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RESEARCH ARTICLE

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Identification of ampullary carcinoma mixed subtype using a panel of six antibodies and its clinical significance

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Objectives: To investigate the function of immunomarkers CK7, CK20, CK17, CDX2, MUC1, and MUC2 in the identification of primary ampullary carcinoma mixed subtype.

Methods: Forty-two cases of primary ampullary carcinoma were performed by immunohistochemical studies. The correlation between the mixed subtype and the other two subtypes and patient survival data was analyzed using the SPSS 16.0 statistical software.

Results: Among 42 cases, 12 (28.6%) cases were classified as mixed subtype, which showed variable expression patterns: 91.7% (11/12) for CK7, 83.3% (10/12) for CK20; 66.7% (8/12) for CK17, CDX2, and MUC1; and 50% (6/12) for MUC2. Ten (83.3%) mixed types coexpressed four or more immunomarkers. Eight (19%) intestinal subtypes mainly showed a positive expression of CK20, CDX2, and MUC2. Twenty-two (52.4%) pancreaticobiliary subtypes showed a positive expression of CK7, MUC1, and CK17. Stages III and IV diseases in mixed subtype (25%) and intestinal subtype (25%) were less than pancreaticobiliary subtype(63.6%) ($p = 0.039$). Follow-up data appeared to show a better survival rate for patients with mixed subtype than those with pancreaticobiliary subtypes.

Conclusion: Immunohistochemical staining provided a more reliable means of diagnosing mixed ampulla carcinoma. Accurate subtyping of ampullary carcinoma is clinically important to select effective chemotherapy regimens and to assess disease prognosis.

KEYWORDS
ampullary carcinoma, immunohistochemistry, immunomarker, mixed subtype, survival rate

1 | INTRODUCTION

Among all tumors of the digestive system, ampullary carcinoma is relatively rare. It is commonly treated by pancreaticoduodenectomy (Whipple procedure), which is one of the most complicated operations in general surgery, carrying high postoperative complications and mortality. Accurate pathologic diagnosis is very important for determining prognosis, selecting the treatment option, and developing a chemotherapy regimen.

The ampulla of Vater is a unique structure formed by the union of two different types of mucosa: the intestinal mucosa of the duodenum and the pancreaticobiliary mucosa from the pancreatic and common bile ducts. It is a hot spot for adenoma and adenocarcinoma.[1] Given the anatomic complexity of the structure, the definition of ampullary carcinoma remains somewhat ambiguous. As a result, carcinomas of the pancreatic head, distal common bile duct, and second portion of duodenum may be misdiagnosed as ampullary carcinoma. On the other hand, heterogeneous histologic

phenotypes (intestinal, pancreaticobiliary, and mixed) are observed in ampullary carcinomas.

In recently established guidelines, a tumor is designated an ampullary primary only if the following criteria are met.[2,3] First, its epicenter is located in the lumen or wall of the distal ends (intra-ampullary component) of the common bile duct and/or pancreatic duct; or at the papilla of Vater (ie, the junction of duodenal and ampullary mucosae, as defined by the College of American Pathologists); or the duodenal-facing surface of the papilla (the ampullary protuberance). In the latter circumstance, a tumor is designated an ampullary primary, rather than duodenal, only if the ampullary orifice is clearly located within the lesion. Second, the tumor epicenter or >75% of the bulk of the tumor is within the ampulla.

A typical ampullary carcinoma may be diagnosed by endoscopy and imaging modalities, but this may not always be the case in clinical practice. Many cases have an insidious clinical course and patients are often admitted due to obstructive jaundice. In addition, tumor tissue origin cannot be precisely determined by endoscopy and imaging, but knowing tissue origin is of great importance to treatment decision-making. The optimal chemotherapy regimen for ampullary carcinoma differs according to the tissue origin. Histologically, ampullary carcinomas are traditionally subclassified into pancreaticobiliary and intestinal subtypes (Figure 1). Some retrospective studies have suggested that the pancreatobiliary subtype is best treated with gemcitabine therapy, while the intestinal subtype is better treated with a 5-fluorouracil-based regimen.[4]

