Review Article

Indian Journal of Pathology: Research and Practice
Volume 11 Number 4, October - December 2022
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.11422.3

Role of Immunohistochemical Typing in Pancreaticobiliary and Intestinal Type Differentiation of Periampullary Adenocarcinomas

Niket Shah[1], S Sankar[2], S Rajendiran[3]

How to cite this article:

Niket Shah, S Sankar, S Rajendiran/Role of Immunohistochemical Typing in Pancreaticobiliary and Intestinal Type Differentiation of Periampullary Adenocarcinomas/Indian Journal of Pathology: Research and Practice 2022;11(4) 147–160.

Abstract

Background: Ampullary cancer is of particular classificatory interest as it may arise either from the intestinal epithelium or the epithelium covering the pancreatobiliary ducts knows as Intestinal type (IT) and Pancreatobiliary type (PBT) respectively. These 2 histological subtypes have different clinical characteristics, in terms of disease stage, recurrence rates and survival. Immunohistochemical staining against specific differentiation markers, including cytokeratins 7 (CK7) and 20 (CK20), mucins 1 (MUC1) and 2 (MUC2), as well as caudal type homeodomain (CDX2) protein, has been proven to be a useful adjunct in determining the exact histological sub type, especially in cases of large or mixed type tumors.

Material and Methods: During the study period (2018–2021), 15 consecutive patients with IT and 15 consecutive patients with PBT adenocarcinoma, based on H&E staining characteristics, were included in analysis. Data on clinical parameters, including age, gender, pre-operative assessment of disease stage, CA 19-9 serum levels and histopathological parameters including tumor size, pT stage, pN stage, LVI, PNI and differentiation were collected in a prospective manner from patients' surgical and pathological records. IHC markers (CK7, CK20, CDX20 and MUC1) were analyzed on all histopathological blocks.

Results: In our analysis, pT Stage, Tumor Stage, CA 19-9 (IU/mL), Tumor size (cm), PNI, MUC1, combination CK7/MUC1, CK20, CDX2, combination CK20/CDX2 were significantly associated with histological subtype. CDX2 expression was found to have highest diagnostic accuracy (96.7%) for histological subtype followed by, in decreasing frequency, high CA 19-9 (93.3%), MUC1 (90%) and CK20/CDX2 combination (90%) and CK20 (86.7%). CDX2 was the only marker that showed highest sensitivity (100%) and high specificity (93.3%) for predicting IT. MUC1 positivity was associated with PBT with highest sensitivity of 100% and high specificity of 80%. The combination of CK7/MUC1 showed 86.7% sensitivity and 80% specificity with 83.3% diagnostic accuracy to identify PBT tumor, whereas combination of CK20/CDX2 showed 100% sensitivity and 80% specificity with 90% diagnostic accuracy to identify IT tumor.

Conclusion: CDX2 and MUC1 have a highest sensitivity (100%) and highest NPV (100%) for IT and PBT differentiation respectively. These immunohistochemical subtypes correlates well with the conventional histomorphological classification. A panel of IHC markers like CK20 and CDX2 together allows better identification of differentiation than the use of single markers alone.

Keyword: Ampullary malignancy; Intestinal type; Pancreaticobiliary type; CK20; CDX2; CK7; MUC1; Immunohistochemistry.

Author Affiliation: [1]Senior Resident, [2]Professor & Head, Department of Surgical Gastroenterology, [3]Professor, Department of Pathology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu 600116, India.

Corresponding Author: Niket Shah, Senior Resident, Dept of Surgical Gastroenterology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu 600116, India.

E-mail: nikk240488@gmail.com

Received on: 08.07.2022, **Accepted on:** 10.08.2022

INTRODUCTION

Carcinomas of the ampulla of Vater represent 0.5% of gastrointestinal carcinomas and 7% of carcinomas arising in the region of the head of the pancreas.[1] Although uncommon, ampullary adenocarcinoma accounts for 90% of ampullary epithelial neoplasms. Ampulla of vater tumours are second most common malignancy of periampullary region.[1-3] As ampullary cancers carry better

prognosis, an independent TNM classification was developed for ampullary tumours.[4]

Ampullary cancer is of particular classificatory interest as it may arise either from the intestinal epithelium or the epithelium covering the pancreatobiliary ducts.[5] It was first described by Kimura et al. in 1994 that adenocarcinomas of the ampulla, which comprise 90% of all its malignancies, are categorized into 2 main histological subtypes, the IT and the PBT.[6] Albores-Saavedra et al.[7] classified the ampullary malignancies into these 2 subtypes. WHO has classified gastrointestinal malignancy based on molecular profile and immunohistochemical staining.[8]

The current classification of ampullary carcinomas relies on this distinction and also includes uncommon subtypes such as mucinous adenocarcinoma.

Both histological subtypes have different histologic characteristics and different clinical characteristics, in terms of disease stage, recurrence rates and survival.[6] Consequently, sub-classification of ampullary adenocarcinomas not only has prognostic and therapeutic implications but could possibly lead to adjustment in TNM staging criteria.[4]

In most studies[6-10] carcinomas of the pancreatobiliary subtype are found to be more aggressive than that of the intestinal subtype (5 yrs disease survival rate of 48% vs. 73%).

Similarly, for chemotherapy response, small retrospective studies have suggested that patients with PBT carcinomas may benefit from gemcitabine therapy, and those with IT tumors may benefit from a 5-fluorouracil (5-FU)-based regimen.

Immunohistochemical staining against specific differentiation markers, including cytokeratins 7 (CK7) and 20 (CK20), mucins 1 (MUC1) and 2 (MUC2), as well as caudal type homeodomain (CDX2) protein, have shown significantly high sensitivity, specificity and diagnostic accuracy in diagnosing the precise histological subtype, especially in case of mixed type and large size tumors.

AIM

To analyze the importance of IHC markers as single or a panel to differentiate between 2 morphological types of periampullary adenocarcinoma and their clinico-pathological implications.

OBJECTIVES

1. To utilize IHC markers (CK7, CK20, CDX2 and MUC1) to assess their ability to differentiate between histological IT and PBT tumor.
2. To evaluate the sensitivity, specificity, PPV, NPV and diagnostic accuracy of each IHC marker in diagnosing histological IT and PBT of tumor.
3. To assess the accuracy of markers' panel in diagnosing IT and PBT tumor.

