RESEARCH ARTICLE

Editorial Process: Submission:09/15/2024 Acceptance:03/23/2025

CD 14 Expression in Urothelial Carcinoma of The Urinary Bladder (Histopathological and Immunohistochemical Study)

Nora T. El-Zohery[1], Yousra R. Shalaby[2]*, Ayman I. Kassem[3], Rasha R. Mostafa[1]

Abstract

Background: Egypt has a significantly higher prevalence of cancer bladder than the rest of the world. CD-14 antigen is involved in Toll-like receptor-mediated signaling pathways in inflammatory tumor microenvironment which promote tumor development and proliferation. There have been few studies on *CD14* antigen effect on urothelial carcinoma of the urinary bladder. This study aimed to evaluate the role of *CD14* in prognosis of uurothelial carcinoma of the urinary bladder and its association with the tumor progression. **Material and methods:** This retrospective cross-sectional study included fifty-one cases of urothelial carcinoma obtained either by cystoscopic biopsies or radical cystectomies. They were immunohistochemically stained using anti- *CD14* antibody. Statistical correlations between *CD14* expression and available clinicopathological data were done. **Results:** Positive immunoexpression of *CD14* was noted in 84.3% of all cases, showing scores 1, 2 & 3 in 9.8%, 29.4%, and 45.1% of cases, respectively. While negative immunoexpression was noted in 15.7% of cases and was scored as 0. A statistically significant correlation was noticed between *CD14* immunohistochemical expression and each of the tumor grade, pathological tumor stage, status of muscle invasion, and pathological lymph node stage (P value=0.045, 0.030, 0.001, and 0.008 respectively). However, no statistically significant correlation was noted between *CD14* immunohistochemical expression and each of the two-year survival rates in radical cystectomy cases and the mortality rate after exclusion of the postoperative complications (P value = 0.114 & 0.156 respectively). **Conclusion:** The intensity of *CD14* expression was weak and even lost in high-grade and late-stage urothelial bladder carcinoma cases, while low-grade urothelial carcinoma cases showed *CD14* overexpression. Accordingly, the significance of therapeutic approaches targeting *CD14* in high-grade and late-stage urothelial bladder carcinoma shall be questionable.

Keywords: *CD14*- urothelial carcinoma- urinary bladder

Asian Pac J Cancer Prev, **26 (3)**, 889-897

Introduction

Bladder cancer (BC) is the second most frequent genitourinary malignancy [1]. Urothelial carcinoma (UC) accounts for 90% of all bladder tumors [2]. The overall 5-year recurrence-free survival rate ranged from 58 to 81% [3]. Egypt has a significantly higher prevalence of cancer bladder than the rest of the world with 43 851 new cases and 24 917 deaths from urinary bladder cancer estimated in 2020 [4].

Solid tumors are a complicated mass of cells that interact with one another to induce a variety of immunological markers that aid in the diagnosis of tumor microenvironment (TME) [5]. Within TME, the cells produce a range of soluble chemicals, which form complicated signaling networks. One of these signaling networks is the tumor-promoting inflammation (TPI) [6].

CD-14 antigen, a glycosyl-phosphatidylinositol (GPI)-linked glycoprotein, is critical in Toll-like receptor (TLR)-mediated signaling pathways. *CD14* plays a crucial role in the signaling pathways of TLRs 2, 3, 4, 7, and 9. The TLRs are well-known for their function involving recognizing pathogen-associated molecular patterns (PAMPs). Additionally, TLRs identify dangerous molecular patterns. The latter has been linked to some disorders, such as cancer [6].

CD14-expressing tumor cells have a critical role in orchestrating TPI to promote tumor development and proliferation. This subgroup of tumor cells produces inflammatory molecules, which stimulate angiogenesis and help build and maintain an immune-suppressive, inflammatory tumor microenvironment. Furthermore, this subpopulation can promote tumor growth by generating substances that stimulate autocrine and paracrine proliferative processes. Moreover, Stronger *CD14* expression was associated with tumor development and

*[1]Pathology Department, Faculty of Medicine, Cairo University, Egypt. [2]Department of Anatomic Pathology, Faculty of Medicine, Kasr Al-Ainy, Cairo University, Cairo, Egypt. [3]Department of Urology, Faculty of Medicine, Kasr Al-Ainy, Cairo University, Cairo, Egypt. *For Correspondence: yousra.raafat@kasralainy.edu.eg*

a worse prognosis. Consequently, therapeutic targeting of *CD14* could be an effective cancer treatment method [7].

