.05/10=0.0050. No variable reached this threshold. BDI, biliary duct invasion; IQR, interquartile range; LVI, lymphovascular invasion.

however, intraductal papillary neoplasm of the bile duct was not specifically suspected preoperatively. Histopathologic and immunohistochemical evaluation supported a diagnosis of metastatic colorectal adenocarcinoma. Among the remaining 18 BDI-positive cases, none was clinically or radiologically suspected to represent iCCA preoperatively. Of the 19 BDI-positive cases, 14 underwent segmentectomy (73.7%) and 5 underwent metastasectomy (26.3%); negative margins were achieved in 12 of 19 cases (63.2%).

Clinicopathologic comparisons between BDI-positive and BDI-negative cases are summarized in Table 1 (full cohort, $n = 133$) and Table 2 (primary tumor subgroup, $n = 100$). In univariate analysis, male sex showed the strongest numerical trend toward BDI: 16 of 19 BDI-positive cases were male (84.2%) compared with 70 of 114 BDI-negative cases (61.4%; $P = 0.096$). LVI was present in 26.3% of BDI-positive cases versus 12.3% of BDI-negative cases ($P = 0.149$). Tumor budding was observed in 31.6% of BDI-positive cases versus 21.1% of BDI-negative cases

Table 2. Primary tumor features by biliary duct invasion status (primary tumor subgroup, *n* = 100)

Variable	BDI (+), *n* = 14 (14.0%)	BDI (–), *n* = 86 (86.0%)	*P* value
Timing of hepatic resection			
Synchronous	6 (42.9%)	53 (61.6%)	0.302
Metachronous	8 (57.1%)	33 (38.4%)	
Primary tumor differentiation			
Low grade	11 (78.6%)	69 (80.2%)	0.850[c]
High grade	1 (7.1%)	8 (9.3%)	
Mucinous	1 (7.1%)	6 (7.0%)	
Not assessable after neoadjuvant therapy	1 (7.1%)	3 (3.5%)	
Lymph node metastasis (primary)			
Present	10 (71.4%)	56 (65.1%)	0.767[b]
Absent	4 (28.6%)	30 (34.9%)	
Primary tumor diameter			
Median [IQR], cm	4.0 [2.4–5.8]	4.5 [3.3–6.0]	0.209[a]

[a]Mann-Whitney *U* test. [b]Fisher's exact test (2 × 2 tables with an expected cell count < 5). [c]Fisher-Freeman-Halton exact test (*r* × *c* tables with an expected cell count < 5). This primary tumor subgroup analysis is exploratory because of the smaller sample size and missing data for 33 patients; *P* values are uncorrected for multiple comparisons. Primary tumor data were unavailable for 33 patients (resection performed at outside institutions or before the institutional archive period). BDI, biliary duct invasion; IQR, interquartile range.

($P = 0.374$). Age, resection type, number of metastatic foci, lesion diameter, differentiation grade, surgical margin status, primary tumor features, and timing of hepatic resection did not differ significantly between groups.

Discussion

In this retrospective analysis of 133 consecutive patients who underwent hepatic resection for colorectal cancer liver metastases, BDI was identified in 14.3% of patients. Western series have reported prevalence estimates of 3.6–10.6%.[9,10] This prevalence contrasts with substantially higher rates of 40–42.6% reported in Japanese surgical cohorts.[11,12] The reasons for this geographic discrepancy remain incompletely understood. Differences in pathologic sampling protocols, the extent of intraoperative cholangiography or bile duct exploration, diagnostic awareness, and histologic classification criteria may contribute. Biological differences, including ethnic variation in the molecular epidemiology of CRC, may also contribute to geographic variation. Regardless of the cause, this variability underscores the importance of standardized, prospectively validated histologic criteria for reporting BDI.