MATERIALS AND METHODS

Patients and Data

Permission for the study was obtained from an institutional review board. It is a prospective study of periampullary adenocarcinoma. During the study period (2018–2021), a standardized protocol was followed for diagnostic workup of pancrea to duodenectomy specimens: every specimen was examined macroscopically by an experienced pathologist. Identifiable tumour masses or suspected areas were measured in three dimensions. Location, size and distance of the tumour to the resection margin were analysed and lymph nodes were evaluated. Inclusion criteria included age >18 years, no history of previous neoadjuvant therapy, periampullary location of tumour, biopsy proven adenocarcinoma and no history of previous biliary stenting. Exclusion criteria included benign tumours or non-invasive (in situ) carcinomas, unusual types (Mucinous, Poorly differentiated) of ampullary adenocarcinomas, neuroendocrine tumours and mixed variety of tumours (IT and PBT mixed phenotype in single tumour). Histological staining assessment was done by 2 independent pathologists. Finally, 15 consecutive patients with IT and 15 consecutive patients with PBT adenocarcinoma, based on H&E staining characteristics, were included in final analysis. Data on clinical parameters, including age, gender, pre-operative assessment of disease stage, CA 19-9 serum levels and histopathological parameters including tumour size, pT stage, pN stage, LVI, PNI and differentiation were collected in a prospective manner from patients' surgical and pathological records.

HISTOLOGY

All tumours were classified as either IT or PBT,

according to the criteria suggested by Kimura et al.[6] and later revised by Albores-Saavedra et al.[7] Tumours with mixed pattern were subtyped according to the dominant component. Cancer staging was performed using the 8th edition of the TNM staging system issued by the American Joint Committee on Cancer.[11,12]

IMMUNOHISTOCHEMISTRY

The most representative blocks were selected for immunohistochemical staining. These blocks were serially sectioned at 3.5 μm thickness, put on slides and stored. The sections were deparaffinized and pre-treated before incubation with following antibodies:

1. CK7 (dilution 1:100, monoclonal mouse antihuman Cytokeratin 7, clone OV-TL 12/30, code AM944-5M, BioGenex)
2. CK20 (dilution 1:20, monoclonal mouse antihuman Cytokeratin 20, clone IT-Ks 20.8, code AM946-5M, BioGenex)
3. MUC1 (Ready-To-Use, rabbit monoclonal antibody MUC1, clone EP85, code AN813-5M, BioGenex)
4. CDX2 (dilution 1:40, monoclonal mouse antihuman CDX2, clone CDX2-88, code AM392-5M, BioGenex)

IHC staining was performed with Biogenics X Matrix immunostainer. Immunoreactivity was evaluated according to published criteria.[10,13,14] Nuclear immunoreactivity for CDX2 was assessed, and samples were regarded as positive if more than 25% of the nuclei were positive. Both membranous and cytoplasmic staining for MUC1, CK7 and CK20 were assessed, and samples were regarded as positive if more than 10% of cells were positive.

Statistical Analysis

Data were coded and recorded in MS Excel spreadsheet program. SPSS v23 (IBM Corp.) was used for data analysis. Descriptive statistics were documentedin the form of frequencies and percentages for categorical variables and in the form of means/standard deviations and medians/ IQRs for continuous variables, and Data were presented in a tabular manner. Group comparisons for continuously distributed data were made using independent sample 't' test when comparing two groups. If data were found to be non-normally distributed, non-parametric tests in the form of Wilcoxon Test was used. Chi-squared test was used for group comparisons for categorical data. In case the expected frequency in the contingency tables was found to be <5 for >25% of the cells, Fisher's Extract test was used instead. Linear association between two continuous variables was explored using Pearson's correlation (if the data were normally distributed) and Spearman's correlation (for non-normally distributed data). Statistical significance was kept at $p < 0.05$.

RESULTS

Fig. 1 and 2 showing H&E staining characteristics and IHC markers' expression in IT and PBT adenocarcinoma tumors in our study.

Table 1 shows summary of clinical and histopathological features of tumors. A total of 30 patients who had undergone pancreato-duodenectomy for periampullary malignancy were included in this study. 15 (50%) cases of IT and 15 (50%) cases of PBT were included based on histopathological analysis.

The mean Age (Years) was 61.13 ± 10.71.

Out of 30 cases, 22 (73.3%) were male and 8 (26.7%) female.

11 cases (36.7%) were found to have pT2 lesion followed by 6 cases (20%) had pT3a lesion, 5 cases (16.7%) had pT3b lesion, 3 cases (10%) had pT1a and pT1b lesion. 2 (6.7%) cases had pT4 lesion.

22 (73.3%) cases had pN0 stage followed by 6 (20.0%) cases had pN1 stage and 2 (6.7%) cases had pN2 stage.

Most frequently found stage was stage IB in 8 (26.7%) followed by 5 cases (16.7%) in each stage IIA, IIB and IIIA.

The median CA 19-9 (IU/mL) was 105 (80 – 172.50).

15 (50.0%) cases had CA 19-9: <100 IU/mL and 15 (50.0%) cases had CA 19-9: ≥100 IU/mL.

Majority of tumors (n=27, 90%) were grade 2 moderately differentiated and 2 patients had grade 3 poorly differentiated tumors (6.7%).

The mean Tumor size (cm) was 2.91 ± 1.56.

10 (33.3%) cases had Tumor size: <2 cm and 20 (66.7%) cases had Tumor size: ≥2 cm.

PNI was present in 9 cases (30%) whereas LVI was present in 2 cases (6.7%).

Table 2 is showing clinical and histopathological variables and their association in predicting histological subtype of tumor.

The following variables were significantly associated ($p<0.05$) with the histological subtype: pT Stage, Tumor Stage, CA 19-9 (IU/mL), Tumor