In addition to the pancreaticobiliary and intestinal subtypes, a mixed subtype is recently recognized.[4-7] This may partially explain why some patients do not respond as predicted to treatment. The prognosis and chemotherapy options for mixed subtype are still being explored, but histopathologic subclassification of ampullary carcinoma becomes more challenging. This is particularly true when the tumor is poorly differentiated, which makes it more difficult to subtype by morphology alone. To date, only scarce data are available regarding the role of tumor markers in the subclassification of ampullary carcinomas, especially for the mixed subtype. The definition of ampullary carcinoma mixed subtype remains unclear.

In the present study, we aimed to subclassify 42 cases of ampullary carcinoma using a panel of six tumor markers, including CK7, CK20, CK17, CDX2, MUC1, and MUC2. In particular, we focused on examining the role of these markers in aiding the histologic diagnosis of mixed subtype. The patient survival data were analyzed to assess the correlation with different subtypes.

2 | MATERIALS AND METHODS

2.1 | Clinicopathologic data

A total of 42 cases of primary ampullary carcinoma from 2012 to 2018 were collected at the Peking University People's Hospital, including 22 males (52.4%) and 20 females (47.6%). All of the patient information was complete and the follow-up data were available. There were 6 well-differentiated adenocarcinomas, 19 moderately differentiated adenocarcinomas, and 17 poorly differentiated adenocarcinomas. The median age of patients was 61.8 ± 10.8 (range 42-88) years. The median size of the tumors was 2.2 ± 1.0 cm in diameter (ranging from 0.8 to 4.5 cm). Using the eighth edition American Joint Committee on Cancer (AJCC) TNM staging system,[8] 6 cases were staged I, 17 staged II, 18 staged III, and 1 staged IV. Twelve cases showed lymph node metastasis (N1 = 10 cases; N2 = 2 cases). The study was approved by the Hospital Ethics Review Committee.

2.2 | Immunohistochemistry

Immunohistochemistry was performed using the conventional streptavidin-perosidase (SP) method. Formalin-fixed, paraffin-embedded tissue sections were deparaffinized and rehydrated with xylene and a series of grades of alcohol. After epitope retrieval and inactivation of endogenous peroxidase, sections were blocked with 10% normal goat serum for 30 minutes and sequentially incubated with the CK7, CK20, CK17, CDX2, MUC1, and MUC2 antibodies (purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) and horseradish peroxidase–conjugated antirabbit and anti-mouse IgG. After washing, slides were developed by the chromogen diaminobenzidine tetrahydrochloride, followed by counterstaining with hematoxylin.

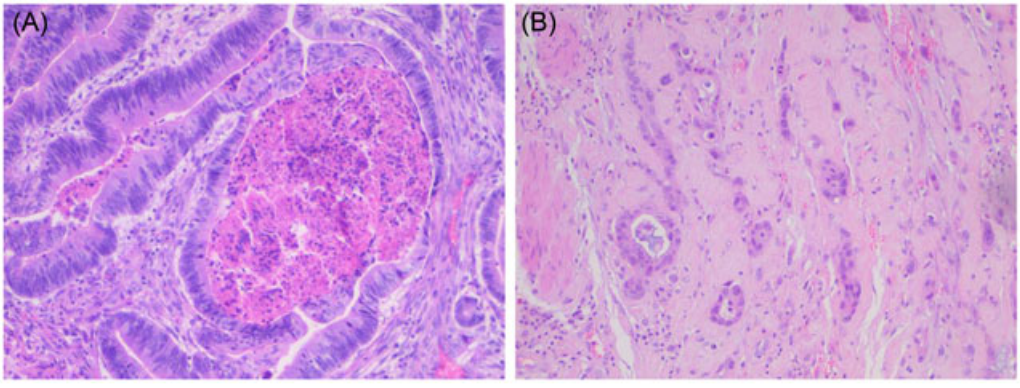

FIGURE 1 Traditionally, ampullary carcinomas are mainly subclassified into intestinal subtype (A) and pancreatobiliary subtype (B). HE, ×100. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Frequency of scattered spotty expression pattern of CK7, CK20, CK17, CDX2, MUC1, and MUC2 in 42 cases of ampullary carcinoma