Bladder cancer is a type of solid tumor that is characterized by the presence of a large number of myeloid cells [8]. *CD14*-high-expressing bladder cancer cells are responsible for a variety of cancer-related characteristics. They produce signaling inflammatory mediators such as cytokines, chemokines, and small molecules, which contribute to the tumor's inflammatory microenvironment. *CD14*-high-expressing bladder cancer cells generate inflammatory chemicals that attract and polarize monocytes and macrophages, resulting in immunosuppressive host characteristics. They are better at down-regulating major histocompatibility complex II (MHC II) on monocytes and macrophages [6].

To conclude, high *CD14* antigen expression by cancer cells proves that the inflammatory microenvironment promotes tumor cell development. However, there have been few studies on *CD14* antigen's effect on the urothelial carcinoma of the urinary bladder.

The aim of this study is to evaluate *CD14* immunohistochemical expression in urothelial carcinoma of the urinary bladder and its correlation with the clinicopathological prognostic parameters to find any association with tumor progression.

The rationale of this work was to; 1- Evaluate the immunohistochemical expression of *CD14* in urothelial carcinoma of urinary bladder. 2- Statistically correlate *CD14* expression with available clinicopathological data to evaluate its role as a prognostic factor in urothelial carcinoma of the urinary bladder and to find any association with tumor progression.

Materials and Methods

After approval by The Research Ethics Committee (REC) (code: N-405-2023) on 11/11/2023, this retrospective cross-sectional study was performed at the Pathology Department, Kasr Al Ainy Hospital, Faculty of Medicine, Cairo University.

Inclusion criteria: The archived paraffin blocks of urothelial carcinoma patients diagnosed between January 2021 and May 2022 were included. The study was conducted in accordance with the Local Ethics Committee requirements of the Faculty of Medicine, Cairo University. The included cases should have complete medical records and a minimum follow-up period of 2 years from diagnosis (or till recurrence/death).

Exclusion criteria: the excluded cases were improperly fixed specimens, biopsies with extensive necrosis, crush or cautery artifacts, or cases of urinary bladder pure squamous cell carcinoma or adenocarcinoma. Based on evidence from an earlier similar study and by considering positive *CD14* expression in urothelial bladder carcinoma as the primary outcome [9], Epi-calc 2000 was used to calculate the sample size of this cross-sectional study. Assuming 80% power, 0.05 level of significance, 78 % null hypothesis value, and an estimated proportion of 60%, the sample size will be = 46 specimens. Considering the drop-out rate of 10%, therefore the final sample size will be 51 specimens.

Histopathological evaluation

Formalin-fixed, paraffin-embedded blocks were sectioned at 5 μm thickness, and stained by hematoxylin and eosin stain. The cases were examined under a microscope and evaluated to detect the histological diagnosis which was performed according to the criteria of the fifth edition of WHO, 2022 [10].

Associated Bilharzial infestation based on the detection of Schistosoma ova in tissues was also recorded.

Immunohistochemical procedure of CD14

The archived 51 paraffin-embedded blocks of urothelial carcinoma cases from cystoscopic biopsies or radical cystectomies were sectioned on adhesive-charged microscopic slides.

Sections of 5 μm were obtained then heat-mediated antigen retrieval was done using citrate buffer pH 6 in an automated water bath (Dako PT link, PT101), according to the Dako standard technique.

The primary antibody was a polyclonal Anti- *CD14* antibody, Catalog number YPA1588 (100 μl) against the human CD 14, manufactured by Chongqing Biospes Co. Ltd (Chongqing, China). Immunohistochemical staining was done in a Dako autostainer (link 48) with a polymer-based detection system. (Dako EnVisionTM FLEX, K8000) (Dako, Colorado, USA) Dako Colorado, Inc. 4850 Innovation Drive Ft. Collins, CO 80525 USA 970-226-2200.

Diaminobenzidine was used as a chromogen, followed by Mayer's hematoxylin as a counterstain. Coverslips and DPX mounting material were used to mount and preserve the sections. The sections were examined using Olympus BX51 light microscope with ×40 objective eyepieces (Olympus, Tokyo, Japan). The positive control used for *CD14* was Kupffer cells lining the hepatic sinusoids.

