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EVALUATION OF TUMOUR BUDDING AND TUMOUR INFILTRATING LYMPHOCYTES IN PANCREATIC CARCINOMA AND ITS SIGNIFICANCE- A RETROSPECTIVE STUDY

Pathology

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ABSTRACT

Background: Pancreatic cancer is lethal with five-year survival rate of less than 10%.Apart from TNM staging and resectability there are no well established histological prognostic factors in pancreatic cancer. Tumour budding is a powerful and independent prognostic factor in colorectal carcinoma in predicting tumour recurrence. Tumour microenvironment, particularly the tumour-infiltrating lymphocytes (TILs), has been shown to have significant role in prognosis of pancreatic cancers. This study aims to analyze tumour budding and TIL in all the pancreatic malignancies and determine its correlation with proven pathological prognostic factors and patient outcome. **Methods:** A retrospective study performed on 30histopathologically diagnosed cases of pancreatic carcinoma. Microscopic evaluation of tumour infiltrating lymphocytes at the tumour centre and advancing front and tumour buds were analysed and compared with various histological parameters and patient survival. **Results:** Ampullary/ periampullary carcinoma(72%) was the most frequent histological type. High tumour bud and low TIL was associated with high tumour grade (p=0.024) and worst histological subtype(p=0.038) respectively. Patients with high tumour bud had low survival while tumors with severe TIL at tumour center showed better survival. **Conclusion:** Tumor budding and TIL assessment helps in prognostication of patients with pancreatic cancer and may be included in the synoptic reporting of pancreatic cancer in routine practice.

KEYWORDS

Tumour Budding, Tumour infiltrating lymphocytes, Pancreatic cancer

INTRODUCTION

Pancreatic cancer in general is lethal with five-year survival rate of less than 10%[1].Most pancreatic tumors are detected late and are usually unresectable.Long term survival in pancreatic cancers is a possibility in early stage and resectable tumours. Therapeutic options in a non resectable tumors are limited and are associated with worse survival.

Tumour budding has been studied in number of tumors and has been proved as a promising, powerful and independent prognostic factor in early stage colorectal carcinoma ,in predicting tumour recurrence, lymph node and distant metastasis[2].Tumor buds are single tumour cells or tiny clusters of up to four cells at the tumor invasive front. Tumor budding is related to epithelial-mesenchymal transition which enhances migration and invasion of tumor cells[3,4].

Tumor infiltrating lymphocytes(TIL) has prognostic role in several malignancies such as head and neck carcinomas,cutaneous melanoma and breast cancer[5]. Tumor infiltrating lymphocytes has strong correlation with immune check point molecules like PDL1 and hence plays role in therapeutic aspects as well[6]. Growing evidence suggests that the tumour microenvironment, particularly the accumulation of tumor-infiltrating lymphocytes (TILs), has significant role in prognosis of pancreatic cancers[7,8].The aim of the current study is to analyze the prognostic significance of tumour budding and TIL in pancreatic cancer by determining its association with various histopathological parameters and patient survival.

MATERIALS AND METHODS

The study commenced after approval from the Institutional Ethics Committee. This was a retrospective study carried on patients diagnosed with pancreatic carcinoma between 2017 and 2022. A total of 30cases of histopathologically proven pancreatic carcinoma including ampullary cancers were studied. Slides from each case were reviewed for microscopic tumour type, lymphovascular emboli, perineural invasion and margin status. Evaluation of tumour infiltrating lymphocytes at tumour centre and advancing front were assessed and graded as Mild: 0-10%, Moderate:15-50%, Severe: 55-100%[9]. For the purpose of statistical analysis Mild and moderate TIL were categorized as Low grade and severe TIL as high grade TIL. (Figure 1)

Tumor budding was assessed at the hot spots at the invasive front of the tumor using 20X objective, microscopic field 0.95mm to identify the maximum number of tumour buds. The scoring was performed based to the average number of buds in 10 fields and graded as follows [10](Figure 2)

G1: average of 0–4 buds
G2: Average of 5-10 buds
G3 : Average of >10 buds

Patients were followed up till December 2022. Minimum follow up was 1 month and maximum was for 4years.The date of primary histopathological diagnosis was considered as the entry point for determining the disease-free survival (DFS) and overall survival (OS). The date of disease progression and date of disease-related death or date of last follow-up was the end-point for DFS and OS, respectively.