Immunomarker	Histopathologic subtype, n%		
	Intestinal (n = 8)	Pancreaticobiliary (n = 22)	Mixed (n = 12)
CK7	0 (0)	0 (0)	1 (8.3)
CK17	5 (62.5)	1 (4.5)	3 (25)
MUC1	0 (0)	0 (0)	0 (0)
CK20	0 (0)	2 (9)	2 (16.7)
CDX2	0 (0)	8 (36.4)	2 (16.7)
MUC2	1 (12.5)	2 (9)	2 (16.7)

A summed score (A × B) was calculated based on the proportion (A) and intensity (B) of positively stained tumor cells. For A, 1 point was assigned if <25% of tumor cells were positively stained, 2 points if 25 to 50%, and 3 points if >50%. Zero (0) point was assigned if there were no positively stained tumor cells. For B, 1 point was assigned for light yellow staining intensity (weak), 2 points for brownish yellow intensity (intermediate), and 3 points for tan coloration (strong). If the sum of A × B was <4, the staining result was considered negative, whereas a sum of ≥4 was considered positive.

2.3 | Statistical analysis

Data were analyzed for statistical significance using the χ^2 test (SPSS, Chicago, IL; Computer Resource Center). The overall survival of patients with ampullary carcinomas in this study was analyzed using a log-rank test based on Kaplan-Meier analyses with data V.16.0 software (SPSS, Chicago, IL; Computer Resource Center). A two-tailed P value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Expressions of CK7, CK20, CK17, CDX2, MUC1, and MUC2 in ampullary carcinoma

We found that five of the six examined immunomarkers exhibited a scattered spotty expression pattern in some ampullary carcinoma cases, staining <25% tumor cells. MUC1 was an exception, which did not show this pattern. CK7 exhibited a spotty expression rate of 8.3% (1/12) only in mixed subtype carcinomas. Both CK17 and MUC2 showed scattered spotty expression in all three subtypes. CK20 and CDX2 were expressed in both pancreaticobiliary and mixed subtypes (Table 1, Figure 2). According to the experimental design of this study, this form of discontinuous scattered focal expression was considered negative (the sum of score [A × B] for this pattern is <4).

