Section: Urology

Original Research Article

PROGNOSTIC SIGNIFICANCE OF CYTOTOXIC T LYMPHOCYTE CD8+ IMMUNOSCORE IN UROTHELIAL CARCINOMA BLADDER

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Received : 08/04/2025
Received in revised form : 25/05/2025
Accepted : 13/06/2025

Keywords:
Urothelial Bladder Cancer, Immunoscore, Immunotherapy, Prognosis.

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DOI: 10.47009/jamp.2025.7.3.129
Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (3); 666-670

ABSTRACT

Carcinoma of the urinary bladder is unique in that immunotherapy is a standard component of its management. Research supports the pivotal role of CD8+ T cells in mounting anti-tumor immune responses. The Immuno-score classification system evaluates the immune infiltrates within the tumor microenvironment and has shown superior prognostic value compared to traditional TNM staging. In our cross-sectional study involving 50 TURBT specimens of urothelial carcinoma, we immune-scored CD8+ lymphocytes and correlated the Immuno-score with tumor grade and prognosis over a three-month follow-up period. Our findings indicate that the Immuno-score holds promise as a predictor of prognosis following TURBT.

INTRODUCTION

Bladder cancer is the second most common genitourinary malignancy next to prostate cancer. The most common system for classifying the extent of spread of cancer is the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) TNM classification.[1] This TNM staging system has stood the test of time but provides incomplete prognostic information.[2] The focus of this classification is solely on the tumor cells and fails to incorporate the effects of the host immune response.[3] A growing body of literature.[4] supports the hypothesis that cancer development is influenced by the host immune system. Immunoscore classification quantifies the in situ immune infiltrates and has been demonstrated to have a prognostic significance superior to that of the AJCC/UICC TNM classification system.[5]

There is growing evidence indicating the involvement of CD8+ T lymphocytes in immune responses against tumors.[6,7] A combination of two markers, CD3+ and CD8+, in two specific tumor regions (CT - center of the tumor and IM - invasive margin) has been identified for validation in standard clinical settings. In this study, our objective is to assess the prognostic importance of CD8+ (cytotoxic T cells) Immuno-score in urothelial carcinoma of the urinary bladder.

Aims and Objectives

1. To perform immune-scoring for cytotoxic T lymphocytes CD8+ lymphocytes in urothelial carcinoma.
2. To correlate the immune-score with tumour grade and calculate its significance in predicting prognosis.

MATERIALS AND METHODS

This cross sectional study was conducted from December 2018 to December 2020 which included 50 cases diagnosed with primary urothelial cancers of any histological types by Transurethral resection of bladder tissue (TURBT) biopsies [Figure 1]. Cases were followed up for a period of three months.

Surgically excised specimens were fixed in 10% buffered formalin overnight and grossly examined. Representative sections were processed, paraffin embedded, cut into sections and stained with Haematoxylin and Eosin. These were microscopically examined and tumours were graded into high grade and low grade based on their histology. Immunohistochemistry (IHC) for CD3 & CD8 was performed on 5 micron sections using standard protocols on all cases [Figure 3-6].

Immuno-scoring was done, as explained below. Three non-contiguous areas of highest lymphocyte density were selected at both CT (centre of the tumor) and IM (invasive margin) regions. Immuno-score (I0 to I4) was calculated based on the density of CD3+ and CD8+ T lymphocytes in both CT and IM regions of the tumour. For example, if both markers were elevated in both CT and IM regions, a highest score of I4 was given. If both markers were low in both regions, then the lowest possible score of I0 was given. Similarly, I1, I2, I3 scores are given based on the regions with elevated markers as show in the table

ISSN (O): 2687-5365; ISSN (P): 2753-6556

[Figure 2, Table 1]. Based on previous studies, the lowest score has been linked to the worst prognosis.