Evaluation of CD14 immunohistochemical expression

The immunohistochemical slides were examined and scored by two pathologists independently, based on staining intensity and the percentage of stained tumor cells that showed membranous positivity only as (Table 1) [9]. Score 0 was considered a negative expression while scores 1-3 were considered a positive expression.

The results of *CD14* immunostaining were correlated with the clinicopathological features and patients' survival.

Statistical methods

Microsoft Excel 2016 will be used for data entry and the statistical package for social science (SPSS version 24) will be used for data analysis. All collected data will be revised for competencies and logical consistency. Data exploration as normal or skewed distribution will be done by Kolmogorov–Smirnov/Shapiro–Wilk's test.

Simple descriptive statistics in the form of arithmetic mean and standard deviation or median and inter-quartile range) will be used for the summary of numerical variables, while frequencies and percentages for categorical ones.

Bivariate relationships will be displayed in cross-tabulations and a comparison of proportions will be performed using the chi-square and Fisher's exact tests where proper Pearson or Spearman's rank correlation

analysis will be used according to data normality.

Finally, regression analysis will be performed to adjust for possible confounders. P value will be calculated to assess statistical significance, a value less than 0.05 will be considered statistically significant. Microscopic photos were captured using a digital camera attached to an Olympus microscope model BX 53.

Results

The patients' ages ranged from 26 to 81, with a mean of 57±12 years and 54.9% of cases were more than 57 years. About 96.1% of patients were males.

Concerning the predominant pathological features of the included cases, they were papillary urothelial carcinoma (52.9%), high grade (68.6%), and non-muscle proper invasive tumor [pTa & pT1] (54.9%) of the included cases. The studied cases were classified as pTa; pT1; pT2a; pT2b; pT3a; pT3b and pT4a representing 27.5%; 27.5%; 5.9%; 13.7%; 2%; 13.7% and 9.8% of the studied cases respectively, pTa and pT1 stages being the most commonly encountered. Table 2 summarizes the clinicopathological features of the included cases.

Regarding the association between the histopathological subtypes and the pathological features of the included cases, there was a significant positive association was detected between the tumor histopathological subtype and tumor pathologic stage (P value = 0.001). Most papillary urothelial carcinoma cases (92.6%) are non-muscle invasive (pTa & pT1) and most conventional urothelial carcinoma cases or those with divergent differentiation are muscle invasive. All cases (100%) of conventional urothelial carcinoma cases or those with divergent differentiation are high grades, so there was a significant positive association detected between tumor histopathological subtype and grade (P value = 0.001). Table 3 summarizes the association between the histopathological subtypes and the pathological features of the included cases.

Positive immunoexpression of *CD14* was noted in 84.3% of all cases, showing scores 1, 2 & 3 in 9.8%, 29.4%, and 45.1% of cases, respectively. While negative immunoexpression was noted in 15.7% of cases and was scored as 0. Table 4 summarizes the immunohistochemical expression of *CD14* (scoring as well as positive & negative expression) (Figure 1).

No statistically significant correlation was detected between *CD14* expression and each of the histopathological subtypes, associated bilharziasis, lymphovascular emboli, perineurial invasion as well as recurrent cases (P value =0.071, 0.572, 0.657, 1, and 0.633 respectively).

A statistically significant correlation was noticed between *CD14* immunohistochemical expression and each of the tumor grade, pathological tumor stage, status of muscle invasion, and pathological lymph node stage (P value=0.045, 0.030, 0.001, and 0.008 respectively). Table 5 summarizes the association between *CD14* immunohistochemical expression (positive & negative expression) and the clinicopathological features of included cases.

Moreover, a significant inverse correlation between tumor histological grade and *CD14* expression intensity was detected where 100 % of low-grade cases showed positive *CD14* expression and 100% of *CD14* negatively expressed cases were high grade (P value = 0.045). About sixty-five percent of *CD14* positively expressed cases were non-muscle invasive and 100% of *CD14* negatively expressed cases were muscle invasive (P value=0.001).

No significant association between *CD14* immunohistochemical expression and two-year survival rate in radical cystectomy cases (P value = 0.114) was observed. Table 6 summarizes the association between *CD14* immunohistochemical expression and two-year survival rate of radical cystectomy cases.