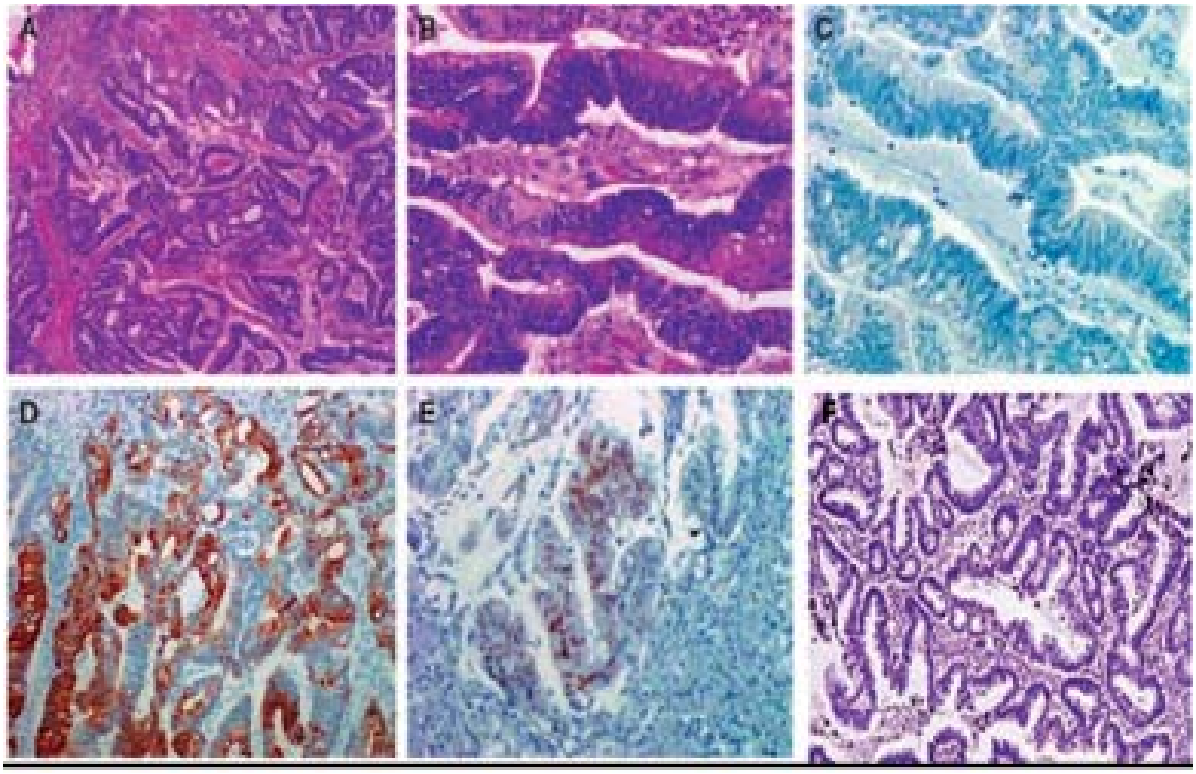

Fig. 1: An Intestinal type adenocarcinoma showing (A) A cribriform architectural pattern and (B) nuclear hyperchromasia and pseudostratification on H&E staining. By IHC, tumor cells are (C) Negative for MUC1, (D) Positive for CK20, (E) Positive for CDX2 and (F) Negative.

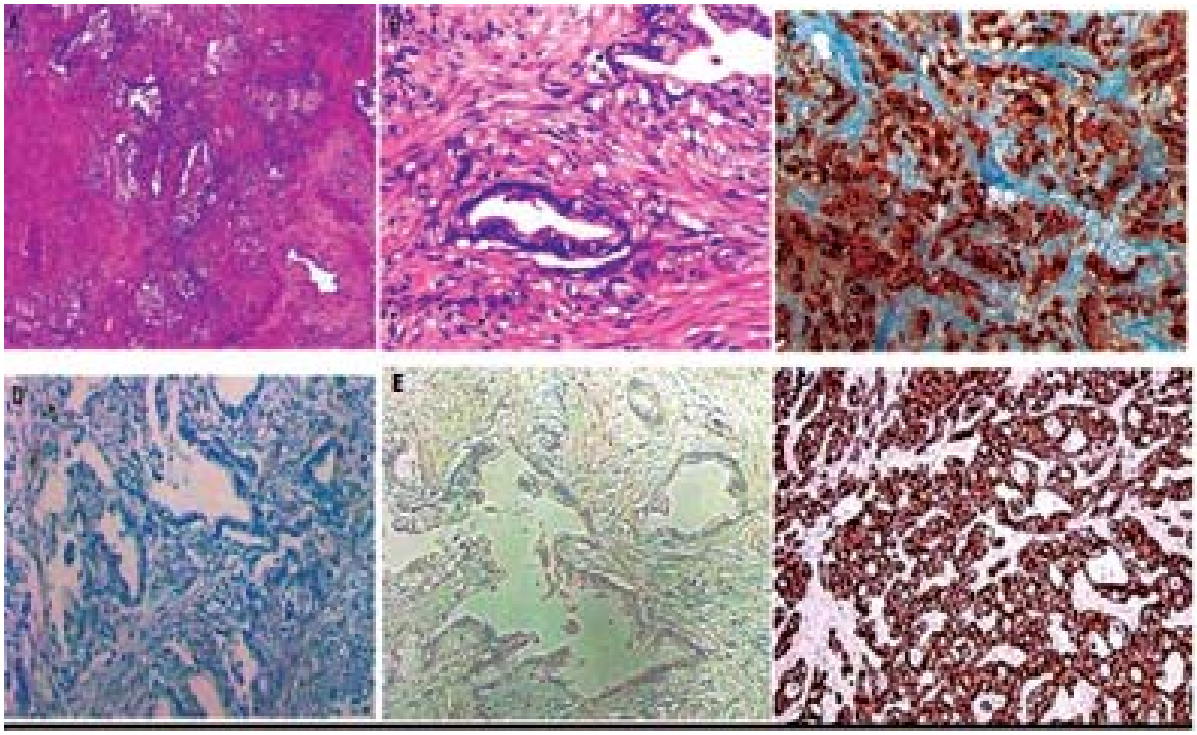

Fig. 2: A Pancreatico biliary type adenocarcinoma showing (A) Simple glands composed of cuboidal cells with rounded nuclei arranged in a single layer and (B) along with small clusters of cells and individual cells, embedded in a desmoplastic stroma on H&E staining. By IHC, tumor cells are (C) Positive for MUC1, (D) Negative for CK20, (E) Negative for CDX2 and (F) Positive for CK7

Table 1: Summary of Clinical And Tumour Details

Clinical And Tumor Details	Mean ± SD || Median (IQR) || Min-Max || Frequency (%)
Age (Years)	61.13 ± 10.71 || 63.00 (54.00-65.75) || 43.00 - 84.00
Gender	
Male	22 (73.3%)
Female	8 (26.7%)
pT Stage	
pT1a	3 (10.0%)
pT1b	3 (10.0%)
pT2	11 (36.7%)
pT3a	6 (20.0%)
pT3b	5 (16.7%)
pT4	2 (6.7%)
pN Stage	
pN0	22 (73.3%)
pN1	6 (20.0%)
pN2	2 (6.7%)
Tumor Stage	
I A	3 (10.0%)
I B	8 (26.7%)
II A	5 (16.7%)
II B	5 (16.7%)
III A	5 (16.7%)
III B	4 (13.3%)
CA 19-9 (IU/mL)	105.00 (80.00-172.50) || 55.00 - 300.00
CA 19-9	
<100 IU/mL	15 (50.0%)
≥100 IU/mL	15 (50.0%)
Differentiation	
G1	1 (3.3%)
G2	27 (90.0%)
G3	2 (6.7%)
Tumor size (cm)	2.91 ± 1.56 || 2.50 (1.50-3.50) || 1.00 - 7.00
Tumor size	
<2 cm	10 (33.3%)
≥2 cm	20 (66.7%)
PNI (Present)	9 (30.0%)
LVI (Present)	2 (6.7%)

Histological Subtype	
Intestinal type	15 (50%)
Pancrea to billiary type	15 (50%)

size (cm), PNI, MUC1, CK7/MUC1, CK20, CDX2, CK20/CDX2.

The mean age in IT group was 58.73 years and PBT group was 63.53 years.

There was no significant difference between the groups in terms of Age (t = -1.239, p = 0.226).