This methodology was selected in accordance with previously established definitions used by Galon et al. in colon cancer.[5]

Table 1: Immunoscoring Method

IHC SCORE	CD3-CT	CD3-IM	CD8-CT	CD8-IM
I0	-	-	-	-
I1	+	-	-	-
I1	-	+	-	-
I1	-	-	+	-
I1	-	-	-	+
I2	+	+	-	-
I2	+	-	+	-
I2	-	+	-	+
I2	-	-	+	+
I3	+	+	+	-
I3	+	+	-	+
I3	+	-	+	+
I3	-	+	+	+
I4	+	+	+	+

IHC – Immunohistochemistry (+) stained positive, (-) stained negative

In the present study, we considered I0, I1 as low scores and I2, I3, I4 as high scores and accordingly documented the results.

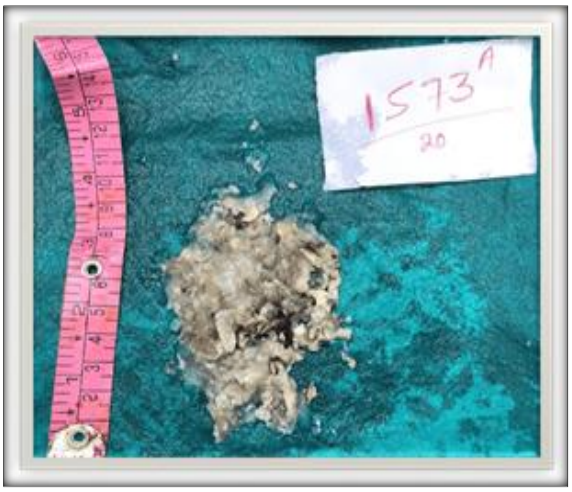

Figure 1: Gross Image of TURBT Specimen

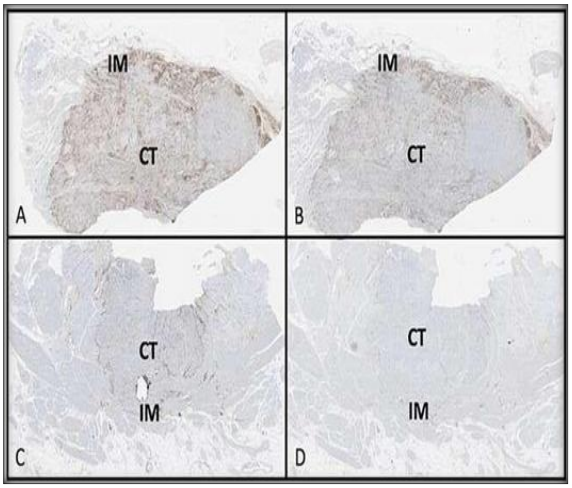

Figure 2: High vs. low density and locations of CD3 & CD8 (A) High concentration CD3 in CT and IM. (B) High concentration CD8+ in CT and IM. (C) Low concentration CD3+ in CT and IM. (D) Low concentration CD8+ in CT and IM