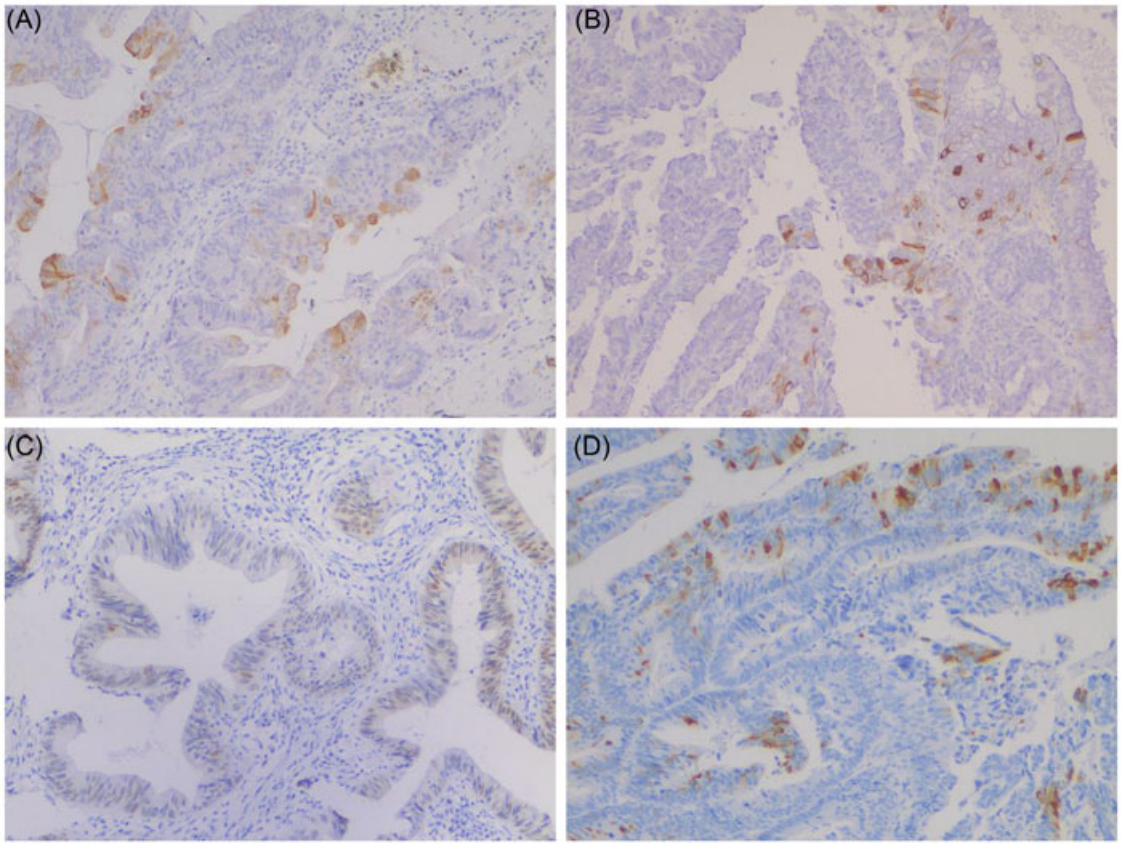

FIGURE 2 Scattered spotty expression pattern of CK17 (A), CK20 (B), CDX2 (C), and MUC2 (D) by immunohistochemistry observed in ampullary carcinoma, which was considered negative. SP, ×100 [Color figure can be viewed at wileyonlinelibrary.com]

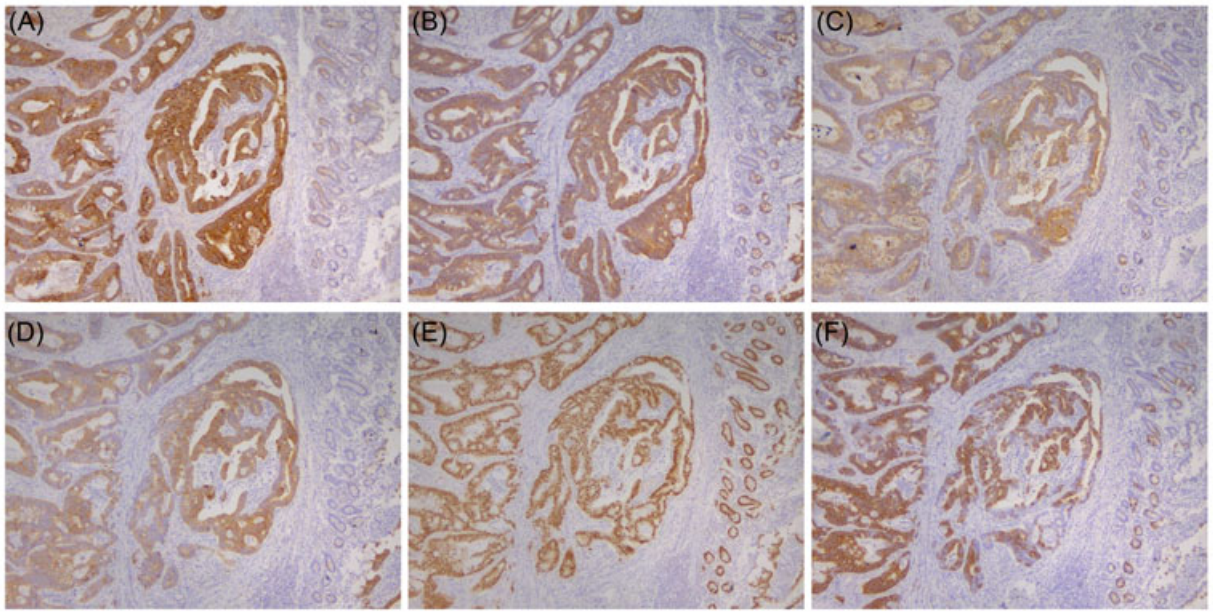

FIGURE 3 An example of mixed subtype of ampullary carcinoma showing simultaneous coexpression of all six immunomarkers, CK7 (A), CK17 (B), MUC1 (C), CK20 (D), CDX2 (E) and MUC2 (F). SP, ×40 [Color figure can be viewed at wileyonlinelibrary.com]

