Original Article

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Pathology Section

Prognostic Value of CD8 and its Association with Stroma in Periampullary Carcinoma: A Cohort Study

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ABSTRACT

Introduction: Periampullary Carcinoma (PAC) is a rare gastrointestinal malignancy originating from within 2 cm of the Ampulla of Vater. It accounts for 0.5-2% of gastrointestinal cancers and includes Ampullary Carcinoma (AC), Pancreatic head Carcinoma (PC), Distal Common bile duct Carcinoma (DCC), and Duodenal Carcinoma (DC). PACs show heterogeneous histopathological features, characterised by intratumoural spatial complexity in cellularity, extracellular matrix, and necrosis. CD8+ Tumour-Infiltrating Lymphocytes (TILs) are cytotoxic T lymphocytes. Their activity, along with that of other immune cells in the tumour microenvironment, affects cancer progression and immune regulation.

Aim: To assess and quantify CD8+ TILs and stromal density using clinicohistopathological features and Immunohistochemistry (IHC).

Materials and Methods: This cohort study was done over a 48-month period (February 2021 to January 2025) at Guntur Medical College, Guntur, Andhra Pradesh, India. Representative Formalin-Fixed, Paraffin-Embedded (FFPE) tissue blocks from ampullary growth were selected, and sections were analysed using H&E, Masson's trichrome special stain, and CD8 and α-SMA IHC. Clinicohistopathological data were recorded. Variables {subtype, grade, stage, Lymphovascular Invasion (LVI), lymph node status} were evaluated using Fisher's exact test, Kaplan-Meier methods, and Cox regression.

Results: A total of 31 cases were evaluated (mean age: 57.4 years). The study comprised 15 patients (48.4%) aged <59 years and 16 (51.6%) aged ≥59 years. Significant associations were identified between high total CD8+ TILs expression and patient age, tumour grading, gemcitabine use, stromal density, Smooth Muscle Actin (SMA) activity, and histological subtype (p-value <0.05 for all). High CD8+ TIL infiltration declined as stromal density increased (loose: 11/15 vs moderate: 4/11 vs strong: 0/5; p-value=0.009). Univariable Cox regression analysis revealed a favourable prognostic trend for patients with higher CD8 expression (HR=0.78, p-value=0.595). The median Overall Survival (OS) was 33 months. In multivariable analysis, high total CD8+ TILs expression was associated with significantly improved OS (HR 0.11, p-value=0.005); however, due to sample size constraints, this adjusted estimate represents an exploratory prognostic trend in patients receiving adjuvant chemotherapy.

Conclusion: A loose stromal microenvironment facilitates robust, protective CD8+ TILs infiltration, whereas a dense stroma often limits CD8+ TILs efficacy. While these exploratory findings highlight total CD8+ TILs as a promising prognostic biomarker, larger multicentre cohort validation is required before clinical integration. Combining standard chemotherapy with targeted stromal remodelling and immune checkpoint inhibitors holds significant potential to enhance CD8+ TIL cytotoxicity and improve clinical outcomes.

Keywords: Ampulla of vater, Prognostic biomarker, Tumour-infiltrating lymphocytes

INTRODUCTION

The PAC is a rare gastrointestinal malignancy originating from within 2 cm of the Ampulla of Vater. It includes AC, PC, DCC, and DC [1-3]. PACs show heterogeneous histopathological features, characterised by intratumoural spatial complexity in cellularity, extracellular matrix, and necrosis [4]. AC originates from the duodenal surface of the major papillae. It can be divided into two main histological categories based on mucosal origin and morphology: Intestinal (I-type) and Pancreatobiliary (PB-type). About 40% of cases show mixed or hybrid phenotypes with features of both types [5]. The tumour microenvironment or stroma is a multicellular system consisting of mesenchymal, endothelial, and haematopoietic cells within the extracellular matrix [6]. The stromal response reduces microvascularity and drug delivery to tumour cells, thereby complicating treatment by promoting progression, metastasis, and chemotherapy resistance [7]. Both the pancreatobiliary and hybrid types elicit a marked fibro-inflammatory (desmoplastic) response, driven by stellate cell activation. This creates dense stroma characteristic of pancreatic cancer [8]. α-Smooth Muscle Actin (SMA) is a standard intracellular marker for myofibroblastic Cancer-Associated Fibroblasts (myCAFs). These α-SMA-positive myCAFs are significantly associated with aggressive tumour behaviour and poorer patient survival [9]. CD8+ TILs cytotoxic T cells identify cancer as a foreign body in a major histocompatibility complex class 1-restricted manner, attacking tumour cells that present tumour-associated antigen peptides [10,11]. Immune cells influence the tumour microenvironment, but this depends on factors such as tumour type, cellular interactions, leucocyte plasticity, and their microenvironmental location [12]. This study aimed to assess the prognostic importance of CD8+TILs and their association with stromal density and activity. Also to evaluate how clinicopathological variables and treatment history impact OS in patients treated with surgery followed by neoadjuvant and adjuvant chemotherapy using Haematoxylin and Eosin staining, Masson's trichrome special stain, CD8 and α-SMA IHC.