The pathogenesis of BDI remains poorly defined. The phenomenon was described by Herbut and Watson in 1946.[15] Two main mechanisms have been proposed. First, parenchymal metastatic deposits may directly invade adjacent bile duct walls by lateral extension, analogous to perineural or vascular invasion at other sites.[6] Second, the peribiliary plexus, a capillary network closely associated with the portal tract and connecting hepatic arterial branches, portal venous branches, and the biliary epithelium, may facilitate periductal spread of tumor cells to the bile duct wall through vascular implantation.[6]

BDI can pose substantial radiologic and histopathologic diagnostic challenges. Radiologically, it may cause bile duct dilatation, intraductal filling defects, or strictures that can be difficult to distinguish from iCCA or intraductal papillary neoplasm of the bile duct, particularly when the associated parenchymal tumor is inconspicuous.[5] The risk of an incorrect pathologic diagnosis may be greatest in small biopsy specimens or lesions with predominantly intraductal spread. Morphologic features favoring metastatic CRC over iCCA include cribriform or tubulovillous glandular architecture, elongated nuclei with pencillate chromatin, and "dirty" intraluminal necrosis; in contrast, abundant desmoplastic stroma and perineural invasion favor iCCA.[10,16-18] When morphology is equivocal, particularly in tumors with extensive intrabiliary growth or poor differentiation, immunohistochemistry can be helpful: a CK20+/CDX2+/SATB2+/CK7− profile supports colorectal origin, whereas iCCA is typically CK7 positive and negative for CDX2 and SATB2.[19-21] However, large-duct iCCA may express intestinal markers such as CK20 and CDX2; therefore, immunohistochemical findings should not be interpreted in isolation. In our practice, morphology remained the primary diagnostic tool, and immunohistochemistry was used as an adjunct in selected cases requiring additional diagnostic support, particularly tumors with extensive intrabiliary growth or poor differentiation. Immunohistochemistry was not routinely performed in all colorectal liver metastases, and none of the cases underwent immunohistochemical evaluation specifically because iCCA was considered in the differential diagnosis. Notably, perineural invasion, a strong indicator of iCCA, was absent in all cases in this series, consistent with prior reports suggesting that it is uncommon in CRC metastases regardless of growth pattern.

In the present series, no clinicopathologic variable was signific-

antly associated with BDI in univariate analyses. Male sex showed the strongest numerical trend ($P = 0.096$), a finding that warrants prospective validation. LVI also showed a numerical trend toward higher BDI rates (26.3% vs. 12.3%), raising the hypothesis that tumors with vascular invasion within the metastatic lesion may have biologic features associated with intrabiliary dissemination. Tumor budding, an established adverse prognostic factor in colorectal pathology, was numerically more frequent in BDI-positive cases, although the difference was not statistically significant. Importantly, LVI identified histologically within the resected hepatic metastasis represents vascular invasion demonstrable in the metastatic lesion and should not necessarily be interpreted as evidence of the earlier vascular dissemination event that led to liver metastasis. These findings should be interpreted cautiously because the relatively small number of BDI-positive cases limits the strength of the observed clinicopathologic associations. Nevertheless, the numerical trends for male sex and LVI may generate hypotheses for future investigation in larger prospective cohorts.

The surgical implications of BDI may be clinically important regardless of statistical associations with other variables.[22] If BDI extends beyond the parenchymal resection margin into the bile duct, microscopically positive ductal margins may not be anticipated by a typical surgical resection plan.[10] In our cohort, 36.8% of patients with BDI had positive surgical margins compared with 47.4% of patients without BDI ($P = 0.546$); this difference was not statistically significant. Nevertheless, intraductal tumor extension may create intraoperative challenges in achieving complete ductal clearance, particularly when biliary involvement is not identified preoperatively. Intraoperative frozen-section examination of bile duct margins could therefore be considered when BDI is suspected preoperatively or identified intraoperatively, as it may help guide the extent of resection needed to achieve clear ductal margins.