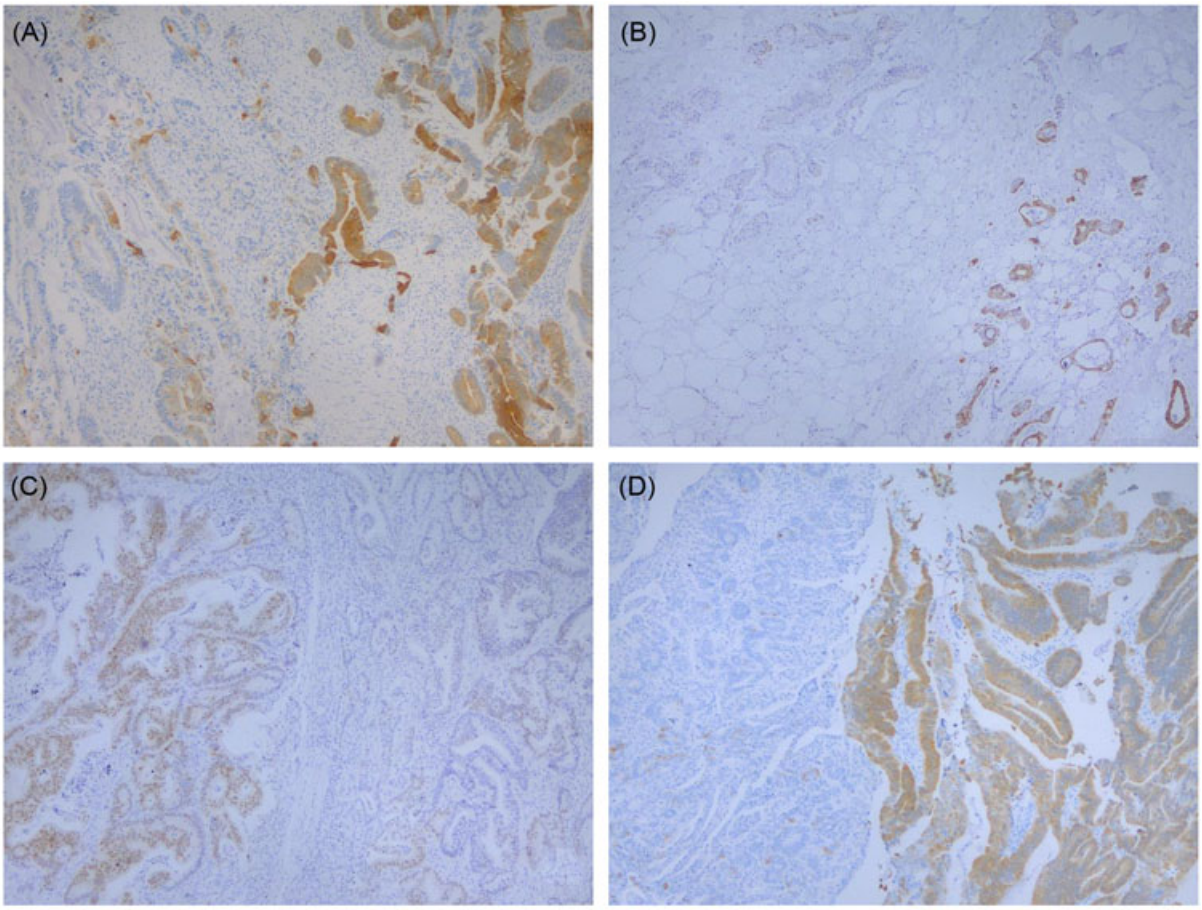

FIGURE 4 An example of the mixed subtype of ampullary carcinoma showing simultaneous coexpression of CK7 (A), CK17 (B), CDX2(C), and MUC2(D), the expression was often detected in only half of the tumor cells. SP, ×40 [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Frequency of CK7, CK20, CK17, CDX2, MUC1, and MUC2 expression in 42 cases of ampullary carcinoma