MATERIALS AND METHODS

This cohort study was done in the Department of Pathology at Guntur Medical College, Guntur, Andhra Pradesh, India, over a 48-month period from February 2021 to January 2025. The study was approved by the Institutional Ethics Committee (GMC/IEC/33/2025).

Inclusion criteria: Ampullary tumours, pancreatoduodenectomy specimen, paraffin block available for IHC were included in the study.

Exclusion criteria: Endoscopic biopsies, paraffin block unavailable, and insufficient for IHC. Tumours with primary origin in the pancreas, duodenum, and distal bile duct were excluded from the study.

Study Procedure

This was a single-centre study in which all diagnosed and surgically archived consecutive PAC cases were screened; all 31 cases of AC were included, and no cases of distal bile duct, duodenal, or primary pancreatic carcinoma were received during the study period. Well-preserved, FFPE tumour tissue blocks were selected for analysis. Serial sections were cut and processed for H&E, Masson's trichrome special stain, and IHC. Following the CAP protocol, H&E-stained slides were reviewed for histopathologic subtype, grade, stage, LVI, Perineural Invasion (PNI), and lymph node status. Treatment for patients was determined based on histopathological subtype and standard protocols for neoadjuvant and adjuvant chemotherapy: gemcitabine-based regimens for pancreatobiliary and mixed subtypes, and 5-FU-based regimens for intestinal subtypes [13]. Nine patients did not receive neoadjuvant therapy due to elevated bilirubin and stent complications. Intestinal-subtype cases received eight to 10 cycles of adjuvant chemotherapy, whereas pancreatobiliary-subtype cases received five to six cycles. All patients received adjuvant chemotherapy, and 22 received both neoadjuvant and adjuvant chemotherapy.

For each patient, clinical details recurrence, and status at last follow-up were recorded. Regular outpatient visits were tracked, laboratory tests, and cross-sectional imaging at scheduled intervals.

Immunohistochemical study: The best FFPE tissue blocks showing tumour characteristics were selected, and 3 µm-thickness sections were cut. CD8 (144B, Mouse monoclonal antibody, cat no: PDM094 10MM, Diagnostic Biosystems, CA, USA; 1:200 dilution) and SMA (1A4, Mouse monoclonal antibody, cat no: PDM003-10MM, Diagnostic Biosystems, CA, USA; 1:200 dilution) antibodies were used for IHC. Antigen retrieval was performed using Diagnostic Biosystems Montage Opus 365. Tonsil tissue was used as a positive control for CD8. Leiomyoma tissue was used as a positive control for αSMA. The Leica Bond-Max (Leica Biosystems, Germany) staining platform was used for immunohistochemical staining.

Immunohistochemical scoring: Samples stained for CD8 were independently assessed by two expert pathologists. The pathologists were blinded to clinical data. They used a semiquantitative scoring system called the H-score. Manual scoring allows pathologists to manually exclude artifacts such as tissue folding, necrotic debris, or non-specific background. If there were discrepancies, the final H-score was determined by consensus between the two pathologists. Cohen's kappa was used to measure inter-rater variability, adjusting for chance agreement.

An H-score (0-300) was calculated for each case. The score was determined by multiplying the percentage of positive cells (0-100%) by the staining intensity. Immunostaining intensity was visually graded as 0 (absent), 1 (weak), 2 (moderate) and 3 (strong). Cells were assessed in three tumour compartments: intratumoural, stromal, and invasive margin. For each case, five to 10 high-power fields (using a 40x objective lens) were evaluated in each compartment. The interpretation of the CD8 staining reaction and the use of the median H-score for binary categorisation (low vs high) were based on the methodology described by Rezagholizadeh F et al., [14]. In the present study cohort, the calculated median H-score cut-offs were 122 for intratumoural CD8, 148 for invasive margin CD8, and 123 for stromal CD8. Furthermore, a median total CD8 H-score of 447 was established as the cut-off for categorising cases into low or high total CD8 expression groups. Stroma was categorised as loose, moderate, or strong based on H&E-stained sections. On the corresponding slides, Masson's trichrome was performed. αSMA was scored as high, moderate, and low based on predefined criteria. While CD8+ TIL H-scores were evaluated independently to establish inter-rater reliability, the categorical grading of stromal density and α-SMA activity was determined by direct consensus review between the two expert pathologists.

STATISTICAL ANALYSIS

Fisher's exact test was used to compare the proportions of categorical outcomes between the groups. OS was measured, and Kaplan-Meier curves were plotted. Univariate analyses used the Mantel-Cox log-rank test. Multivariate analyses were done using a Cox proportional hazards model. Two blinded, expert pathologists independently evaluated CD8+ TILs, dichotomising continuous H-scores into "low" and "high" expression based on the median. Inter-rater reliability was established using percentage agreement and Cohen's Kappa (κ). Analyses were done using the Jamovi software (Version 2.6). A p-value <0.05 was considered statistically significant.

RESULTS

A total of 31 cases were evaluated (mean age: 57.4 years). The study comprised 18 males (58.1%) and 13 females (41.9%). Histologically, 16 cases (51.6%) were Intestinal subtype, 11 cases (35.5%) were Pancreatobiliary subtype, and four cases (12.9%) were mixed subtype. All 31 cases (100%) had negative (R0) margins. No local or distant recurrences were observed prior to death; thus, OS is reported as the primary endpoint.