This study has several limitations. First, its retrospective, single-center design introduces the possibility of selection bias and may limit the generalizability of the findings. In addition, only patients who underwent hepatic resection for colorectal liver metastases were included; therefore, the observed prevalence of BDI may not be representative of all patients with colorectal liver metastases, particularly those with unresectable disease or those managed nonsurgically. Second, the BDI-positive cohort ($n = 19$) was relatively small and did not permit reliable multivariable inference. Accordingly, the clinicopathologic associations identified in this study should be regarded as hypothesis-generating rather than definitive. Third, immunohistochemical markers were not evaluated systematically across the entire cohort because staining was performed according to routine diagnostic requirements rather than a predefined study protocol. Consequently, the diagnostic performance of these markers, including sensitivity and specificity, could not be formally assessed. Fourth, survival and recurrence data were incomplete or unavailable for a substantial proportion of patients; therefore, the prognostic significance of BDI could not be determined despite its potential importance for surgical margin assessment reported in the surgical literature. In addition, clinicopathologic data from the primary colorectal tumor were unavailable for 33 patients (25%), which may have introduced bias into subgroup analyses of primary tumor characteristics. Standardized reassessment of primary tumor budding and detailed subclassification of vascular invasion also could not be performed retrospectively because of limitations in the availability and consistency of the original pathologic material and documentation. These features therefore could not be evaluated using standardized contemporary criteria across the entire cohort. Another limitation was the absence of a comparative intrahepatic cholangiocarcinoma cohort. Direct comparison of the histopathologic and immunohistochemical features of colorectal liver metastases with those of primary intrahepatic cholangiocarcinoma, including large-duct iCCA, was therefore not possible, limiting the extent to which our findings can define the diagnostic distinction between these entities. Finally, molecular data, including *RAS* and *BRAF* status and mismatch repair status, were not consistently available because of the retrospective study design. Future prospective, multicenter studies with standardized histopathologic criteria, comprehensive immunohistochemical and molecular characterization, and long-term follow-up are warranted to clarify the clinicopathologic and prognostic significance of BDI in colorectal liver metastases.

Conclusions

BDI occurs in approximately 14% of surgically resected CRC liver metastases in this cohort. Reported prevalence varies substantially across published series. No clinicopathologic variable is significantly associated with BDI. The relatively small number of BDI-positive cases may have limited statistical power to detect modest associations; therefore, the observed numerical trends should be considered hypothesis-generating and require confirmation in larger, adequately powered prospective studies. Male sex and LVI show nonsignificant numerical trends toward association with BDI. The principal contribution of this study is its morphologic characterization of BDI, complemented by selective immunohistochemical assessment in diagnostically challenging cases, which highlights morphologic and immunohistochemical features that may aid the differential diagnosis of BDI and iCCA. Awareness of potential ductal extension may facilitate surgical planning, and intraoperative frozen-section margin assessment may be considered in selected cases when BDI or the extent of intraductal tumor growth is suspected.

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Conflict of interest

Funda Yilmaz is an Associate Editor of the *Journal of Clinical and Translational Pathology*. The author was not involved in the editorial handling, peer review, or decision-making process for this manuscript. The remaining authors declare no competing interests.

Author contributions

Study conception and design (F Yildirim, F Yilmaz, AA, DN), data acquisition (F Yildirim, F Yilmaz, AA, AU, MS, BD, MZ, DN), data analysis and interpretation (F Yildirim), drafting of the manuscript (F Yildirim, AA), critical revision of the manuscript for important intellectual content (AA, AU, MS, BD, MZ, DN), administrative, technical, or material support (BD, MZ, DN), and study supervision (DN, BD, MZ). All authors made significant contributions to the study and approved the final manuscript.

Ethical statement

This study was conducted in accordance with the World Medical Association Declaration of Helsinki. The study protocol was approved by the Clinical Research Ethics Committee of Ege University (approval no. 18-7.1/19; August 3, 2018). Written informed consent was obtained from all patients preoperatively, including consent for the use of clinical data and tissue for research purposes. As this was a retrospective study involving previously collected data and tissue and did not require any additional intervention or procedure, the institutional ethics committee did not require additional written informed consent specifically for this study.

Data sharing statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Presentation

Part of this study was presented at the 30th European Congress of Pathology (September 8–12, 2018, Bilbao, Spain), and the abstract was published in *Virchows Archiv* (volume 473, supplement 1, page S124, abstract PS-14-016).

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