	Intestinal, n (%)		Pancreaticobiliary, n (%)		Mixed, n (%)	
Immunomarker	Positive	Negative	Positive	Negative	Positive	Negative
CK7	1 (12.5)	7 (87.5)	22 (100)	0 (0)	11 (91.7)	1 (8.3)
CK17	1 (12.5)	7 (87.5)	20 (90.9)	2 (0.09)	8 (66.7)	4 (33.3)
MUC1	0 (0)	8 (100)	22 (100)	0 (0)	8 (66.7)	4 (33.3)
CK20	8 (100)	0 (0)	0 (0)	22 (100)	10 (83.3)	2 (16.7)
CDX2	8 (100)	0 (0)	0 (0)	22 (100)	8 (66.7)	4 (33.3)
MUC2	5 (62.5)	3 (37.5)	0 (0)	22 (100)	6 (50)	6 (50)

Among the 42 cases, eight (19%) were classified as the intestinal subtype. These tumors showed positive expression of both CK20 and CDX2 in 100% (8/8) of cases, and MUC2 expression in 62.5% (5/8). Both CK7 and CK17 were weakly expressed in one case (12.5%). No positive expression of MUC1 was observed in this subtype.

A total of 22 cases (52.4%) were classified as the pancreaticobiliary subtype. These tumors showed positive expression of both CK7 and MUC1 in 100% of cases and positive expression of CK17 in 90.9% (20/22). No positive expression of CK20, CDX2, and MUC2 was observed in the pancreaticobiliary subtype.

Twelve cases (28.6%) were classified as the mixed subtype, which contained various amounts of pancreaticobiliary and intestinal components. In cases with poor tumor differentiation, it is difficult to accurately classify on the basis of hematoxylin-eosin (HE) morphology, but we were able to detect simultaneous coexpression of various immunomarkers by immunohistochemistry. Specifically, CK7 was positively expressed in 91.7% (11/12) of mixed subtype carcinomas; CK20 in 83.3% (10/12); CK17, CDX2, and MUC1 each in 66.7% (8/12); and MUC2 in 50% (6/12). Among mixed subtypes, 25% (3/12) coexpressed all six immunomarkers (Figure 3), 25% (3/12) coexpressed five markers, 33.3% (4/12) coexpressed four markers, and 16.7% (2/12) coexpressed two markers. All coexpressed immunohistochemical markers were from both the intestinal-derived expression group and the pancreaticobiliary-derived expression group. In tumors that coexpressed CK7, CK17, CDX2, and MUC2, the expression was often detected in only half of the tumor cells (Figure 4). The detailed immunohistochemical findings are presented in Table 2.

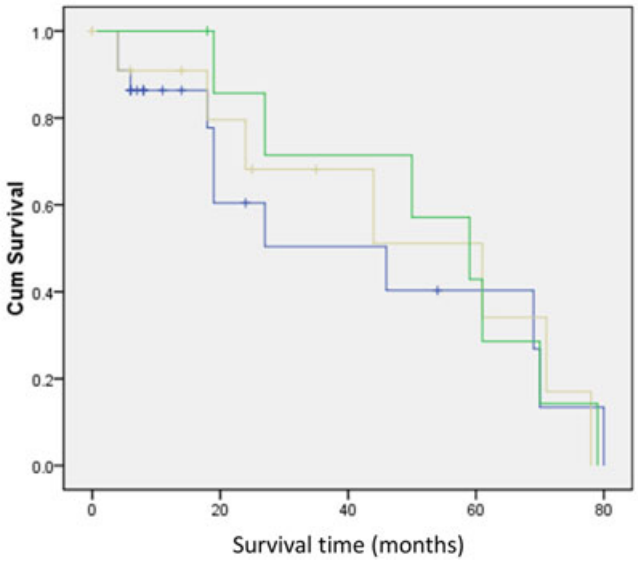

FIGURE 5 Comparison of survival rates of patients with different subtypes of ampullary carcinoma (blue—pancreaticobiliary subtype; yellow—mixed subtype; green—intestinal subtype) [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | Clinicopathological relationship and prognosis of different pathological subtypes in 42 patients with primary ampullary carcinoma