Over a median follow-up of 32 months (range: 6-48 months), 19 mortality events occurred. The median OS for the cohort was 33.0 months (95% CI: 26.0-Not Reached), with an estimated 4-year OS rate of 37.2% (95% CI: 23.2%-59.6%).

Independent classification of CD8+ TILs across the 31 cases demonstrated high inter-rater reproducibility with an 87.1% absolute agreement. Cohen's Kappa confirmed substantial, statistically significant agreement between the two pathologists (κ=0.742, Z=4.13, p-value<0.001) [Table/Fig-1,2]. [Table/Fig-1] summarises the baseline distribution of categorical variables, demonstrating the proportional spread of high and low CD8 scores across all three tumour compartments. [Table/Fig-2] details the cross-tabulation of these expression levels against clinicopathological features, highlighting the statistically significant inverse association between total CD8 infiltration and stromal density.

Variable	Category	n (%)
Intratumoural CD8	Low (≤122)	17 (54.8)
	High (>122)	14 (45.2)
Invasive margin CD8	Low (≤148)	16 (51.6)
	High (>148)	15 (48.4)
Stromal CD8	Low (≤123)	16 (51.6)
	High (>123)	15 (48.4)
Total CD8 score	Low (≤447)	16 (51.6)
	High (>447)	15 (48.4)
Stroma density	Loose	15 (48.4)
	Moderate	11 (35.5)
	Strong	5 (16.1)
SMA activity	Low	15 (48.4)
	Moderate	11 (35.5)
	High	5 (16.1)

[Table/Fig-1]: Distribution of CD8 expression and stromal characteristics (n=31). CD8 high/low categorisation is based on sample medians. α-SMA was graded based on predefined criteria.

Univariable Cox regression revealed a consistent, favourable prognostic trend for higher CD8+ TILs expression. Patients with high intratumoural (HR 0.65), invasive margin (HR 0.71), and total CD8 (HR 0.78) exhibited reduced mortality hazards, indicating a protective antitumour immune response [Table/Fig-3]. Furthermore, loose stromal density and low SMA activity were associated with improved survival, suggesting that a less desmoplastic microenvironment facilitates immune infiltration. Although the Mixed subtype trended toward a higher hazard and gemcitabine exposure showed no significant survival effect (p-value=0.981), the consistently reduced hazard ratios across all high CD8 compartments support its utility as a favourable prognostic biomarker in adjuvant-treated cases.

Multivariable Cox regression confirmed age ≥59 years as an independent predictor of poorer survival (adjusted HR 13.56,

Variable	Category	Intratumoural CD8			Invasive margin CD8			Stromal CD8			Total CD8		
		Low n (%)	High n (%)	p-value	Low n (%)	High n (%)	p-value	Low n (%)	High n (%)	p-value	Low n (%)	High n (%)	p-value
Age (median= 59 years)	<59	7 (41.2)	8 (57.1)	0.479	10 (62.5)	5 (33.3)	0.156	11 (68.8)	4 (26.7)	**0.032**	11 (68.8)	4 (26.7)	**0.032**
	≥59	10 (58.8)	6 (42.9)		6 (37.5)	10 (66.7)		5 (31.2)	11 (73.3)		5 (31.2)	11 (73.3)	
Sex	Female	5 (29.4)	8 (57.1)	0.157	8 (50.0)	5 (33.3)	0.473	7 (43.8)	6 (40.0)	1.000	8 (50.0)	5 (33.3)	0.473
	Male	12 (70.6)	6 (42.9)		8 (50.0)	10 (66.7)		9 (56.2)	9 (60.0)		8 (50.0)	10 (66.7)	
T stage	T1-2	9 (52.9)	5 (35.7)	0.473	4 (25.0)	10 (66.7)	0.032	6 (37.5)	8 (53.3)	0.479	5 (31.2)	9 (60.0)	0.156
	T3	8 (47.1)	9 (64.3)		12 (75.0)	5 (33.3)		10 (62.5)	7 (46.7)		11 (68.8)	6 (40.0)	
N stage	N0	6 (35.3)	4 (28.6)	1.000	3 (18.8)	7 (46.7)	0.135	6 (37.5)	4 (26.7)	0.704	4 (25.0)	6 (40.0)	0.458
	N+	11 (64.7)	10 (71.4)		13 (81.2)	8 (53.3)		10 (62.5)	11 (73.3)		12 (75.0)	9 (60.0)	
Grading	G1	10 (58.8)	6 (42.9)	0.479	4 (25.0)	12 (80.0)	**0.004**	5 (31.2)	11 (73.3)	**0.032**	4 (25.0)	12 (80.0)	**0.004**
	G2/G3	7 (41.2)	8 (57.1)		12 (75.0)	3 (20.0)		11 (68.8)	4 (26.7)		12 (75.0)	3 (20.0)	
Perineural Invasion (PNI)	No	8 (47.1)	13 (92.9)	**0.009**	12 (75.0)	9 (60.0)	0.458	10 (62.5)	11 (73.3)	0.704	10 (62.5)	11 (73.3)	0.704
	Yes	9 (52.9)	1 (7.1)		4 (25.0)	6 (40.0)		6 (37.5)	4 (26.7)		6 (37.5)	4 (26.7)	
Lymphovascular Invasion (LVI)	No	5 (29.4)	4 (28.6)	1.000	3 (18.8)	6 (40.0)	0.252	5 (31.2)	4 (26.7)	1.000	3 (18.8)	6 (40.0)	0.252
	Yes	12 (70.6)	10 (71.4)		13 (81.2)	9 (60.0)		11 (68.8)	11 (73.3)		13 (81.2)	9 (60.0)	
Gemcitabine	No	8 (47.1)	8 (57.1)	0.722	6 (37.5)	10 (66.7)	0.156	6 (37.5)	10 (66.7)	0.156	4 (25.0)	12 (80.0)	**0.004**
	Yes	9 (52.9)	6 (42.9)		10 (62.5)	5 (33.3)		10 (62.5)	5 (33.3)		12 (75.0)	3 (20.0)	
Stroma density	Loose	7 (41.2)	8 (57.1)	0.112	6 (37.5)	9 (60.0)	0.396	3 (18.8)	12 (80.0)	**0.001**	4 (25.0)	11 (73.3)	**0.009**
	Moderate	5 (29.4)	6 (42.9)		6 (37.5)	5 (33.3)		8 (50.0)	3 (20.0)		7 (43.8)	4 (26.7)	
	Strong	5 (29.4)	0 (0.0)		4 (25.0)	1 (6.7)		5 (31.2)	0 (0.0)		5 (31.2)	0 (0.0)	
SMA activity	Low	7 (41.2)	8 (57.1)	0.112	6 (37.5)	9 (60.0)	0.396	3 (18.8)	12 (80.0)	**0.001**	4 (25.0)	11 (73.3)	**0.009**
	Moderate	5 (29.4)	6 (42.9)		6 (37.5)	5 (33.3)		8 (50.0)	3 (20.0)		7 (43.8)	4 (26.7)	
	High	5 (29.4)	0 (0.0)		4 (25.0)	1 (6.7)		5 (31.2)	0 (0.0)		5 (31.2)	0 (0.0)	
PAC type	Intestinal	8 (47.1)	8 (57.1)	0.184	6 (37.5)	10 (66.7)	0.272	6 (37.5)	10 (66.7)	0.237	4 (25.0)	12 (80.0)	**0.005**
	Mixed	4 (23.5)	0 (0.0)		3 (18.8)	1 (6.7)		2 (12.5)	2 (13.3)		3 (18.8)	1 (6.7)	
	PB	5 (29.4)	6 (42.9)		7 (43.8)	4 (26.7)		8 (50.0)	3 (20.0)		9 (56.2)	2 (13.3)	