Statistical Analysis

Chi-square test was used to investigate the statistical correlation between TIL and Tumour budding with various histopathological parameters (site, histological type,histological grade, pathological stage, lymphovascular emboli and perineural invasion). Kaplan–Meier method was used to calculate the DFS and OS among various grades of tumor budding and TIL.Log rank test was applied to know the strength of association between the DFS and OS with each of the parameters. p value less than 0.05 was considered statistically significant. The data were analyzed using SPSS version 27.

RESULTS

A total of 30 patients,histologically diagnosed as pancreatic carcinoma were included in the study. Patient's age ranged from 30 – 75 years and mean age at diagnosis was 53 years. There was male preponderance with Men : women ratio of 1.5:1.

Ampullary/ periampullary carcinomas(72%) were frequent compared to pancreatic ductal carcinoma(28%). Pancreaticobiliary (PB) was the most common histological subtype and more than half(52%) of the tumors were of histological grade 2 (moderately differentiated).

Evaluation of tumor budding and tumor infiltrating lymphocytes:

High grade TIL in tumor center was seen in the Ampullary/ periampullary carcinomas compared to PDAC though the difference was not statistically significant. There was significant association of severe TIL with Pancreaticobiliary subtype (p=0.038). Tumors with lower grade TIL had higher lymphovascular emboli, perineural invasion and higher histological grade though not statistically significant.

Grade 3 tumor budding was seen significantly in histological grade 3 tumors. Grade 3 tumors buds were also seen associated with stage 3 as compared to stage 2/ 3 and pancreatobiliary subtype of tumors as compared to intestinal subtype, the difference was not statistically

significant. Tumor budding did not show any association with the tumor location/ lymphovascular emboli and perineural invasion. TIL at the invasive front did not show any significant association with any of the histological parameters.(Table 1)

However grade3 tumor budding at the invasive front significantly correlated with lower grade TIL at tumor center (p=0.01).(Figure 3)

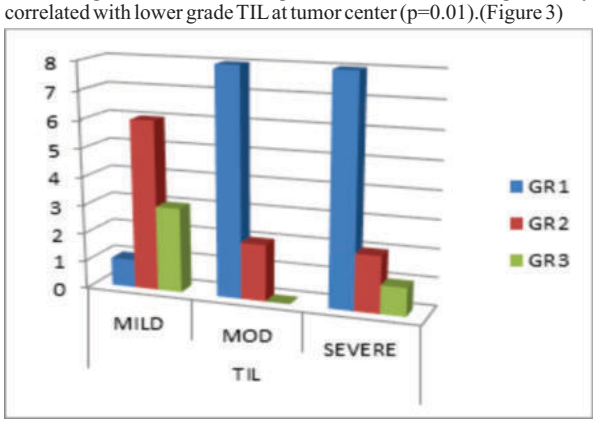

Figure 3: Correlation of Tumour Infiltrating Lymphocytes and Tumour Budding (MOD- Moderate, GR-Grade, TIL: Tumour Infiltrating Lymphocytes)

Table 1: Association of various histopathological parameters with Tumour Infiltrating Lymphocytes and Tumour Budding

		TIL MILD/ MOD	TIL SEVERE	P Value	TB Gr1	TB Gr2	TB Gr3	P Value
SITE of tumour	Ampullary/ Periampullary	9	12	0.210	7	7	7	1.00
	PDAC	6	3		3	3	3	
Stage	1	2	6	0.22	4	3	1	0.67
	2	4	4		3	3	2	
	3	9	5		4	4	6	
Histo type	INT	2	5	0.038	5	2	0	0.063
	PB	7	14		5	7	9	
	Mucinous	2	0		0	3	0	
Histo grade	G1	6	5	0.13	1	3	5	0.033
	G2	6	9		8	3	6	
	G3	4	0		0	0	4	
LVI	Present	7	6	0.13	5	2	6	0.139
	Absent	8	8		5	8	3	
PNI	Present	13	2	0.13	5	4	6	0.139
	Absent	12	2	0.13	5	4	6	0.139