Table 2: Association between Histological Subtype and Parameters

Parameters	Histological Subtype		p value
	Intestinal (n = 15)	Pancreatobilliary (n = 15)	
Age (Years)	58.73 ± 10.61	63.53 ± 10.61	0.226
Gender			0.682
Male	12 (80.0%)	10 (66.7%)	
Female	3 (20.0%)	5 (33.3%)	
pT Stage* **			0.011
pT1a	3 (20.0%)	0 (0.0%)	
pT1b	2 (13.3%)	1 (6.7%)	
pT2	8 (53.3%)	3 (20.0%)	
pT3a	2 (13.3%)	4 (26.7%)	
pT3b	0 (0.0%)	5 (33.3%)	
pT4	0 (0.0%)	2 (13.3%)	
pN Stage			0.401
pN0	11 (73.3%)	11 (73.3%)	
pN1	4 (26.7%)	2 (13.3%)	
pN2	0 (0.0%)	2 (13.3%)	
Tumor Stage* **			<0.001
I A	3 (20.0%)	0 (0.0%)	
I B	7 (46.7%)	1 (6.7%)	
II A	1 (6.7%)	4 (26.7%)	
II B	0 (0.0%)	5 (33.3%)	
III A	4 (26.7%)	1 (6.7%)	
III B	0 (0.0%)	4 (26.7%)	
CA 19-9 (IU/mL)* **	82.33 ± 22.27	190.33 ± 69.29	<0.001
CA 19-9***			<0.001
<100 IU/mL	14 (93.3%)	1 (6.7%)	
≥100 IU/mL	1 (6.7%)	14 (93.3%)	
Differentiation			1.000
G1	1 (6.7%)	0 (0.0%)	
G2	13 (86.7%)	14 (93.3%)	

G3	1 (6.7%)	1 (6.7%)	
Tumor size (cm)* **	2.01 ± 0.79	3.80 ± 1.65	0.001
Tumor size* **			0.002
<2 cm	9 (60.0%)	1 (6.7%)	
≥2 cm	6 (40.0%)	14 (93.3%)	
PNI (Present)* **	0 (0.0%)	9 (60.0%)	<0.001
LVI (Present)	0 (0.0%)	2 (13.3%)	0.483
CK7 (Positive)	11 (73.3%)	13 (86.7%)	0.651
MUC1 (Positive)***	3 (20.0%)	15 (100.0%)	<0.001
CK7/MUC1 (Positive)***	3 (20.0%)	13 (86.7%)	<0.001
CK20 (Positive)***	13 (86.7%)	2 (13.3%)	<0.001
CDX2 (Positive)***	14 (93.3%)	0 (0.0%)	<0.001
CK20/CDX2 (Positive)***	12 (80.0%)	0 (0.0%)	<0.001

***Significant at p<0.05, 1: t-test, 2: Fisher's Exact Test, 3: Wilcoxon-Mann-Whitney U Test, 4: Chi-Squared Test

There was no significant difference between the various groups in terms of distribution of Gender ($\chi 2 = 0.682$, $p = 0.682$).

There was a significant difference between the various groups in terms of distribution of pT Stage ($\chi 2 = 13.273$, $p = 0.011$).

The most common T stage seen with IT group was pT2 (53.3%) followed by pT1a (20%). The most common T stage seen with PBT group was pT3b (33.3%) followed by pT3a (26.7%). The tumors in PBT group was found to be of higher pT stage compared with IT group, which was statistically significant (p=0.011).

The most common overall tumor stage observed in IT group was stage IB (46.7%) followed by stage IIIA (26.7%), whereas most common overall tumor stage seen with PBT group was stage IIB (33.3%) followed by stage IIA and IIIB (each 26.7%). The higher overall stage was seen to be more commonly associated with PBT group, which was found to be statistically significant (p <0.001).

The mean and median CA 19-9 was 190.33 IU/mL and 180 IU/mL in PBT group, whereas it was 82.33 IU/mL and 80 IU/mL in IT group respectively. Statistically significant difference was seen between 2 groups in terms of CA 19-9 (IU/mL) (W = 7.500, p = <0.001), with the mean and median CA 19-9 (IU/mL) being higher in PBT group.

In IT group, 93.3% cases had CA 19-9 was less than 100 IU/mL whereas in PBT group, 93.3% cases had CA 19-9 was more than 100 IU/mL.

CA 19-9 levels ≥ 100 IU/mL was significantly associated with PBT group (Odds Ratio 196 (11.12-3453.72), p<0.001).

The mean Tumor size in IT group was 2.01 cm and PBT group was 3.8 cm. There was a statistical significant difference between the 2 groups in terms of Tumor size (W = 32.500, p = 0.001), with the median Tumor size being highest in the PBT group.

60% of the patients in IT group had Tumor size <2 cm whereas 93.3% of the patients in PBT group had Tumor size ≥2 cm.

Larger tumor size (≥2 cm) was associated with PBT group, which was statistically significant (p <0.002).

There was a significant difference between the various groups in terms of distribution of PNI ($\chi 2 = 12.857$, p = <0.001). 60% PBT tumors had shown PNI, whereas none of the IT tumors was associated with PNI.

73.3% tumors from IT group and 86.7% tumors from PBT group showed CK7 positivity. CK7 positivity did not differentiate between IT and PBT tumors significantly.

All tumors from PBT group showed MUC1 expression (100%) whereas only 20% tumors from IT group showed MUC1 expression. MUC1 expression was significantly associated with PBT tumors (p = <0.001).

On analyzing combination of markers, 86.7% tumors in PBT group showed positivity for CK7/MUC1 expression, whereas this combination was found negative in 80% of IT group tumors. This

Table 3: Association Between Histological Subtype and CK7 (n = 30)

CK7	Histological Subtype			Fisher's Exact Test	
	Intestinal	Pancreatobilliary	Total	$\chi 2$	P Value
Positive	11 (73.3%)	13 (86.7%)	24 (80.0%)		
Negative	4 (26.7%)	2 (13.3%)	6 (20.0%)	0.833	0.651
Total	15 (100.0%)	15 (100.0%)	30 (100.0%)		

Table 4: Association Between Histological Subtype and MUC1 (n = 30)

MUC1	Histological Subtype			Chi-Squared Test	
	Intestinal	Pancreatotbilliary	Total	$\chi 2$	P Value
Positive	3 (20.0%)	15 (100.0%)	18 (60.0%)		
Negative	12 (80.0%)	0 (0.0%)	12 (40.0%)	20	<0.001
Total	15 (100.0%)	15 (100.0%)	30 (100.0%)		

combination association with the type of tumor was statistically significant (Odds ratio 26 (3.69-183.42); p <0.001).

86.7% tumors from IT group showed CK20 expression, whereas only 2 tumors from PBT group (13.3%) showed CK20 expression. CK20 positivity was significantly associated with IT type tumor (Odds ratio 42.25 (5.15-346.87) p = <0.001).