[Table/Fig-2]: Association of CD8 expression with clinicopathological and stromal features (n=31).

Variable	Levels	n (%)	Hazard ratio (95% CI)	p-value
Age (median=59)	<59	15 (48.4)	Reference	**0.004**
	≥59	16 (51.6)	4.35 (1.62-11.71)	
Sex	Female	13 (41.9)	Reference	0.805
	Male	18 (58.1)	1.13 (0.44-2.87)	
T stage	T1-T2	14 (45.2)	Reference	0.598
	T3	17 (54.8)	0.78 (0.32-1.94)	
N stage	N0	10 (32.3)	Reference	0.444
	N+	21 (67.7)	1.49 (0.53-4.17)	
Grade	G1	16 (51.6)	Reference	0.900
	G2/G3	15 (48.4)	1.06 (0.43-2.61)	
PNI	No	21 (67.7)	Reference	0.760
	Yes	10 (32.3)	0.86 (0.33-2.27)	
LVI	No	9 (29.0)	Reference	0.302
	Yes	22 (71.0)	1.80 (0.59-5.48)	
Gemcitabine	No	16 (51.6)	Reference	0.981
	Yes	15 (48.4)	0.99 (0.40-2.44)	
Intratumoural CD8 (Low vs High)	Low	17 (54.8)	Reference	0.366
	High	14 (45.2)	0.65 (0.26-1.65)	
Invasive margin CD8	Low	16 (51.6)	Reference	0.462
	High	15 (48.4)	0.71 (0.29-1.77)	
Stromal CD8	Low	16 (51.6)	Reference	0.787
	High	15 (48.4)	1.13 (0.46-2.79)	
Total CD8	Low	16 (51.6)	Reference	0.595
	High	15 (48.4)	0.78 (0.31-1.94)	
Stroma density	Strong	5 (16.1)	Reference	
	Moderate	11 (35.5)	0.42 (0.12-1.47)	0.176
	Loose	15 (48.4)	0.66 (0.22-1.98)	0.458
SMA activity	High	5 (16.1)	Reference	
	Moderate	11 (35.5)	0.42 (0.12-1.47)	0.176
	Low	15 (48.4)	0.66 (0.22-1.98)	0.458
PAC type	Intestinal	16 (51.6)	Reference	
	PB	11 (35.5)	0.93 (0.35-2.51)	0.891
	Mixed	4 (12.9)	1.15 (0.31-4.26)	0.835

[Table/Fig-3]: Univariable Cox regression analysis of clinicopathological and stromal variables (n=31).