IHC of CD8 in HGPUC

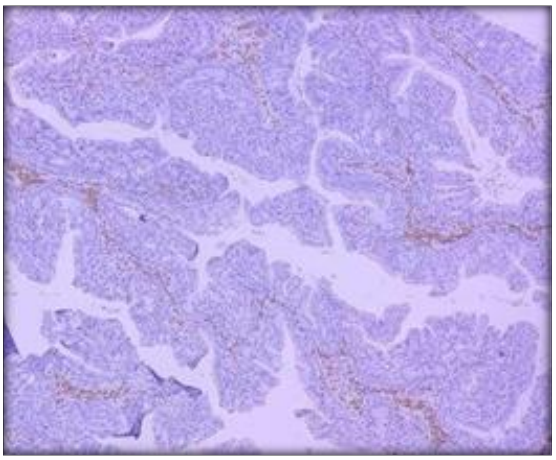

Figure 3: 10x image Focal positivity of CD8+ HGPUC

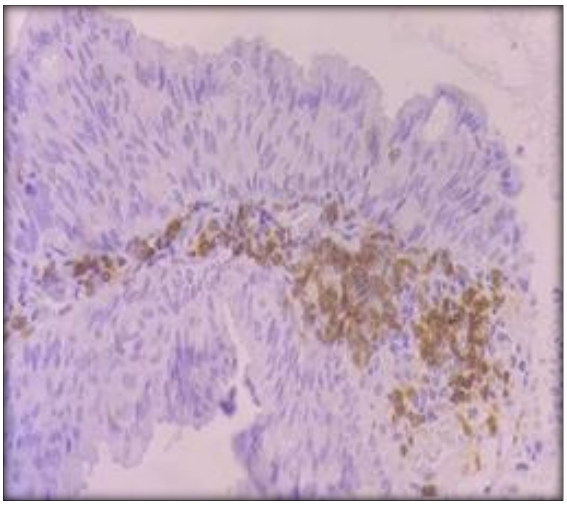

Figure 4: 40x image Focal positivity of CD8+ in HGPUC

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IHC of CD8 in LGPUC

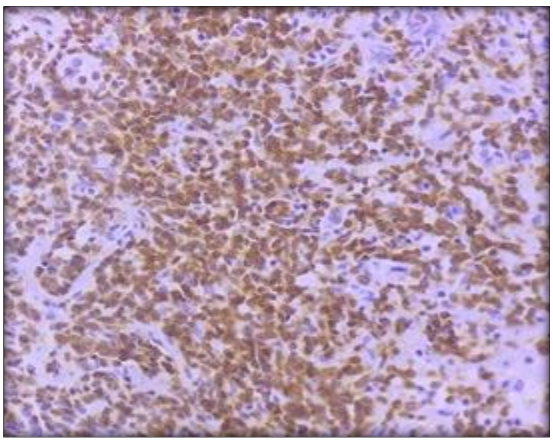

Figure 5: 40x image; High expression of CD3+ in LGPUC

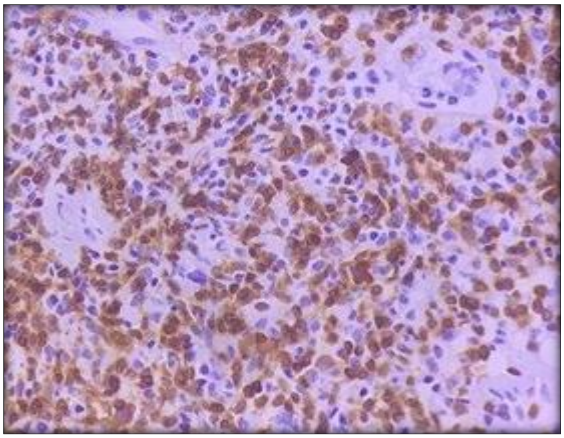

Figure 6: 40x image; High expression of CD8+ in LGPUC

Statistical Analysis: Data was entered in Microsoft Excel and analysis was done using IBM SPSS version 20.0 Armonk, New York, USA. Descriptive statistical analysis was done. Results on categorical measurements are presented as Percentages. Significance is assessed at 5 % level of significance. $P < 0.05$ is statistically significant and $P < 0.001$ is statistically highly significant. Fisher's exact test was used to find out the significance of study parameters on a categorical scale between two groups.

RESULTS

We had a total number of 50 cases. Mean age of the study population was 56 years and those in their 6th decade formed the dominant age group, contributing to 30% of the total number of cases [Chart 1]. There was a male preponderance of 80%.