No significant association between *CD14* immunohistochemical expression and the mortality rate after the exclusion of the postoperative complications (only one patient died due to postoperative complications) (P value = 0.156) was detected. Table 7 summarizes the association between *CD14* immunohistochemical expression and the late mortality rate in radical cystectomy cases.

Discussion

Bladder cancer is the tenth most prevalent cancer globally. More than 600,000 persons were diagnosed with bladder cancer worldwide in 2022, with over 220,000 dying because of the disease. Bladder cancer is among the most difficult and expensive tumors to diagnose and cure. Its diagnosis is primarily based on cystoscopy, an invasive and costly technique. Most bladder cancers are detected early when they are highly curable. However, approximately 25% of bladder tumors are detected at a later stage [11]. BC is the second most frequent malignancy among Egyptian men with a 4:1 male-to-female ratio up on the GLOBOCAN appraisal [4].

CD14-high bladder cancer cells are responsible for a variety of cancer-related characteristics. They produce signaling inflammatory mediators such as cytokines, chemokines, and small molecules, which contribute to the tumor's inflammatory microenvironment. *CD14*-high bladder cancer cells generate inflammatory chemicals that attract and polarize monocytes and macrophages, resulting in immunosuppressive host characteristics [9].

In the following discussion, we correlate the clinicopathological variables of our study with the most recent studies done on Egyptian patients by Amin et al. [12] and Ragab et al. [13].

In the present study, the patients' ages ranged from 26 to 81, with a mean of 57±12 years and 54.9% of cases were more than 57 years which was in accordance to the study done by Ragab et al., 2021 where the age of the

Table 1. *CD14* Immunohistochemical Expression Scoring

Score	Staining Intensity & Percentage
Score 0	No staining
Score 1	Weak staining in <25% of tumor cells
Score 2	Moderate staining in 25%-75% of tumor cells
Score 3	Strong staining in >75% of tumor cells

Table 2. Demographic and Clinicopathological Features of The Included Urothelial Carcinoma Cases

		Number (Percentage)
Age groups	Less than or equal 57 years	23 (45.1%)
	More than 57 years	28 (54.9%)
Sex	Male	49 (96.1%)
	Female	2 (3.9%)
Tumor site	Dome	9 (17.6%)
	Posterior	6 (11.8%)
	Left lateral	5 (9.8%)
	Right lateral	7 (13.7%)
	Whole	8 (15.7%)
	Anterior	16 (31.4%)
Tumor size (cm) grouping	Less than or equal 4 cm	30 (58.8%)
	More than 4 cm	21 (41.2%)
Tumor gross appearance	Infiltrating	10 (19.6%)
	Ulcerating	9 (17.6%)
	Polypoid	26 (51.0%)
	Fungating	6 (11.8%)
Tumor histopathological subtype	Papillary urothelial carcinoma	27 (52.9%)
	Conventional urothelial carcinoma	12 (23.5%)
	Urothelial carcinoma with squamous differentiation	7 (13.7%)
	Urothelial carcinoma with glandular differentiation	1 (2.0%)
	Urothelial carcinoma with micropapillary differentiation	3 (5.9%)
	Urothelial carcinoma with lymphoepithelioma differentiation	1 (2.0%)
Tumor grade	Low	16 (31.4%)
	High	35 (68.6%)
Invasion	Invasive(≥T1)	37 (72.5%)
	Non-Invasive(=Ta)	14 (27.5%)
Pathological tumor stage	PTa	14 (27.5%)
	pT1	14 (27.5%)
	pT2a	3 (5.9%)
	pT2b	7 (13.7%)
	pT3a	1 (2.0%)
	pT3b	7 (13.7%)
	pT4a	5 (9.8%)
Muscle proper invasion	Non muscle invasive(<T2)	28 (54.9%)
	Muscle invasive(≥T2)	23 (45.1%)
Pathological lymph node stage	pNx (Could not be assessed)	31 (60.8%)
	pN0	14 (27.5%)
	pN1	4 (7.8%)
	pN2	2 (3.9%)
Associated bilharziasis	Present	6 (11.8%)
	Absent	45 (88.2%)
Lymphovascular emboli	Present	14 (60.9%)
	Absent	9 (39.1%)
Perineural invasion	Present	12 (52.2%)
	Absent	11 (47.8%)
Operation	Transurethral resection of bladder tumor [TURBT]	28 (54.9%)
	Radical cystectomy	23 (45.1%)
Two-year recurrence	Yes	11 (21.6%)
	No	27 (52.9%)
	Not available	13 (25.5%)
Adjuvant therapy	No: Non-compliant	23 (45.1%)
	Yes: BCG+Chemotherpy + Chemo and radiotherapy	18 (35.3%)
	Not available	10 (19.6%)
Two-year survival	No	7 (13.7%)
	Yes	34 (66.7%)
	Not available	10 (19.6%)