TIL -Tumour Infiltrating Lymphocytes, TB Tumour Budding, Histo: Histological, PDAC-Pancreatic ductal adenocarcinoma, INT-Intestinal PB Pancreaticobiliary, LVI-Lympho vascular Invasion, PNI-Perineural Invasion, G- Grade

Survival analysis:

Patient with high grade TIL in tumour centre had higher overall survival (28.4 ± 8.4 months) and DFS (25.9± 5.6months) compared to patients with tumor showing low grade TIL (OS =13.5 ± 3.3 months and DFS=15.7± 6.7 months) (Table 2)(Figure 4)

Table 2: Comparison of median disease-free survival and overall survival with Tumour Infiltrating Lymphocytes in tumour centre

TIL in tumour centre	OS (months)	p value	DFS (months)	p value
Low grade	13.5± 3.3	0.390	15.7 ± 2.2	0.473
High grade	28.4 ± 5.6		25.9 ± 5.6	

TIL-Tumour Infiltrating Lymphocytes, OS- Overall Survival, DFS-Disease Free Survival

Likewise patients with tumor showing high grade TIL at invasive margin had longer overall survival compared to lower grade TIL (Table3)

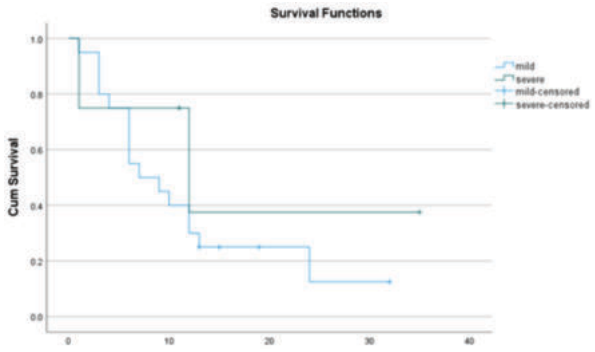

Figure 4: Overall Survival curve of Tumour Infiltrating Lymphocytes in tumour centre(Cum- Cumulative, OS- Overall Survival, TIL: Tumour Infiltrating Lymphocytes)

Table 3: Comparison of mean disease-free survival and overall survival with Tumour Infiltrating Lymphocytes in invasive margin

TIL in invasive margin	OS	p value	DFS	p value
MILD/MODERTATE	15.808 ± 2.185	0.237	14.618 ± 2.153	0.219
SEVERE	35.997± 6.937		26.725 ± 5.166	

TIL-Tumour Infiltrating Lymphocytes, OS- Overall Survival, DFS-Disease Free Survival

Patients showing Grade 1 tumor budding had higher overall survival and DFS as compared to tumors showing grade 3 tumor budding (Table 4)(Figure 5)

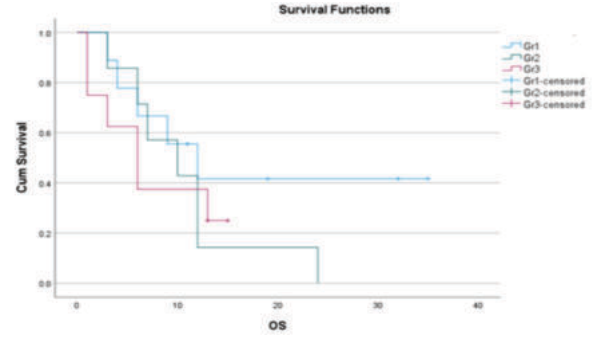

Figure 5: Overall survival curve of Tumour Budding (Cum-Cumulative,OS- Overall Survival)

Table 4: Comparison of median disease-free survival and overall survival with Tumour Budding

TB	OS	p value	DFS	p value
Grade1	19.321±3.735	0.432	17.765 ± 4.472	0.425
Grade2	17.699±3.928		9.566 ± 1.309	
Grade 3	10.026±2.054		10.026 ± 2.054	

TB: Tumour Budding, OS- Overall Survival, DFS-Disease Free Survival

The differences in survival did not reach the statistical significance due to the variation in survival time and small sample size.