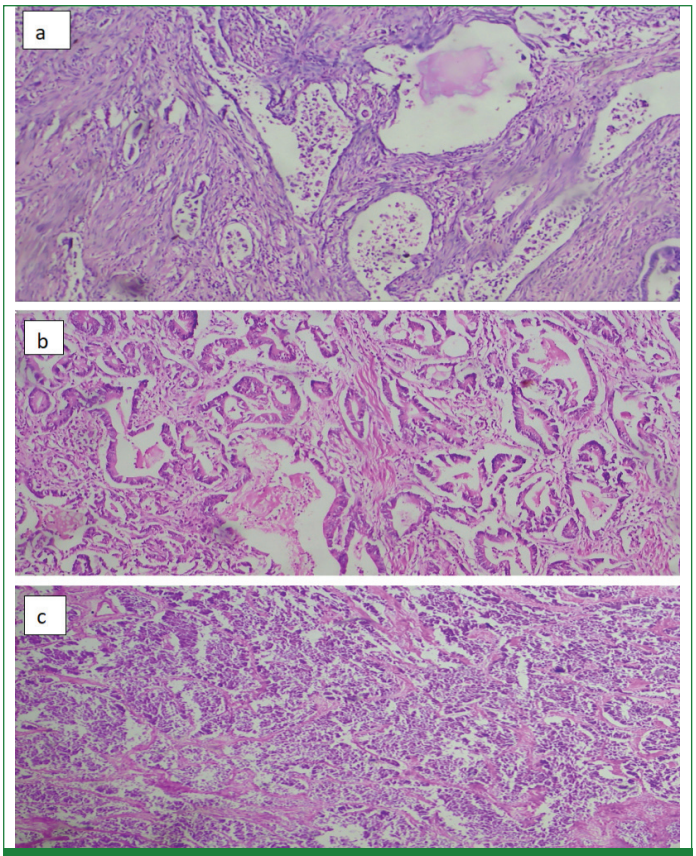
95% CI: 3.79-48.56, p-value <0.001). Crucially, high total CD8+ TILs expression emerged as a powerful, independent favourable prognostic biomarker, demonstrating a markedly reduced mortality hazard (HR 0.11, 95% CI: 0.02-0.52, p-value=0.005) [Table/Fig-4].

Variable	Levels	n (%)	HR (univariable)	HR (multivariable)
Age (median=59)	<59	15 (48.4)	-	-
	≥59	16 (51.6)	4.35 (1.62-11.71, **p=0.004**)	13.56 (3.79-48.56, **p<0.001**)
T stage	T1-T2	14 (45.2)	-	-
	T3	17 (54.8)	0.78 (0.32-1.94, p=0.598)	1.05 (0.37-2.98, p=0.924)
Grade	G1	16 (51.6)	-	-
	G2/G3	15 (48.4)	1.06 (0.43-2.61, p=0.900)	0.43 (0.14-1.31, p=0.139)
Gemcitabine	No	16 (51.6)	-	-
	Yes	15 (48.4)	0.99 (0.40-2.44, p=0.981)	0.66 (0.18-2.40, p=0.530)
Total CD8	Low	16 (51.6)	-	-
	High	15 (48.4)	0.78 (0.31-1.94, p=0.595)	0.11 (0.02-0.52, **p=0.005**)

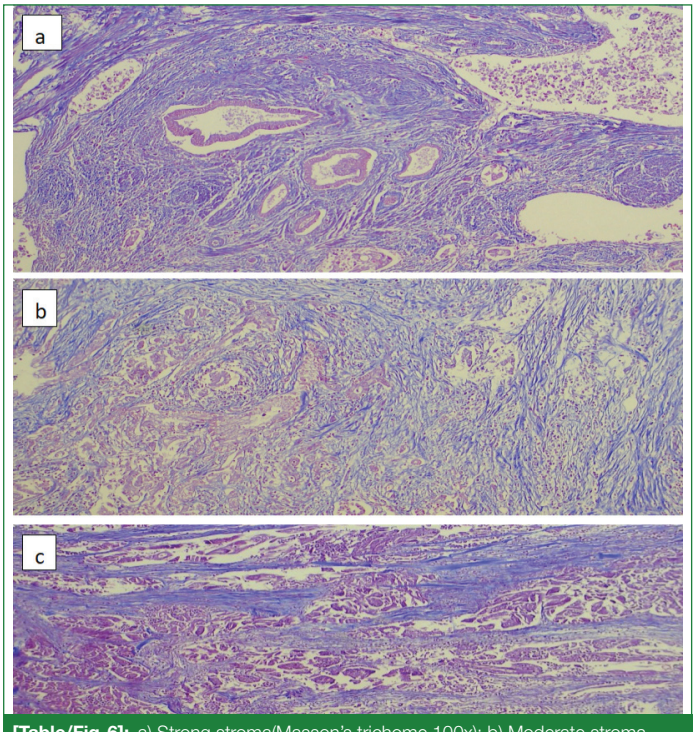
[Table/Fig-4]: Multivariable survival analysis.
*Footnote: HR= hazard ratio; A higher HR indicates increased hazard, whereas an HR below 1 indicates lower hazard; Study size: n=31

However, due to the small cohort size and limited number of events, this multivariable model carries an inherent risk of statistical overfitting; therefore, the magnitude of these adjusted hazard ratios should be interpreted strictly as an exploratory trend.