patients included in their study ranged from 23: 83 years and mean (±SD) patient age was 59.73 (±12.43) [13], while the mean age was slightly younger than reported by Amin et al. [12] whereas the mean age for their cases was reported to be 61.56 [12].

In the present study, 96.1% of the patients were males which was higher than that reported by Amin et al. [12] and Ragab et al. [13] where 85.5% and 82.5% of their cases were males respectively.

In general, there is male predominance in urinary bladder carcinoma which may be attributed to men's greater exposure to tobacco and occupational carcinogens than women [14]. Animal models and epidemiological research support the hypothesis that estrogens [15] and anti-androgens are protective against this malignancy, particularly the urothelial type [16].

Concerning the predominant pathological features of the included cases, they were papillary urothelial carcinoma representing 52.9% of all cases -as our study included only cases of urothelial carcinoma-, high grade (68.6%) and the pathological tumor stages 1, 2, 3 & 4 represented 54.9%, 19.6 %, 15.7% & 9.8% respectively.

Amin et al. [12] reported that transitional cell carcinoma (TCC) was the most common histopathological pattern in their study representing 79.3% of all cases as the study included different types of urinary bladder carcinoma, 43.6% of cases were high grade and the pathological tumor stages 1, 2, 3 & 4 represented 43.6%, 41.4%, 9.1% & 5.4% respectively [12].

Ragab et al. [13] reported that TCC was also the most common histopathological pattern in their study representing 65% of all cases as the study included different types of urinary bladder carcinoma, 62.5% of cases were high grade and the pathological tumor, stages 1, 2, 3, 4 represented 24.9%, 21.6%, 29.1%, 24.1% respectively [13].

Accordingly, the differences between the three studies were not so significant, which may be attributed to different sample sizes. Regarding the association between the histopathological subtypes and the pathological features of the included cases, there was a significant positive association detected between the tumor histopathological subtype and tumor pathologic stage (P value = 0.001). Most urothelial carcinoma cases with divergent differentiation were muscle invasive. All urothelial carcinoma cases

Table 3. The Association between the Histopathological Subtypes and the Pathological Features of The Included Urothelial Carcinoma Cases

		Histopathological subtype								
		Papillary urothelial carcinoma		Conventional urothelial carcinoma		Urothelial carcinoma with squamous differentiation		Urothelial carcinoma with glandular differentiation		Urothelial carcinoma with micropapillary differentiation		Urothelial carcinoma with lymphoepithelioma differentiation		P value
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	
Tumor grade	Low	16 (59.3%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		0.001
	High	11 (40.7%)		12 (100.0%)		7 (100.0%)		1 (100.0%)		3 (100.0%)		1 (100.0%)		
Pathological tumor stage	pTa	14 (51.9%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		0.001
	pT1	11 (40.7%)		1 (8.3%)		2 (28.6%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		
	pT2a	1 (3.7%)		2 (16.7%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		
	pT2b	1 (3.7%)		2 (16.7%)		1 (14.3%)		0 (0.0%)		2 (66.7%)		1 (100.0%)		
	pT3a	0 (0.0%)		1 (8.3%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		
	pT3b	0 (0.0%)		4 (33.3%)		2 (28.6%)		1 (100.0%)		0 (0.0%)		0 (0.0%)		
	pT4a	0 (0.0%)		2 (16.7%)		2 (28.6%)		0 (0.0%)		1 (33.3%)		0 (0.0%)		
Muscle proper invasion	Non muscle invasive(<T2)	25 (92.6%)		1 (8.3%)		2 (28.6%)		0 (0.0%)		0 (0.0%)		0 (0.0%)		<0.001
	Muscle invasive(≥T2)	2 (7.4%)		11 (91.7%)		5 (71.4%)		1 (100.0%)		3 (100.0%)		1 (100.0%)		
Lymphovascular emboli	Present	1 (50.0%)		6 (54.5%)		3 (60.0%)		1 (100.0%)		2 (66.7%)		1 (100.0%)		0.9
	Absent	1 (50.0%)		5 (45.5%)		2 (40.0%)		0 (0.0%)		1 (33.3%)		0 (0.0%)		
Perineural invasion	Present	0 (0.0%)		7 (63.6%)		4 (80.0%)		0 (0.0%)		0 (0.0%)		1 (100.0%)		0.088
	Absent	2 (100.0%)		4 (36.4%)		1 (20.0%)		1 (100.0%)		3 (100.0%)		0 (0.0%)		