93.3% tumors from IT group expressed CDX2 positivity whereas none of the PBT group tumor showed CDX2 expression. CDX2 was found to be negative in all tumors from PBT group. CDX2 expression was statistically significant in differentiating IT from PBT tumors (Odds ratio 299.67 (11.28-7961.75) p < 0.001).

Table 5: Association Between Histological Subtype and CK7/MUC1 (n = 30)

CK7/MUC1	Histological Subtype			Chi-Squared Test	
	Intestinal	Pancreatobilliary	Total	$\chi 2$	P Value
Positive	3 (20.0%)	13 (86.7%)	16 (53.3%)		
Negative	12 (80.0%)	2 (13.3%)	14 (46.7%)	13.393	<0.001
Total	15 (100.0%)	15 (100.0%)	30 (100.0%)		

Table 6: Association Between Histological Subtype and CK20 (n = 30)

CK20	Histological Subtype			Chi-Squared Test	
	Intestinal	Pancreatobilliary	Total	$\chi 2$	P Value
Positive	13 (86.7%)	2 (13.3%)	15 (50.0%)		
Negative	2 (13.3%)	13 (86.7%)	15 (50.0%)	16.133	<0.001
Total	15 (100.0%)	15 (100.0%)	30 (100.0%)		

The combination of the markers CK20/CDX2 was found to be positive for 80% of IT tumors. All PBT tumors were negative for CK20/CDX2 expression. This combination of CK20/CDX2 expression was statistically significant in differentiating IT from

Table 7: Association Between Histological Subtype and CDX2 (n = 30)

CDX2	Histological Subtype			Chi-Squared Test	
	Intestinal	Pancreatobilliary	Total	$\chi 2$	P Value
Positive	14 (93.3%)	0 (0.0%)	14 (46.7%)		
Negative	1 (6.7%)	15 (100.0%)	16 (53.3%)	26.25	<0.001
Total	15 (100.0%)	15 (100.0%)	30 (100.0%)		

Table 8: Association Between Histological Subtype and CK20/CDX2 (n = 30)

CK20/CDX2	Histological Subtype			Chi-Squared Test	
	Intestinal	Pancreatobilliary	Total	$\chi 2$	P Value
Positive	12 (80.0%)	0 (0.0%)	12 (40.0%)		
Negative	3 (20.0%)	15 (100.0%)	18 (60.0%)	20	<0.001
Total	15 (100.0%)	15 (100.0%)	30 (100.0%)		

Table 9: Performance of Study Parameters for Predicting Histological Subtype: Pancreatobilliary vs Intestinal Type

Primary Diagnostic Parameters

Variable	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
CA 19-9 (IU/mL) (Cutoff: 115 by ROC)	93.3% (68-100)	93.3% (68-100)	93.3% (68-100)	93.3% (68-100)	93.3% (78-99)
CA 19-9	93.3% (68-100)	93.3% (68-100)	93.3% (68-100)	93.3% (68-100)	93.3% (78-99)
Tumor size (cm) (Cutoff: 3 by ROC)	73.3% (45-92)	86.7% (60-98)	84.6% (55-98)	76.5% (50-93)	80.0% (61-92)
Tumor size	93.3% (68-100)	60.0% (32-84)	70.0% (46-88)	90.0% (55-100)	76.7% (58-90)
PNI	60.0% (32-84)	100.0% (78-100)	100.0% (66-100)	71.4% (48-89)	80.0% (61-92)
LVI	13.3% (2-40)	100.0% (78-100)	100.0% (16-100)	53.6% (34-72)	56.7% (37-75)
CK7	86.7% (60-98)	26.7% (8-55)	54.2% (33-74)	66.7% (22-96)	56.7% (37-75)
MUC1	100.0% (78-100)	80.0% (52-96)	83.3% (59-96)	100.0% (74-100)	90.0% (73-98)
CK7/MUC1	86.7% (60-98)	80.0% (52-96)	81.2% (54-96)	85.7% (57-98)	83.3% (65-94)
CK20	86.7% (60-98)	86.7% (60-98)	86.7% (60-98)	86.7% (60-98)	86.7% (69-96)
CDX2	100.0% (78-100)	93.3% (68-100)	93.8% (70-100)	100.0% (77-100)	96.7% (83-100)
CK20/CDX2	100.0% (78-100)	80.0% (52-96)	83.3% (59-96)	100.0% (74-100)	90.0% (73-98)

PBT tumors (Odds ratio 110.71 (5.21-2350.49) p <0.001).

Diagnostic accuracy: Based on the performance of the study parameters, CDX2 expression had the highest diagnostic accuracy (96.7%) for histological subtype followed by, in decreasing frequency, high

CA 19-9 (93.3%), MUC1 (90%) and CK20/CDX2 combination (90%) and CK20 (86.7%).

Sensitivity: Highest sensitivity for diagnosing histological subtype was seen with expression of MUC1 (100%), CDX2 (100%) and combination CK20/CDX2 (100%) followed by high CA 19-9 >115 IU/mL (93.3%) and high tumor size >3 cm (93.3%).

Specificity: From the IHC markers used for this study, Highest specificity for diagnosing histological subtype was seen with expression of CDX2 (93.3%) followed by CK20 (86.7%) and combination CK20/CDX2 (80%).

PPV and NPV: From IHC markers used for this study, Highest PPV for diagnosing histological subtype was seen with expression of CDX2 (93.8%) followed by CK20 (86.7%). Similarly, highest NPV was seen with expression of MUC1 (100%), CDX2 (100%) and combination CK20/CDX2 (100%).

DISCUSSION

Patients with periampullary adenocarcinoma demonstrate a wide range of outcomes, with ampullary and duodenal tumours as well as tumours with intestinal morphology displaying a much better survival than pancreatic head and distal bile duct tumours or tumours with pancreatobiliary differentiation.[15-17]

However, exact origin of malignancy is not always possible to determine because majority of periampullary tumors would have grown to such large size to involve multiple potential sites of origin (pancreas, bile duct, ampulla, and duodenum). Furthermore, recent data suggest that histological subtype is a superior prognostic factor in periampullary adenocarcinoma, outperforming other parameters such as tumor location as even some poorly differentiated cancers preserve the histomolecular profile of their mucosa of origin.[2,9,10]

Bronsten et al.[18] and Westgaard et al.[19] reported in their respective studies that the PBT of differentiation was independently associated with a poor prognosis, while tumor origin did not significantly predict survival when adjusting for other histopathologic prognostic factors such as tumor margins and lymph node status. Hence differentiation into IT or PBT have become of paramount importance in view of its prognostic significance. Herein, we attempted to study whether this subtyping can be achieved with high sensitivity and specificity with IHC markers alone or in combination.

Recently multiple studies have attempted and proved the significant role of certain IHC markers in subtyping of periampullary malignancies into IT and PBT as well as their prognostic significance and patients' survival. Few important studies are mentioned here.