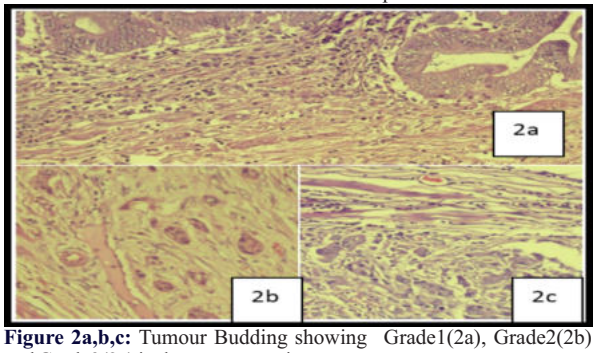

Figure 2a,b,c: Tumour Budding showing Grade1(2a), Grade2(2b) and Grade3(2c) in the tumour section.

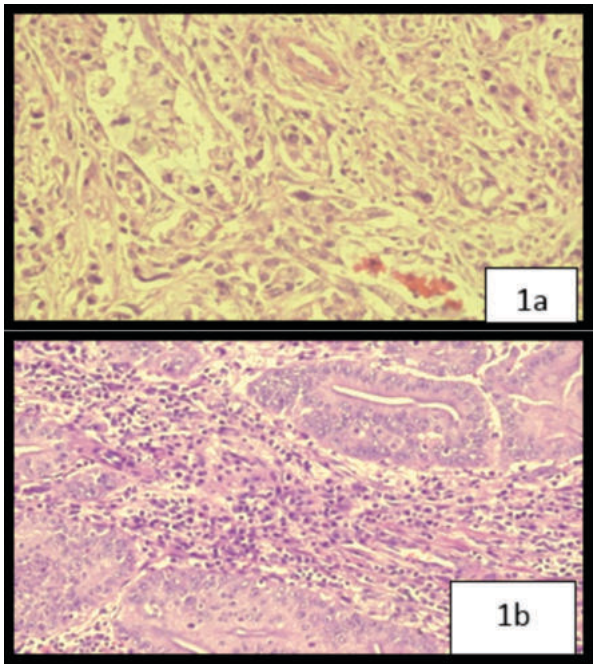

Figure 1a,b: Tumour Infiltrating Lymphocytes showing mild(1a) and severe(1b) in the tumour section.

DISCUSSION

Pancreatic carcinoma is a highly aggressive cancer that escapes early detection and resists treatment[11]. TNM staging and tumor resectability remains to be the most important prognostic indicators in pancreatic cancer.

Tumour budding biologically is a type of defense mechanism by tumor cells to fight through the peritumoural connective tissue and elude the host's defense to facilitate lymphovascular invasion and cause local and distant metastasis. Tumor budding has been observed in most Pancreatic ductal adenocarcinoma (PDAC) and has been proposed as an independent prognostic marker in recent studies[11] .There is no consensus scoring system for tumor budding in pancreatic tumors.[2]We have used the scoring recommended by international tumor budding consensus conference(ITBCC) in our study. We found that high grade tumour budding was significantly associated with worser histological grade, which was similar to study by Sadozi et al.[1] Most of the studies of tumor budding are on PDAC. Lawlor et al found an increased risk of all-cause mortality and recurrence in PDAC patients with high-grade TB[12].We have studied its presence in both ampullary and PDAC and found that tumor budding is common in pancreaticobiliary subtype than the intestinal subtype; Pancreaticobiliary subtype being the worst tumor subtype amongst the pancreatic tumors[13].