Among the 42 cases of primary ampullary carcinoma included in this study, the pancreaticobiliary, intestinal, and mixed subtypes were not associated with patient's gender, patient's age, tumor size, histologic differentiation, pancreatic invasion, bile duct invasion, or depth of duodenal invasion. However, the histologic subtypes significantly differed with regard to the TNM stages of the tumors. Stage III + IV tumors were more significantly rare in pancreaticobiliary subtype (63.6%, 14/22) compared with intestinal subtype (25%, 2/8) and mixed subtype (25%, 3/12) ($\chi^2 = 6.508$, $P = 0.039$).

During an average of 80 months of follow-up after surgery, the median survival time was 33.8 months (± 23.8 months) for all patients. Survival rates were 87.5% (7/8) for the intestinal subtype, 50% (11/22) for the pancreaticobiliary subtype, and 58.3% (5/7) for the mixed subtype. Although an overall statistical analysis did not show significant differences in survival rates among different subtypes, analysis of follow-up data during the first 60 months revealed the following survival ranking from the lowest to the highest: pancreaticobiliary subtype < mixed subtype < intestinal subtype (Figure 5). It is highly likely that the analysis of a larger number of cases would yield statistically significant results (Table 3, Figure 5).

4 | DISCUSSION

Ampullary carcinoma reportedly accounts for about 0.5% of malignant tumors of the digestive system, but the actual

TABLE 3 Comparison of clinicopathologic features among different subtypes of ampullary carcinoma (n = 42)

Feature	Histopathologic subtype			χ^2	*P* value
	Intestinal (n = 8)	Pancreaticobiliary (n = 22)	Mixed (n = 12)		
Sex					
Male	3	14	5	2.402	0.301
Female	5	8	7		
Age, y					
≤ 60	4	10	7	0.517	0.772
> 60	4	12	5		
Tumor size, cm					
≤2	4	14	5	1.603	0.449
>2	4	8	7		
Histologic differentiation					
Well	2	2	2	7.486	0.112
Moderate	4	7	8		
Poor	2	13	2		
TNM Staging					
Stage I + II	6	8	9	6.508	0.039
Stage III + IV	2	14	3		
Pancreatic invasion					
Yes	2	10	4	1.230	0.541
No	6	12	8		
Common bile duct invasion					
Yes	1	1	2	1.440	0.4879
No	7	21	10		
Depth of duodenal invasion					
No	0	2	2	9.373	0.154
Mucosa and submucosa	1	2	0		
Muscularis propria	4	5	7		
Serosa and subserosa	3	13	3		
Follow-up				3.864	0.145
Alive	1	11	5		
Died	7	11	7		
Survival time, mo	52.1 ± 8.3	42.2 ± 8.0	48.4 ± 9.1	47.245	0.504

frequency may be higher. With a better understanding of the definition of the ampulla of Vater, advances in endoscopic and imaging techniques and more accurate pathologic diagnosis, some of the cases that were previously misdiagnosed as carcinomas of the pancreatic head or duodenum have now been reclassified as ampullary carcinomas. Typical ampullary carcinoma can be diagnosed by imaging, particularly magnetic resonance imaging (MRI) that has a high soft tissue resolution. It can be combined with thin-layer scanning to achieve a diagnostic accuracy of 92.63%.[9] However, imaging cannot determine tissue origin and thus cannot help subclassification of ampullary carcinoma. Ampullary carcinomas with typical histopathology may be subclassified based on morphologic features recognized on conventional HE slides. It is generally agreed that the intestinal subtype of ampullary carcinoma is morphologically similar to colorectal carcinoma—exhibiting interconnected tubules, elongated glands, complex cribriform structures, or solid nests, and commonly showing necrotic debris in the glandular lumens.[4,10,11] Tumor cells are typically columnar with hyperchromatic and pseudostratified nuclei, and basophilic cytoplasm, and may contain goblet cells. On the other hand, the pancreaticobiliary subtype usually shows a wider spectrum of histomorphology. Typical cases show simple or branching glands or small solid cell nests. Tumor cells are typically cuboidal to low columnar with rounded vesicular nuclei arranged in a single layer in a desmoplastic stroma (Figure 1).