In the present study, CDX2 was the only marker that showed highest sensitivity (100%) and high specificity (93.3%) for predicting intestinal subtype (considering 10% staining of tumor cells as positive). Chu et al.[1] found CDX2 expression sensitivity 100% and specificity 83.3% for IT differentiation. Westgaard et al.[19] found CDX2 to be positive in 54% IT tumours and 15% PBT, with a sensitivity of 54% and specificity of 85% for IT. In a study by Hansel et al.[14] in 2004, less than 5% of pancreatic and biliary adenocarcinomas expressed CDX1/2. Sessa et al.[20] found 100% sensitivity and 70% specificity of CDX2 for IT differentiation, considering 10% cut-off for positive staining. In a study by N Bakshi et al.[21] in 2019, CDX2 showed high sensitivity (93.2%) and specificity (94.9%) for IT tumours with statistical significant correlation ($p<0.05$). De Paiva Haddad et al.[22] showed 86% sensitivity and 78% specificity of CDX2 expression for IT differentiation.

In our study, CK7 was expressed in majority of PBT tumors with high sensitivity (86.7%) but it has low specificity (26.7%), low PPV and low NPV as it has been found to be a rather common finding in IT tumors as well.[15] Morini et al.[23] have also shown in a very recent multicentric retrospective Italian study that more than 50% of IT adenocarcinomas were positive for CK7. Other studies have also noted that though CK7 is associated with pancreatobiliary origin in other locations, periampullary and ampullary intestinal adenocarcinoma also expresses CK7, often in a strong and diffuse manner.[15] We believe therefore that CK7 has limited utility in subtyping periampullary tumors as it is a nonspecific marker. Chu et al.[1] found CK7 expression sensitivity 83.3% and specificity 81.8% for PBT. In a study by P Bronsert et al.[18] in 2013, PBT marker CK7 showed the inverse pattern with high median expression level in PDAC / DBDAC (90%/ 95%), decreased with AMPAC (85%) and 0% in DUOAC. These correlations were highly significant ($p < 0.001$). In a study by DC Ang et al.[15] in 2014 CK7 showed positivity in 70% IT tumors and in 91%PBT tumors. The correlation was statistically significant ($P< 0.0001$). In a study by CF Moro et al.[16] in 2016, CK7 was expressed in approximately 70% of tumors of the intestinal type. In a study by N Bakshi et al.[21] in 2019, CK7 was positive in 91% of PBT tumours and 61% of IT tumours. This finding showed that CK7 immuno reactivity was not specific for either subtype.

Another marker CK20 showed moderate sensitivity of 86.7% and a specificity of 86.7% for IT tumor. CK20 and CDX2 positivity were found to be predictive of IT differentiation. In a study by P Bronsert et al.[18] in 2013, CK20 expression was highest 70% in DUOAC, 10% for AMPAC and negative (0%) for DBDAC and PDAC. In a study by bakshi et al.[21] in 2019, CK20 showed sensitivity of 82.2% and specificity of 89.9% for IT tumours. N Kumari et al.[24] in 2013, CK 20 had a moderate sensitivity of 50% and a specificity of 86.8% for predicting IT.

In our study, MUC1 positivity was associated with PBT with highest sensitivity of 100% and high specificity of 80%. In a study by Sessa et al.[20] in 2007, A significantly higher frequency of MUC1 and MUC5AC expression was detected in PBT tumours ($p<0.0001$), whereas a significantly higher percentage of positive cases for MUC2 was found among IT ACs ($p<0.023$). In a study by N Bakshi et al.[21] in 2019, MUC1 expression was positive in all the 79 PBT (100%) cases, 20% intestinal and 4% indeterminate cases, and the specificity of this marker was therefore 62%. In a study by Moriya T et al.[25] they evaluated MUC1 and MUC2 markers and showed that the positivity of MUC1 in the PBT was 93% and the positivity of MUC2 in the IT was 67%.

Westgaard et al.[19] found that CK7positivity ($P = 0.009$), CDX2 negativity ($P = 0.002$), and MUC4 positivity ($P = 0.026$) were independent markers in their study to predict PBT.

N Kumari et al.[24] in 2013 showed that MUC1 and CK7 were the markers with the least sensitivity and specificity (100% and 0%; 90.5% and 21% respectively).

In a study by I Perysinakis et al.[26] in 2017, On IHC staining, CK20 ($P < .0005$), MUC2 ($P = 0.054$), and CDX 2 ($P < 0.0005$) expression were more prevalent in IT tumours, while MUC1 ($P < 0.0005$) was more frequently expressed in PBT tumours.

A study by de Paiva Haddad et al.[22] used CK7, CK17, CK20, MUC1, MUC2, MUC5AC, MUC6, CDX2 and CD10 for subtyping periampullary carcinoma. The markers that showed significantly higher frequency of positivity for IT were MUC2, CK20, CD10, and CDX2, and for PBT were CK7 and MUC1.

The cut-off for positive staining for different markers has been variable in different studies ranging from any cell positive to as high as 25% to 30%. Therefore, the sensitivity for CDX2 in the literature ranges from 54% to 100% and for MUC1 ranges from 49% to 100%.[1,10,27,28,29] The specificity for CDX2 as reported in the literature is 70% to 85% and for MUC1 ranges from 46.5% to 81.8% in different studies.[1,10,19,20,22]

Little is known, however, about the combined expression of IHC markers in ACs. The chance of finding an original site of the malignancy is increased when combined expression of IHC markers has been utilized as compared with single marker.

In our study, combination of CK7/MUC1 showed 86.7% sensitivity and 80% specificity with 83.3% diagnostic accuracy to identify PBT tumor, whereas combination of CK20/CDX2 showed 100% sensitivity and 80% specificity with 90% diagnostic accuracy to identify IT tumor.

In a study by Bakshi N et al.[21], authors showed that specificity for diagnosing the IT, was further improved to 100% when using a panel of markers (i.e., CDX2 and CK20) as compared with individual markers (i.e., CDX2 or CK20) alone.

In a study by Kawabata et al.[10] significant differences were noted in the expression levels of CK 20 and MUC1 in histological IT and histological PBT, with the sensitivity being 100% for CK20 and 94% for MUC1 expression, respectively. These results indicate that the combination CK20 +/MUC1- pattern fully corresponds to the immunohistochemical IT (100%) and that the CK20-/MUC1+ pattern fully corresponds to the immunohistochemical PBT (94%).

In a study by DC Ang et al.[15] in 2014, they showed that combination staining patterns like "IHC positive for CK20/CDX2/MUC2 and negative for MUC1" or "IHC positive for CK20, CDX2, and MUC2, irrespective of MUC1 expression" would equate an IT phenotype (i.e., IHC-IT); and "IHC positive for MUC1 and negative for CDX2 and MUC2" would equate a PBT phenotype (i.e., IHC-PBT). They showed that such combination can predict histologic subtype with 90% sensitivity and 94% specificity. The utility of our IHC panel becomes most significant in the less common subtypes and in tumors that are difficult to classify on H&E. The prognostically more favourable IT can be distinguished confidently from the more aggressive PBT using combination IHC panel.