Tumor budding is the result of an interplay between epithelial and stromal elements at the tumour front influenced by mesenchymal-derived growth signals, resulting in an epithelial-mesenchymal transition (EMT)causing drug resistance, invasion, and metastasis in pancreatic cancer. Tumors with high grade tumor budding had advanced stage and worst OS and DFS in our study. Like wise Jiang H et al[14], found significant association of TB with lower OS. Another study found its association with higher pT classification, lymphatic invasion, and decreased DFS and Os[11]

On the contrary, immune cells within the tumor may play a role in fighting cancer growth by eliciting tumoricidal response sand also promote the expansion of tumours by reducing immune response[15] .TILs is believed to play a role in the recruitment, maturation, and activation of immune cells that inhibit tumour growth. In our study,TIL in tumourcentre was common amongst all ampullary tumors compared to PDAC and associated with pancreaticobiliary subtype. Higher TIL was seen with lower rate of LVI, and lower disease stage. Few otherstudies have shown higher TILs in PDAC [16,17]. Higher TIL is a bad prognostic factor in breast cancer and melanomas while is a good prognostic indicator in colorectal cancers. The role of TIL in pancreatic cancer is yet to be established. Literature shows better survival of patients with higher TIL[7].Likewise in our study lower TIL at invasive margin and tumour centre was associated to decreased OS and DFS.

There was no relationship between TIL in tumor center and TIL at advancing front in our study. However low grade TIL was significantly associated with grade 3 Tumor budding.

TILs are recognized as potential targets to fight cancer cells. Immune checkpoint inhibitors bypass immune system barriers and allow immune cells to attack cancer.TIL immunotherapy as PDL1/ PD1 and CTLA-4 inhibitors have been successfully used to treat head and neck squamous cell carcinoma, melanoma, lung cancer and genitourinary cancers to some extent[18,19].

Assessment of TIL in pancreatic cancer and more studies in this respect could pave way for targeted therapy pancreatic cancer.

Limitation:

Larger sample size could provide better information on the utility of assessing tumour budding and TIL in pancreatic carcinoma.

CONCLUSIONS

Our study shows that tumour budding is frequent in pancreatic cancer and is associated with poor survival and could play a role as measure of tumour aggressiveness. Tumour budding is inversely related to tumour infiltrating lymphocytes at tumor center. Practice of assessment of TIL in pancreatic cancer as a routine supports better prognostication of the patients and could pave way for future targeted therapy.