To date, the definition of mixed ampullary carcinoma remains controversial. Different authors have proposed that the mixed subtype be defined as when the pancreaticobiliary and intestinal subtypes each constitute >10%,[6] >20%,[7] or >25% of the tumor.[4] In our present study, we found that the proportions of pancreaticobiliary and intestinal components in mixed subtype ampullary carcinomas varied widely among different cases, challenging the utility of a unified quantitative standard. However, some pathologists have pointed out that it is difficult to identify mixed ampullary cancer and to obtain a consistent diagnosis between different observers.[7] Several immunohistochemical markers have been investigated for their usefulness in the subclassification of ampullary carcinomas,[5,12,13] but there is no clear conclusion.

We propose that it may be more reliable to define mixed ampullary carcinoma based on the expression patterns of immunomarkers by our study, especially for poorly differentiated tumors. But the key issue is our findings demonstrate that the methodology of evaluating immunohistochemical staining has an important impact on the interpretation of staining results. We observed that some markers exhibited varying degrees of scattered spotty expression, which should not be interpreted as the positive expression because this pattern differs from the "normal expression pattern" that is more confluent and contiguous (the sum of score (A + B) ≥ 4). It is also important to distinguish tumor tissue from benign glands of the ampullary region and to avoid interpreting positive staining in irregular benign glands as tumorous. Bearing these in mind, we were able to classify ampullary carcinomas into different subtypes based on their immunostaining profiles. Specifically, CK7, CK17, and MUC1 are mainly markers of the pancreaticobiliary subtype, while CK20, CDX2, and MUC2 are primarily intestinal markers. All coexpressed immunohistochemical markers were from both the intestinal-derived expression group and the pancreaticobiliary-derived expression group indicated a mixed subtype. Kohler et al[5] found that the mixed subtypes simultaneously express CK7, CK20, and CDX2. We found at least two immunomarkers coexpressions in the mixed subtypes; they were MUC1 and MUC2, CK7 and MUC2, respectively. No combination of CK7 and CK20 was found. It should be mentioned that 40% of our cases included in this study are poorly differentiated. A clear separation between pancreaticobiliary and intestinal subtypes is very difficult based on histopathologic examination. Immunohistochemical stains using the six antibody panels are of great help in this regard. Immunohistochemistry thus provides a more reliable means to aid in the subclassification of ampullary carcinoma, especially for poorly differentiated mixed subtypes. This is the first time we have proposed the identification of mixed subtypes by immunohistochemistry.

Of the 42 patients with ampullary carcinoma, only 4 (9.5%) were under the age of 50 years, indicating that ampullary carcinoma usually occurs among older patients. Statistical analysis of our data showed that different subtypes of ampullary carcinoma were significantly associated with the TNM staging of tumors ($\chi^2 = 6.508$, $P = 0.039$). These results suggest that the pancreaticobiliary subtype is potentially more aggressive biologically than mixed subtype. We have also observed different survival rates in patients with different subtypes, although the differences were not statistically significant possibly due to the relatively small sample size. Nevertheless, the survival curves for the first 60 months after surgery showed patients with mixed subtype in the intermediate survival rate, consistent with Kohler's report.[7]

5 | CONCLUSIONS

In summary, our study provides additional evidence to support immunohistochemistry using a panel of selected antibodies (CK7, CK20, CK17, CDX2, MUC1, and MUC2) as a useful tool to aid in the identification of ampullary carcinomas mixed subtypes. Accurate subclassification is of great importance to patient care and prognosis assessment.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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