Table 4. The Immunohistochemical Expression of *CD14* (Scoring) and (Positive & Negative Expression)

CD14 Immunohistochemical Expression (Scoring)		Number (Percentage)
	Score 0	8 (15.7%)
	Score 1	5 (9.8%)
	Score 2	15 (29.4%)
	Score 3	23 (45.1%)
CD14 Immunohistochemical Expression (Positive & Negative Expression)		Number (Percentage)
	Negative	8 (15.7%)
	Positive	43 (84.3%)

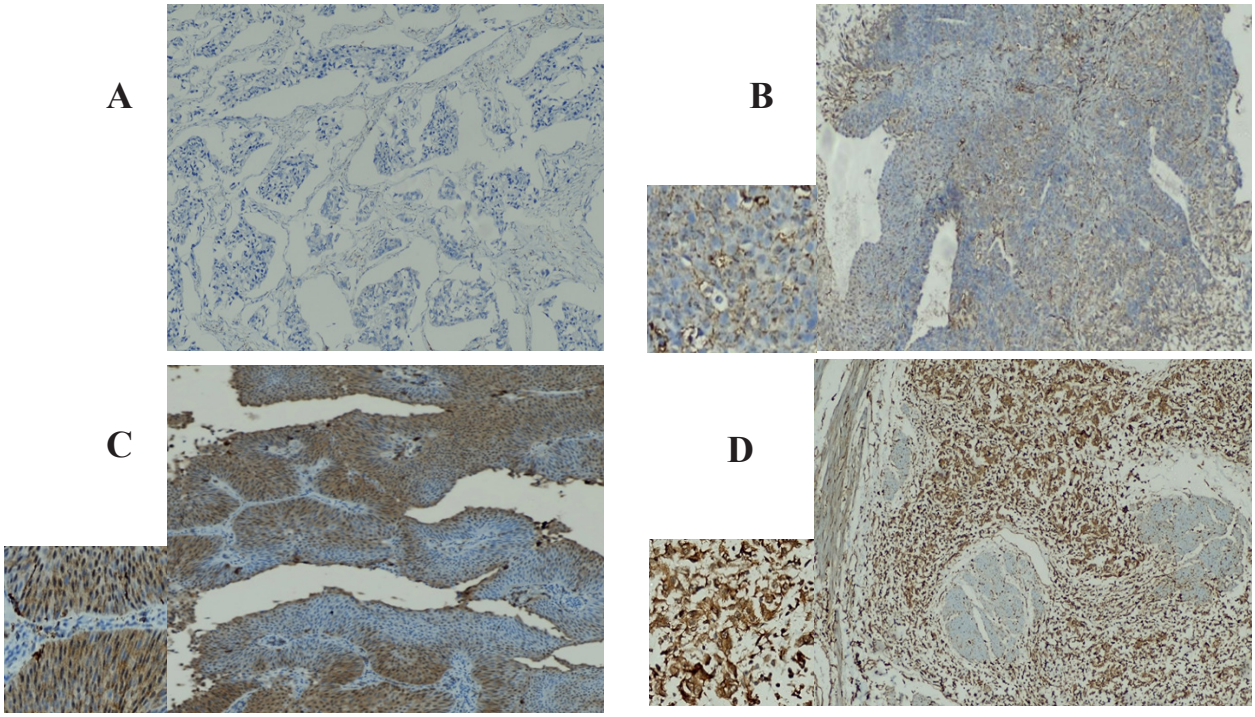

Figure 1. Scoring for *CD14* Immunohistochemical Study Exhibiting Membranous Staining of the Tumor cells: A: Negative (10x magnification); no staining, B: Score 1 (10x magnification); weak staining in <25% of tumor cells, C: Score 2 (x10 magnification); moderate staining in about 60% of tumor cells, D: Score 3 (10x magnification); strong staining in >75% of tumor cells.

with divergent differentiation are high grade, so there was a significant positive association detected between tumor histopathological subtype and grade (P value = 0.001), which agrees with the meta-analysis performed by Mori et al. [17] who showed that patients with urothelial carcinoma with variant histology are usually associated with advanced stage. Also, Prijovic et al. [18] reported that variant histology of urothelial carcinoma is associated with a higher grade and more advanced stage of BC.