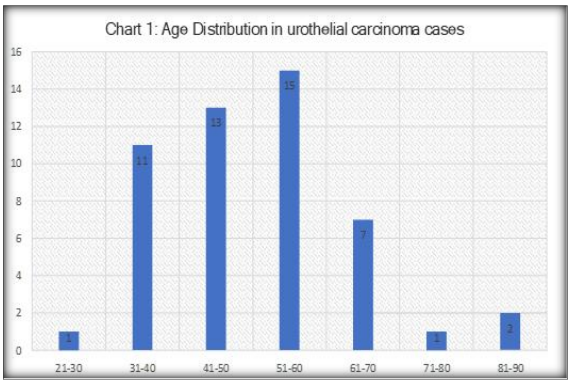

Chart 1: Age distribution in urothelial carcinoma cases

Out of 50 cases, 21 cases were HGPUC (high grade papillary urothelial carcinoma), contributing to up to 42% of the study cases. 15 cases were LGPUC (low grade papillary urothelial carcinoma), 5 were SCC (squamous cell carcinoma), 4 were PUC with squamous differentiation, 3 were adenocarcinoma and 2 were PUNLMP (papillary urothelial neoplasm of low malignant potential) [Table 2].

Table 2: Correlation Between Histological Types, Grades & Immunoscoring in the Present Study

Sl No.	Histological Types	Histological Grades	Immunoscoring of CD3, CD8	No. of Cases (%)	%
1	PUNLMP	Low Grade	High	2 (4)	4
2	LGPUC		High	8 (16)	16
		Low Grade	Low	7 (14)	14
3	HGPUC	High Grade	Low	21 (42)	42
4	PUC with Sq. Diff	High Grade	Low	4 (8)	8
5	SCC	High Grade	Low	5 (10)	10
6	AdenoCa	Low Grade	Low	3 (6)	6

Table 3: Immunoscores of Urothelial Carcinoma Cases

IMMUNOSCORE	NO. OF CASES (%)
LOW (I0,I1)	NN 40 (80)
HIGH (I2,I3,I4)	10 (20)

80% of the study cases have shown low Immunoscores, whereas 20% shown high Immunoscores [Table 3]. 30 cases (60%) of the study cases are HGPUC whereas 20 cases (40%) are LGPUC [Table 4].

10 cases (20%) have shown high expression of CD3+ and CD8+ cytotoxic T lymphocytes which were diagnosed as Low-grade tumors on HPE (PUNLMP & LGPUC). 30 cases (60%) have shown low expression of CD3+ and CD8+ cytotoxic T lymphocytes which were diagnosed as High-grade tumors on HPE (HGPUC, SCC, PUC with Sq diff). 10 cases (20%) have shown low expression of CD3 positivity and CD8 positivity which are diagnosed as Low grade tumours on HPE (AdenoCa) [Table 4].

From these results, it is observed that -

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- High Immunoscores were seen in low grade tumours
- Low Immunoscores were seen in high grade tumours
- Low Immunoscores were also seen in low grade tumours

Table 4: Association of Immunoscore and Tumour Grade

	LOW SCORE	HIGH SCORE	TOTAL	
LOW GRADE (I0, I1)	10 (25. 0 %)	10 (100.0 %)	20 (40.0 %)	P < 0.001 Highly Significant
HIGH GRADE (I2, I3,I4)	30 (75.0 %)	0 (0.0 %)	30 (60.0 %)	
TOTAL	40 (100.0 %)	10 (100.0 %)	50 (100.0 %)	

P < 0.001 * Highly Significant

From the above Table 4 it is observed that the majority of the cases with Low Immunoscores (75 %) are High Tumour Grades and also the majority of the cases with High immunoscores (100 %) are exhibiting Low Tumour Grades. This association between Immunoscores and Tumour Grades was found statistically highly significant (P < 0.001).

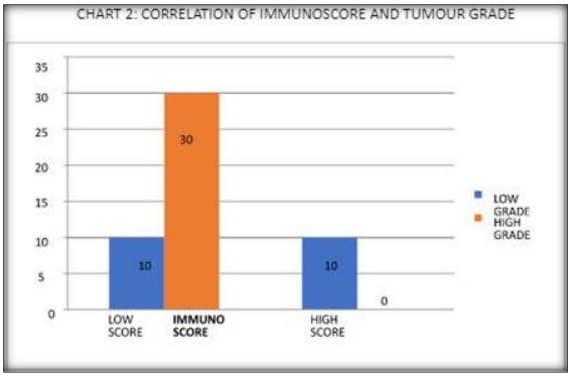

Chart 2: Correlation of Immunoscore and tumour grade

Out of 50 cases, 10 cases were lost to follow-up and the rest of the 40 (80%) were followed up for a period of 3 months.