Correlation with prognosis and survival

The IHC markers have also shown correlation with prognosis.

Kitamura et al.[30] found that ampullary carcinoma with stronger MUC1 stains and weaker MUC2

staining had worse prognosis.

In a study by A westgaard et al.[19] in 2008, PBT ACs significantly more often showed presence of histopathologic features associated with a poor prognosis like perineural infiltration, areas with poor differentiation, advanced pT stage, and pancreatic tumour origin ($p < 0.001$). The histologic type of differentiation was found to be an independent predictor of survival ($p = 0.03$). They also showed an increased median survival in IT than PBT (60 months versus 17 months) irrespective of anatomical location.

In a study by P Bronsert et al.[18] in 2013, high tumor grade, high T and N Stage, lymph node ratio (LNR), PNI and LVI were significantly less frequent with IT adenocarcinoma. In univariate analysis, only histological subtype ($p = 0.006$) and LNR ($p = 0.019$) were found to be independent predictors of survival. Their study confirms that histopathological differentiation, in contrast to tumor location, is an independent prognostic factor.

Hansel et al.[14] demonstrated expression of CDX2as an independent marker of better outcome in periampullary carcinoma. In a study by Sessa et al.[20] in 2007, A longer survival was correlated with the expression of CDX2 as well as with an intestinal type of AC ($p=0.08$).

In a study by Kumari N et al.[24], CDX2 positive tumours showed a statistically significant low tumour stage, more T1/T2 stage tumours, and less PNI and LVI. In CDX2-positive tumours, pathological T stage, tumour stage, PNI, LVI and median survival were statistically significant. CDX2 was the only marker associated with patients' survival and showed a median survival of 44 months in CDX2-positive tumors compared with 22 months in CDX2-negative tumors ($P = 0.03$). In a study by CF Moro et al.[16] in 2016, IT phenotype was associated with better overall survival compared with PBT phenotype (Median OS 54 months v/s 24 months) with p value < 0.001. The IT phenotype, pT4 and pN0 were independent predictors of favourable OS.

A study by Iraklis Perysinakis et al.[17] in 2016, Expression of CK20 ($p = 0.065$) and CDX2 ($p = 0.008$) predicted a more favourable prognosis. Only MUC1 and CK20 identified PBT and IT tumours, respectively, with sufficient sensitivity and specificity. The overall survival was adversely influenced by LNI, elevated serum Ca19-9 levels, jaundice, poor differentiation, advanced T (T4) stage, advanced N stage, advanced overall TNM (III) stage and histological subtype ($p=0.005$). There was a clear trend toward prolonged survival in patients with IT tumours as compared to those with PBT tumours (median = 168 v/s 27 months).

In a study by Werling et al.[31], they showed that CDX2-positive cancers had a median survival of 45 months versus 20 months for CDX2- negative cancers. The 5-year survival rate in their study was 76% in CDX2-positive cancers and 26% in CDX2-negative cancers.

CONCLUSION

We studied the expression of CK7, CK20, MUC1 and CDX2 in periampullary adenocarcinomas. On the basis of the histological classification of periampullary adenocarcinomas, we found that CDX2 and MUC1 have a highest sensitivity (100%) and highest NPV (100%) for IT and PBT differentiation respectively. These immunohistochemical subtypes correlated well with the conventional histomorphological classification. A panel of IHC markers like CK20 and CDX2 together allows better identification of differentiation than the use of single markers alone. Using an IHC scheme will further objectify subtyping of these tumors and yield consistent, accurate results leading to better prediction of prognosis and improved patient outcome.

REFERENCES

1. Chu PG, Schwarz RE, Lau SK, Yen Y, Weiss LM. Immunohistochemical staining in the diagnosis of pancreatobiliary and ampulla of Vater adenocarcinoma: application of CDX2, CK17, MUC1, and MUC2. Am J Surg Pathol. 2005 Mar;29(3):359-67. doi: 10.1097/01.pas.0000149708.12335.6a. PMID: 15725805.
2. Santini D, Tonini G, Vecchio FM, Borzomati D, Vincenzi B, Valeri S, Antinori A, Castri F, Coppola R, Magistrelli P, Nuzzo G, Picciocchi A. Prognostic value of Bax, Bcl-2, p53, and TUNEL staining in patients with radically resected ampullary carcinoma. J Clin Pathol. 2005 Feb;58(2):159-65. doi: 10.1136/jcp.2004.018887. PMID: 15677536; PMCID: PMC1770581.
3. Perysinakis I, Margaris I, Kouraklis G. Ampullary cancer-a separate clinical entity? Histopathology. 2014 May;64(6):759-68. doi: 10.1111/his.12324. Epub 2014 Jan 24. PMID: 24456259.
4. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010 Jun;17(6):1471-4. doi: 10.1245/s10434-010-0985-4. PMID: 20180029.