Regarding *CD14* immunohistochemical expression, there was no significant association between *CD14* immunohistochemical expression and demographic characteristics which was in accordance with what was reported by Ahmad et al. [9] who reported that there was no significant association between *CD14* immunohistochemical expression and demographic characteristics. The results may indicate that demographic factors have no effect on *CD14* expression .

Also, there was no statistically significant correlation detected between *CD14* expression and each of the histopathological subtypes, associated bilharziasis, lymphovascular emboli, and perineurial invasion (P value =0.071, 0.572, 0.657, and 1 respectively).

In the current study, there was a significant inverse correlation noted between tumor histological grade and *CD14* expression intensity where 100 % of low-grade cases showed positive *CD14* expression and 100% of *CD14* negatively expressed cases were high grade (P value = 0.045) which indicates that with increasing the tumor grade, the expression of *CD14* decreased until it was completely lost in high tumor grade. This was in agreement with Al-Hindi et al. [19] (P-value = 0.001) who noted that low-grade bladder cancer cells showed *CD14* over-expression was highly related to low-grade tumors in an inverse correlation [19].

These findings were different from those reported by Ahmad et al. [9] who also showed a significant association between tumor grade and *CD14* expression (Rho=0.264, P-value = 0.043), but found intense *CD14* expression (Score 3) mostly in grade III. Also, Mousa et al. [20]

Table 5. The Association between *CD14* Immunohistochemical pression and The Demographic and Clinicopathological Features of Included Urothelial Carcinoma Cases

		CD14 Immunohistochemical Expression		
		Positive Number Percentage	Negative Number Percentage	P value
Age groups	Less than or equal 57 years	18 (41.9%)	5 (62.5%)	0.442
	More than 57 years	25 (58.1%)	3 (37.5%)	
Sex	Male	41 (95.3%)	8 (100.0%)	1
	Female	2 (4.7%)	0 (0.0%)	
Tumor size (cm) grouping	Less than or equal 4 cm	26 (60.5%)	4 (50.0%)	0.702
	More than 4 cm	17 (39.5%)	4 (50.0%)	
Tumor gross appearance	Infiltrating	6 (14.0%)	4 (50.0%)	0.118
	Ulcerating	8 (18.6%)	1 (12.5%)	
	Polypoid	24 (55.8%)	2 (25.0%)	
	Fungating	5 (11.6%)	1 (12.5%)	
Tumor histopathological subtype	Papillary urothelial carcinoma	26 (60.5%)	1 (12.5%)	0.071
	Conventional urothelial carcinoma	7 (16.3%)	5 (62.5%)	
	Urothelial carcinoma with squamous differentiation	6 (14.0%)	1 (12.5%)	
	Urothelial carcinoma with glandular differentiation	1 (2.3%)	0 (0.0%)	
	Urothelial carcinoma with micropapillary differentiation	2 (4.7%)	1 (12.5%)	
	Urothelial carcinoma with lymphoepithelioma differentiation	1 (2.3%)	0 (0.0%)	
Tumor grade	Low	16 (37.2%)	0 (0.0%)	0.045
	High	27 (62.8%)	8 (100.0%)	
Invasion	Invasive(≥T1)	29 (67.4%)	8 (100.0%)	0.088
	Non-Invasive(=Ta)	14 (32.6%)	0 (0.0%)	
Tumor pathological stage	pTa	14 (32.6%)	0 (0.0%)	0.03
	pT1	14 (32.6%)	0 (0.0%)	
	pT2a	2 (4.7%)	1 (12.5%)	
	pT2b	4 (9.3%)	3 (37.5%)	
	pT3a	1 (2.3%)	0 (0.0%)	
	pT3b	4 (9.3%)	3 (37.5%)	
	pT4a	4 (9.3%)	1 (12.5%)	
Muscle proper invasion	Non muscle invasive(<T2)	28 (65.1%)	0 (0.0%)	0.001
	Muscle invasive(≥T2)	15 (34.9%)	8 (100.0%)	
Pathological lymph node stage	pNx (Could not be assessed)	30 (69.8%)	1 (12.5%)	0.008
	pN0	9 (20.9%)	5 (62.5%)	
	pN1	2 (4.7%)	2 (25.0%)	
	pN2	2 (4.7%)	0 (0.0%)	
Associated bilharziasis	Present	6 (14.0%)	0 (0.0%)	0.572
	Absent	37 (86.0%)	8 (100.0%)	
Lymphovascular emboli	Present	10 (66.7%)	4 (50.0%)	0.657
	Absent	5 (33.3%)	4 (50.0%)	
Perineural invasion	Present	8 (53.3%)	4 (50.0%)	1
	Absent	7 (46.7%)	4 (50.0%)	
Operation	Transurethral resection of bladder tumor [TURBT]	28 (65.1%)	0 (0.0%)	0.001
	Radical cystectomy	15 (34.9%)	8 (100.0%)	
Two-year recurrence	Yes	10 (23.3%)	1 (12.5%)	0.633
	No	23 (53.5%)	4 (50.0%)	
	Not available	10 (23.3%)	3 (37.5%)	
Adjuvant therapy	No: Non-compliant	21 (48.8%)	2 (25.0%)	0.456
	Yes: BCG+Chemotherpy + Chemo and radiotherapy	14 (32.6%)	4 (50.0%)	
	Not available	8 (18.6%)	2 (25.0%)	
Two-year survival	No	7 (16.3%)	0 (0.0%)	0.463
	Yes	28 (65.1%)	6 (75.0%)	
	Not available	8 (18.6%)	2 (25.0%)	