DISCUSSION

In our research, we examined a total of 50 cases of urothelial carcinomas, with 30 cases (60%) categorized as high grade and the remaining 20 cases (40%) as low grade carcinoma. Among these cases, 40 (80%) exhibited a low immune-score (I0, I1), while 10 cases (20%) showed a high immune-score (I2, I3, and I4), all of which were associated with PUNLMP and LGPUC (Chart 2).

Our findings indicate a noteworthy correlation between tumor grade and immune-score, particularly in patients with low-grade carcinoma who displayed a high immune-score characterized by CD3+ and CD8+ lymphocyte expression. This association is statistically highly significant, highlighting the potential importance of the Immuno-score in predicting outcomes based on tumor grade. One limitation of our study is the relatively short follow-up period of only 3 months, which precludes any survival analysis. This represents a drawback that could be addressed in future research to further elucidate the clinical implications of our findings.

Urinary bladder carcinoma stands out as the sole neoplasm where immunotherapy is integrated into standard management protocols. Tumor cells possess the capacity to engage and regulate the immune system, resulting in an imbalance between tumor proliferation and the body's surveillance mechanisms. These immune system adjustments are pivotal in the clinical handling of cancer, as they are linked to the emergence of resistance to anti-tumor drugs. Activated T cells, natural killer cells, and a group of lymphocytes known as tumor infiltrating lymphocytes (TILs), which includes non-T and non-B lymphocytes, demonstrate cytotoxic capabilities, and migrate to the bladder tumor site to impede the malignant cells [9]. This aspect holds significance in bladder cancer management, especially considering that immunotherapy, exemplified by BCG, is an integral component of care for high-risk non-muscle invasive cancer. Additionally, the advent of immune checkpoint blockade therapies like PD-L1 inhibitors has proven beneficial for metastatic bladder cancer patients who have not responded to chemotherapy. TILs specifically refer to the lymphocytes infiltrating the tumor epithelium, playing a crucial role in immune response against bladder tumors.

Our results are indeed supported by a wealth of existing literature. Mansure et al. (2018) demonstrated that a high density of CD8 IM TILs was correlated with improved disease-free survival (DFS) and overall survival (OS), while CD3 IM TILs were associated with better OS.[8] They also found that a high Immuno-score was linked to enhanced DFS and OS, aligning with the findings of your study.

Liakou et al. highlighted in their review the significance of high infiltration of T-lymphocytes as indicative of host immune-competence and a favorable prognosis in bladder cancer patients. They suggested that T-cell infiltration could serve as a surrogate marker for a positive outcome.[9]

Moreover, Offerson et al,[10] reported a strong correlation between peritumoral neo-angiogenesis and improved survival in superficial transitional cell carcinoma (TCC). This correlation was attributed to the angiogenic stimulation of a local inflammatory

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reaction generated by the host against superficial bladder cancer.

Sjodahl et al. (2014) conducted a study focusing on tumour biopsies from Transurethral resection of bladder specimens in 296 patients with urinary carcinoma. Their findings indicated that a high CD3+ score was a favorable prognostic factor in cases of muscle-invasive urinary carcinoma.[11]

These studies collectively underscore the crucial role of immune response and tumor microenvironment in determining prognosis and outcomes for patients with bladder cancer.

CONCLUSION

Our study stands out as one of the initial investigations to establish a clear relationship between prognosis
and cytotoxic T lymphocytes in urothelial carcinomas. The Immuno-score emerges as a promising predictor of prognosis
following Transurethral Resection of Bladder Tumor (TURBT). Specifically, high Immuno-scores are notably linked with
low-grade tumors, correlating with a more favorable prognosis.

Additionally, our findings highlight that a robust immune response at the tumor margin independently predicts a better prognosis. This emphasizes the crucial role of immune surveillance and response in influencing outcomes for patients with bladder cancer.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556