5. Schirmacher, P., Büchler, M.W. Ampullary adenocarcinoma – differentiation matters. BMC Cancer 8, 251 (2008). https://doi.org/10.1186/1471-2407-8-251
6. Kimura W, Futakawa N, Yamagata S, Wada Y, Kuroda A, Muto T, Esaki Y. Different clinicopathologic findings in two histologic types of carcinoma of papilla of Vater. Jpn J Cancer Res. 1994 Feb;85(2):161-6. doi: 10.1111/j.1349-7006.1994.tb02077.x. PMID: 7511574; PMCID: PMC5919425.
7. Albores-Saavedra J, Henson DE, Klimstra DS. Tumors of gallbladder, extrahepatic bile ducts, and ampulla of Vater. In: Atlas of Tumor Pathology. Washington, DC: Armed Forces Institute of Pathology; 2000:259-316.
8. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020 Jan;76(2):182-188. doi: 10.1111/his.13975. Epub 2019 Nov 13. PMID: 31433515; PMCID: PMC7003895.
9. Albores-Saavedra J, Schwartz AM, Batich K, Henson DE. Cancers of the ampulla of vater: demographics, morphology, and survival based on 5,625 cases from the SEER program. J Surg Oncol. 2009 Dec 1;100(7):598-605. doi: 10.1002/jso.21374. PMID: 19697352.
10. Kawabata Y, Tanaka T, Nishisaka T, Inao T, Nishi T, Yano S. Cytokeratin 20 (CK20) and apomucin 1 (MUC1) expression in ampullary carcinoma: Correlation with tumor progression and prognosis. Diagn Pathol. 2010 Nov 25;5:75. doi: 10.1186/1746-1596-5-75. PMID: 21106111; PMCID: PMC3003636.
11. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, eds. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer; 2010.
12. Adsay NV, Bagci P, Tajiri T, et al. Pathologic staging of pancreatic, ampullary, biliary, and gallbladder cancers: pitfalls and practical limitations of the current AJCC/UICC TNM staging system and opportunities for improvement. Semin Diagn Pathol. 2012;29(3):127-141.
13. Zhou H, Schaefer N, Wolff M, Fischer HP. Carcinoma of the ampulla of Vater: comparative histologic/immunohistochemical classification and follow-up. Am J Surg Pathol. 2004 Jul;28(7):875-82. doi: 10.1097/00000478-200407000-00005. PMID: 15223956.
14. Hansel DE, Maitra A, Lin JW, Goggins M, Argani P, Yeo CJ, Piantadosi S, Leach SD, Biankin AV. Expression of the caudal-type homeodomain transcription factors CDX 1/2 and outcome in carcinomas of the ampulla of Vater. J Clin Oncol. 2005 Mar 20;23(9):1811-8. doi: 10.1200/JCO.2005.03.068. PMID: 15774774.
15. Ang DC, Shia J, Tang LH, Katabi N, Klimstra DS. The utility of immunohistochemistry in subtyping adenocarcinoma of the ampulla of vater. Am J Surg Pathol. 2014 Oct; 38(10):1371-9. doi: 10.1097/PAS.0000000000000230. PMID: 24832159.
16. Fernández Moro C, Fernandez-Woodbridge A, Alistair D'souza M, Zhang Q, Bozoky B, Kandaswamy SV, Catalano P, Heuchel R, Shtembari S, Del Chiaro M, Danielsson O, Björnstedt M, Löhr JM, Isaksson B, Verbeke C, Bozóky B. Immunohistochemical Typing of Adenocarcinomas of the Pancreatobiliary System Improves Diagnosis and Prognostic Stratification. PLoS One. 2016 Nov 9;11(11):e0166067. doi: 10.1371/journal.pone.0166067. Erratum in: PLoS One. 2017 Jan 26;12 (1):e0171283. PMID: 27829047; PMCID: PMC5102456.
17. Perysinakis I, Minaidou E, Mantas D, Sotiropoulos GC, Leontara V, Tsipras H, Zografos GN, Margaris I, Kouraklis G. Differentiation and prognostic markers in ampullary cancer: Role of p53, MDM2, CDX2, mucins and cytokeratins. Pathol Res Pract. 2016 Nov;212(11):1039-1047. doi: 10.1016/j.prp.2016.09.004. Epub 2016 Sep 22. PMID: 27688085.
18. Bronsert P, Kohler I, Werner M, et al. Intestinal-type of differentiation predicts favourable overall survival: confirmatory clinicopathological analysis of 198 periampullary adenocarcinoma of pancreatic, biliary, ampullary and duodenal origin. BMC Cancer. 2013;13:428.
19. Westgaard A, Tafjord S, Farstad IN, Cvancarova M, Eide TJ, Mathisen O, Clausen OP, Gladhaug IP. Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma. BMC Cancer. 2008 Jun 11;8:170. doi: 10.1186/1471-2407-8-170. PMID: 18547417; PMCID: PMC2430209.
20. Sessa F, Furlan D, Zampatti C, Carnevali I, Franzi F, Capella C. Prognostic factors for ampullary adenocarcinomas: tumor stage, tumor histology, tumor location, immunohistochemistry and microsatellite instability. Virchows Arch. 2007 Sep;451(3):649-57. doi: 10.1007/s00428-007-0444-1. Epub 2007 Jul 26. PMID: 17653761.
21. Bakshi N, Dhawan S, Nundy S, Rao S, Chopra P, Bhalla S. Role of Immunohistochemistry in the Subtyping of Periampullary Adenocarcinoma. Int J Surg Pathol. 2019 Sep;27(6):598-608. doi: 10.1177/1066896919837606. Epub 2019 Apr 3. PMID: 30942099.
22. de Paiva Haddad LB, Patzina RA, Penteado S, Montagnini AL, da Cunha JE, Machado MC, Jukemura J. Lymph node involvement and not the histophatologic subtype is correlated with outcome after resection of adenocarcinoma of the ampulla of vater. J Gastrointest Surg. 2010 Apr;14(4):719-28. doi: 10.1007/s11605-010-1156-4. Epub 2010 Jan 27. PMID: 20107918.
23. Morini S, Perrone G, Borzomati D, Vincenzi B, Rabitti C, Righi D, Castri F, Manazza AD, Santini D, Tonini G, Coppola R, Onetti Muda A. Carcinoma

of the ampulla of Vater: morphological and immunophenotypical classification predicts overall survival. Pancreas. 2013 Jan;42(1):60-6. doi: 10.1097/MPA.0b013e318258fda8. PMID: 22889982.

24. Kumari N, Prabha K, Singh RK, Baitha DK, Krishnani N. Intestinal and pancreatobiliary differentiation in periampullary carcinoma: the role of immunohistochemistry. Hum Pathol. 2013 Oct;44(10):2213-9. doi: 10.1016/j.humpath.2013.05.003. Epub 2013 Jul 5. PMID: 23834763.
25. Moriya T, Kimura W, Hirai I, Takasu N, Mizutani M. Expression of MUC1 and MUC2 in ampullary cancer. Int J Surg Pathol. 2011 Aug;19(4):441-7. doi: 10.1177/1066896911405654. Epub 2011 Jun 23. PMID: 21700631.
26. Perysinakis I, Minaidou E, Leontara V, Mantas D, Sotiropoulos GC, Tsipras H, Zografos GN, Margaris I, Kouraklis G. Differential Expression of β-Catenin, EGFR, CK7, CK20, MUC1, MUC2, and CDX2 in Intestinal and Pancreatobiliary-Type Ampullary Carcinomas. Int J Surg Pathol. 2017 Feb;25(1):31-40. doi: 10.1177/1066896916664987. Epub 2016 Aug 20. PMID: 27543509.
27. Friess H, Wang L, Zhu Z, Gerber R, Schröder M, Fukuda A, Zimmermann A, Korc M, Büchler MW. Growth factor receptors are differentially expressed in cancers of the papilla of vater and pancreas. Ann Surg. 1999 Dec;230(6):767-74; discussion 774-5. doi: 10.1097/00000658-199912000-00005. PMID: 10615931; PMCID: PMC1420940.
28. Kimura W, Futakawa N, Zhao B. Neoplastic diseases of the papilla of Vater.J Hepatobiliary Pancreat Surg. 2004;11(4):223-231.
29. Heinrich S, Clavien PA. Ampullary cancer. Curr Opin Gastroenterol. 2010;26(3):280-285.
30. Utsunomiya T, Yonezawa S, Sakamoto H, Kitamura H, Hokita S, Aiko T, Tanaka S, Irimura T, Kim YS, Sato E. Expression of MUC1 and MUC2 mucins in gastric carcinomas: its relationship with the prognosis of the patients. Clin Cancer Res. 1998 Nov;4(11):2605-14. PMID: 9829723.
31. Werling RW, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. Am J Surg Pathol. 2003 Mar;27(3):303-10. doi: 10.1097/00000478-200303000-00003. PMID: 12604886.