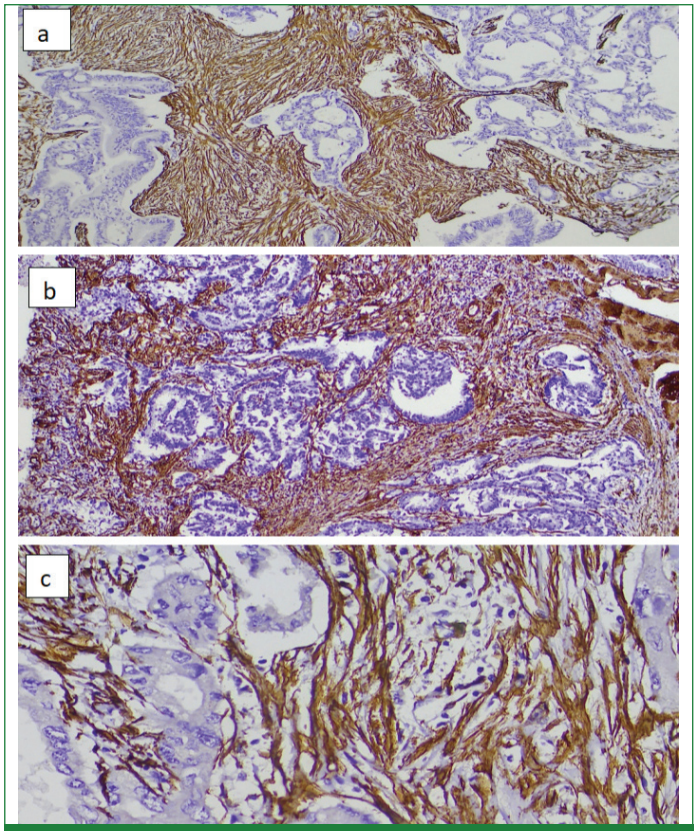
Three cases of pancreatobiliary subtype and two cases of mixed subtype showed strong stromal density and stromal activity. Five cases of pancreatobiliary subtype and six cases of intestinal subtype showed moderate stromal density and stromal activity. Three cases of pancreatobiliary subtype, two cases of mixed subtype and 10 cases of intestinal subtype showed loose stromal density and stromal activity [Table/Fig-5-7].



[Table/Fig-5]: a) Strong stroma (H&E 100x); b) Moderate stroma (H&E 100x); c) Low stroma (H&E 100x).

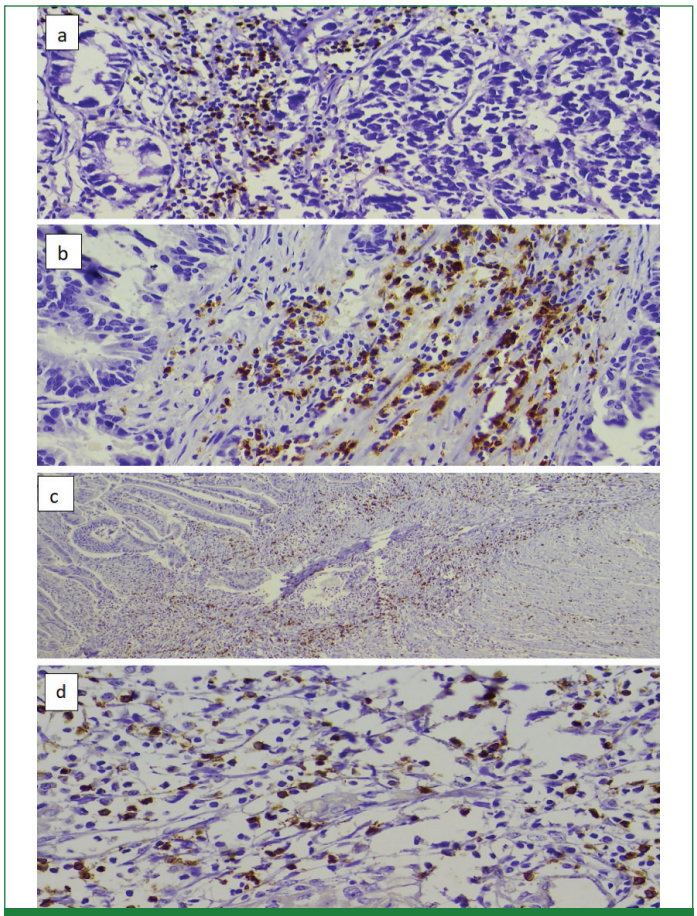


[Table/Fig-6]: a) Strong stroma(Masson's trichome 100x); b) Moderate stroma (Masson's trichome 40x); c) Low stroma (Masson's trichome 100x).