REFERENCES

1. Sadozai H, Acharjee A, Gruber T, Gloor B, Karamitopoulou E. Pancreatic cancers with high grade tumor budding exhibit hallmarks of diminished anti-tumor immunity. Cancers. 2021;13(5).
2. Lugli A, Karamitopoulou E, Zlobec I. Tumour budding: a promising parameter in colorectal cancer.Br. J. Cancer. 2012;106(11):1713-7.
3. Petrova E, Zielinski V, Bolm L, Schreiber C, Knief J, Thorns C, Bronsert P, Timme-Bronsert S, Bausch D, Perner S, Keck T. Tumor budding as a prognostic factor in pancreatic ductal adenocarcinoma. Virchows Arch. 2020;476(4):561-8.
4. Chouat E, Zehani A, Chelly I, Njima M, Maghrebi H, Bani MA, Njim L, Zakhama A, Haouet S, Kchir N. Tumor budding is a prognostic factor linked to epithelial mesenchymal transition in pancreatic ductal adenocarcinoma. Study report and literature review. Pancreatology. 2018: Jan 1;18(1):79-84.
5. Yang J, Lian JW, Chin YP, Wang L, Lian A, Murphy GF, Zhou L. Assessing the Prognostic Significance of Tumor-Infiltrating Lymphocytes in Patients With Melanoma Using Pathologic Features Identified by Natural Language Processing. JAMA. 2021;4(9)
6. Spector ME, Bellile E, Amlani L, Zarins K, Smith J, Brenner JC, Rozek L, Nguyen A, Thomas D, McHugh JB, Taylor JM. Prognostic value of tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma. JAMA Otolaryngol Head & Neck Surg. 2019;145(11):1012-9.
7. Miksch RC, Schoenberg MB, Weniger M, Bösch F, Ormanns S, Mayer B, Werner J, Bazhin AV, D'Haese JG. Prognostic impact of tumor-infiltrating lymphocytes and neutrophils on survival of patients with upfront resection of pancreatic cancer. Cancers. 2019;11(1).
8. Fukunaga A, Miyamoto M, Cho Y, Murakami S, Kawarada Y, Oshikiri T, Kato K, Kurokawa T, Suzuoki M, Nakakubo Y, Hiraoka K. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. Pancreas. 2004;28(1).
9. Fuchs TL, Sioson L, Sheen A, Jafari-Nejad K, Renaud CJ, Andrici J, Ahadi M, Chou A, Gill AJ. Assessment of tumor-infiltrating lymphocytes using International TILs Working Group (ITWG) system is a strong predictor of overall survival in colorectal carcinoma: a study of 1034 patients. The Am.J. Surg Pathol. 2020;44(4):536-44.
10. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, El Zimaity H, Fléjou JF, Hansen TP, Hartmann A, Kakar S. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod pathol. 2017;30(9):1299-311.
11. Karamitopoulou E, Zlobec I, Born D, Kondi-Pafiti A, Lykoudis P, Mellou A, Gennatas K, Gloor B, Lugli A. Tumour budding is a strong and independent prognostic factor in pancreatic cancer. European journal of cancer. 2013 Mar 1;49(5):1032-9.
12. Lawlor RT, Veronese N, Nottegar A, Malleo G, Smith L, Demurtas J, et al. Prognostic Role of High-Grade Tumor Budding in Pancreatic Ductal Adenocarcinoma: A Systematic Review and Meta-Analysis With a Focus on Epithelial to Mesenchymal Transition. Cancers (Basel) (2019) 11:1–11. doi: 10.3390/cancers11010113
13. Ohike N, Coban I, Kim GE, Basturk O, Tajiri T, Krasinskas A, Bandyopadhyay S, Morohoshi T, Shimada Y, Kooby DA, Staley CA. Tumor budding as a strong prognostic indicator in invasive ampullary adenocarcinomas. The Am. J. Surg. Pathol.2010;34(10).
14. Jiang H, Yang Y, Qian Y, Shao C, Lu J, Bian Y, Zheng J. Tumor budding score is a strong and independent prognostic factor in patients with pancreatic ductal adenocarcinoma: an evaluation of whole slide pathology images of large sections. Frontiers in Oncol. 2021: 5002.
15. D'Angelo A, Sobhani N, Roviello G, Bagby S, Bonazza D, Bottin C, Giudici F, Zanconati F, De Manzini N, Guglielmi A, Generali D. Tumour infiltrating lymphocytes and immune-related genes as predictors of outcome in pancreatic adenocarcinoma. PLoS One. 2019;14(8).
16. Orhan A, Vogelsang RP, Andersen MB, Madsen MT, Hölmich ER, Raskov H, Gögenur I. The prognostic value of tumour-infiltrating lymphocytes in pancreatic cancer: a systematic review and meta-analysis. Eur.J.Cancer. 2020;132:71-84.
17. Hall, MacLean, et al. "Expansion of tumor-infiltrating lymphocytes (TIL) from human pancreatic tumors." Journal for immunotherapy of cancer 4 (2016): 1-12.
18. Zhao Y, Deng J, Rao S, Guo S, Shen J, Du F, Wu X, Chen Y, Li M, Chen M, Li X, Li W, Gu L, Sun Y, Zhang Z, Wen Q, Xiao Z, Li J. Tumor Infiltrating Lymphocyte (TIL) Therapy for Solid Tumor Treatment: Progressions and Challenges. Cancers (Basel). 2022 ;14(17):4160.
19. Larkin J, Sarnaik A, Chesney JA, Khushalani NI, Kirkwood JM, Weber JS, Lewis KD, Medina TM, Kluger HM, Thomas SS, Domingo-Musibay E. Lifileucel (LN-144), a cryopreserved autologous tumor infiltrating lymphocyte (TIL) therapy in patients with advanced melanoma: Evaluation of impact of prior anti–PD-1 therapy. J Clin Oncol. 2021 20;39(15_suppl):9505.