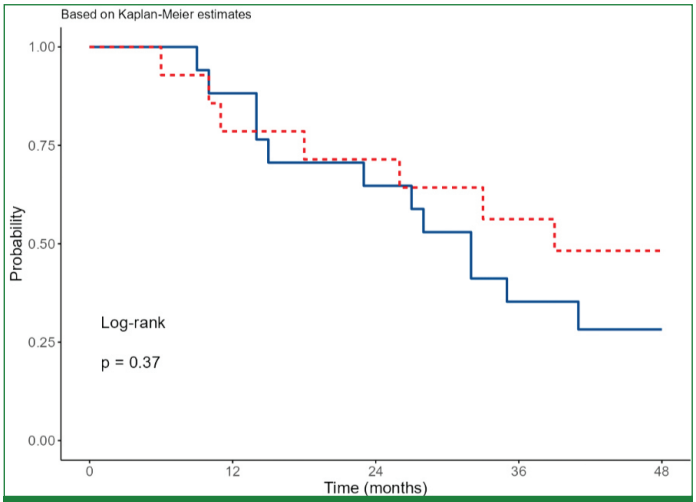
[Table/Fig-7]: a) αSMA high expression (IHC 100x); b) αSMA moderate expression (IHC 100x); c) αSMA low expression (IHC 100x).

Nine cases of pancreatobiliary subtype, three cases of mixed subtype and four cases of intestinal subtype showed low CD8+TILs expression. Two cases of pancreatobiliary subtype, one case of mixed subtype and 12 cases of intestinal subtype showed high CD8+TILs expression [Table/Fig-8].

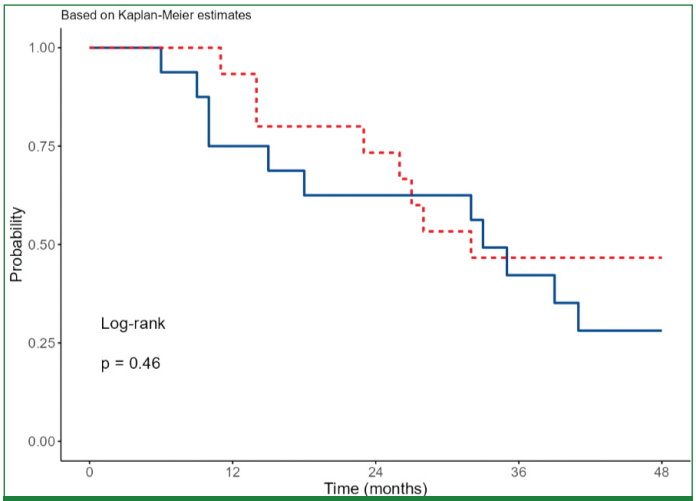


[Table/Fig-8]: a) Intratumoral compartment, CD8 showing high expression (IHC 100x); b) Stroma, CD8 showing high expression (IHC 100x); c) Invasive margin, CD8 showing high expression (IHC 40x); d) Stroma, CD8 showing low expression (IHC 100x).

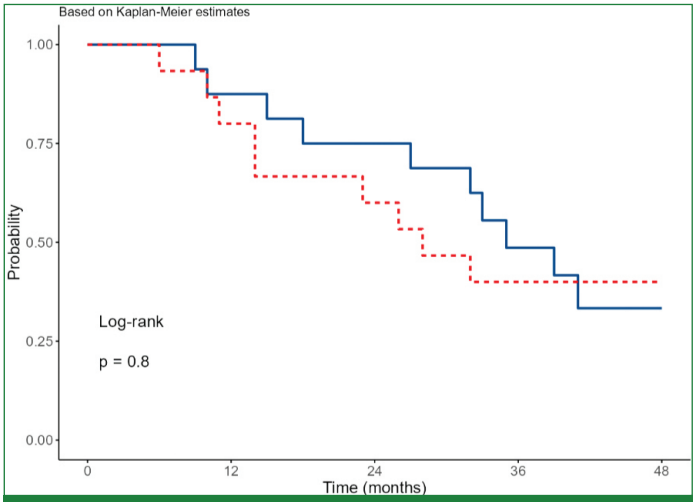
Though univariably non-significant, multivariable analysis confirmed the protective value of total CD8+ TILs (HR 0.11, p-value=0.005), as older age (≥59 years) initially masked this benefit [Table/Fig-9-13].



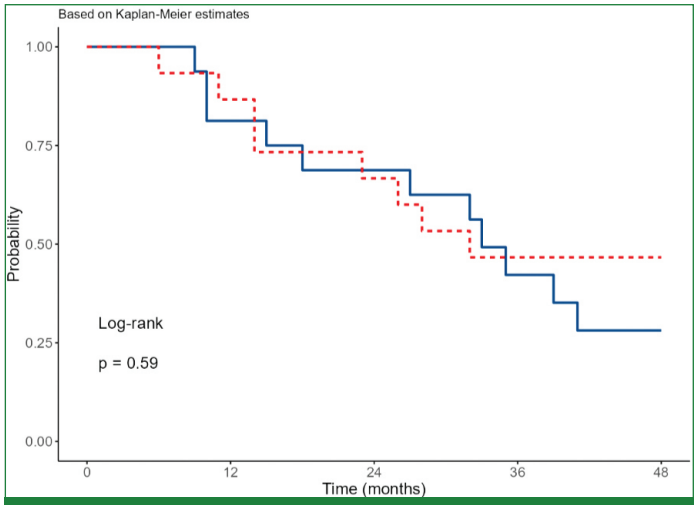
[Table/Fig-9]: Survival plot- intratumoral CD8 (low vs high).



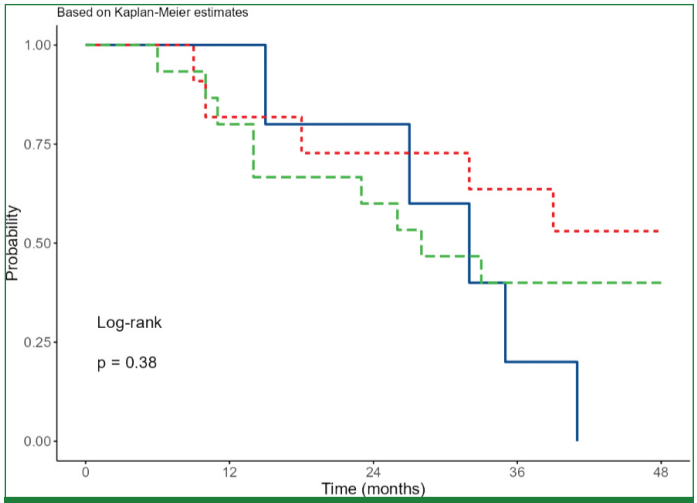
[Table/Fig-10]: Survival plot- invasive margin CD8 (low vs high).



[Table/Fig-11]: Survival plot- Stromal CD8 (low vs high).



[Table/Fig-12]: Survival plot- Total CD8 (low vs high).



[Table/Fig-13]: Survival Plot- SMA activity (high vs moderate vs low).

DISCUSSION

Various studies have assessed the impact of TILs in Pancreatic ductal adenocarcinoma. However, for patients with other types of PAC, the prognostic value of CD8 and its association with TILs remains largely unexplored [15]. The distinct origins of periampullary cancers result in varied reported survival rates, emphasising the challenges in achieving favourable outcomes across different types [16,17]. Pancreatobiliary subtype of AC shows an immune-exclusion profile, indicating an increased abundance of immunosuppressive elements, such as CAFs and regulatory T cells (Tregs), and sparse, less effective immune infiltration [18]. The intestinal subtype of AC shows higher immunogenicity; these tumours display a dense infiltration of CD8+ TILs within the tumour nests and natural killer cells, a stronger immune response, and a higher, spatially uneven concentration of T-cells and immune markers, which associate with a more favourable prognosis [19,20].

In the present study, heterogeneous features, high stromal density and stromal activity showed low CD8+TILs and low stromal density and activity showed high CD8+TILs [Table/Fig-5-8]. Ino Y et al., previously reported better outcomes in patients with high stromal CD8 expression [21], however, this was not observed in the present study, where high stromal CD8 alone did not confer a survival advantage (HR 1.13, p-value=0.787), whereas the total composite CD8 was highly prognostic, this discrepancy was because of tumour's biological microenvironment differing between pancreatobiliary and intestinal subtypes dictates whether these immune cells are active attackers or trapped, dysfunctional or bystanders [22]. In line with the present study, several studies have shown better survival in patients with high intratumoural CD8+ TILs expression in oesophageal, colorectal, head and neck, breast, ovarian, renal, lung, and PDAC [15]. In this study, all the 31 cases showed R0 status due to early symptom presentation and anatomically favourable location, which was in concordance with the study done by Kim HS et al., where, out of 17 intestinal subtype cases, 15 cases (88.2%) showed R0 status, and out of 93 pancreatobiliary subtype, 78 cases (83.9%) showed R0 status [23]. In this study, statistically significant differences between the low and high total CD8+TILs groups were observed for patient age (p-value=0.032), tumour grading (p-value=0.004), gemcitabine use (p-value=0.004), stromal density (p-value=0.009), SMA activity (p-value=0.009), and histological subtype (p-value=0.005). High CD8+ TILs infiltration in the intratumoural and invasive margins

consistently trended toward improved OS. Though univariably non-significant, multivariable analysis confirmed the protective value of total CD8+ TILs (HR 0.11, p-value=0.005), as older age (≥59 years) initially masked this benefit. Mixed-subtype had a higher hazard (HR 1.15, p-value=0.835) when compared with the pancreatobiliary and intestinal subtype. In this study, seven cases (two pancreatobiliary subtype and five intestinal subtype) with high CD8 counts received both neoadjuvant and adjuvant chemotherapy, with a favourable prognosis and OS. In concordance with the present study, a US study of 121 patients showed that patients receiving 5-FU had a longer median OS and improved survival (p-value=0.046) than those receiving gemcitabine [24].

Limitation(s)

The study used manual, semiquantitative CD8 assessment, acknowledging that automated digital image analysis offers higher throughput, though independent pathologist evaluation provided high interobserver reliability to mitigate subjectivity. The small sample size (n=31) reflects the rarity of this malignancy and the inherent risk of selection bias associated with the single-centre retrospective observational cohort design. Crucially, the limited number of events constrains the multivariable Cox regression, leading to statistical overfitting, inflated hazard ratios, and wide confidence intervals. Therefore, all multivariable estimates are strictly exploratory and require validation in larger, prospectively powered multicentre cohorts to definitively establish the independent prognostic utility of CD8+ TILs.

CONCLUSION(S)

CD8 and stromal markers are independent prognostic factors that predict survival and highlight the immunosuppressive nature of the tumour microenvironment. These insights strongly support the integration of CD8 profiling to optimise patient selection for chemotherapy and future immune-based therapies.

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