Table 6. The Association Between *CD14* Immunohistochemical Pression and The Two-Year Survival Rate in Radical Cystectomies Cases

		CD14 Immunohistochemical Expression		P value
		Positive Number (Percentage)	Negative Number (Percentage)	
Two-year survival	No	6 (40.0%)	0 (0.0%)	
	Yes	7 (46.7%)	6 (75.0%)	
	Not available	2 (13.3%)	2 (25.0%)	0.114

Table 7. The Association Between *CD14* Immunohistochemical Pression and The Late Mortality Rate in Radical Cystectomies Cases

		CD14 Immunohistochemical Expression		P value
		Positive Number (Percentage)	Negative Number (Percentage)	
Late Mortality Rate (Mortality Rate After Exclusion Postoperative Complications)	Yes	5 (35.7%)	0 (0.0%)	
	No	7 (50.0%)	6 (75.0%)	0.156
	Not available	2 (14.3%)	2 (25.0%)	

reported a significant association between tumor grade and *CD14* expression (P-value <0.01), where most high-grade tumors showed positive immunohistochemical *CD14* expression (93.8%), whereas only 61.1% of low-grade tumors showed positive immunohistochemical *CD14* expression.

Moreover, another significant inverse association between the pathological tumor stage and *CD14* immunohistochemical expression (P value= 0.030) was detected. About sixty-five percent of *CD14* positively expressed cases were non-muscle invasive and 100% of *CD14* negatively expressed cases were muscle invasive (P value=0.001), thus, the more the tumor is capable of invasion, the less the expression of *CD14*. However, Ahmad et al. [9] reported that there was no significant association between *CD14* expression and the pathological tumor stage (Rho=0.159, P-value = 0.230).

To sum up, these two inverse significant associations were in opposition to what is known about immunoexpression of *CD14*, as higher expression of *CD14* is associated with worse prognostic factors as higher tumor grading and high ability of invasion according to the interpretation of *CD14* high expresser cancer cells express higher levels of numerous inflammatory mediators, increasing tumor growth compared to those of *CD14* low expresser cells.

It can be explained by the role of the patient's bladder tissue immune cells, which then work to redirect the type of immune response through the secretion of growth factors and cytokines, which suppress or stimulate anti-tumor function and control the inflammatory state. It is thought that local tumor microenvironment and immune response carry different protumoral or antitumoral roles in different tumors. Despite the accumulating evidence for the role of *CD14* in several cancers, the lack of functional and mechanistic studies that explain it renders it doubtful. In addition to taking into consideration that only few studies have investigated *CD14* expression in bladder urothelial carcinoma.

In the current study, there was no significant association between *CD14* immunoexpression and tumor recurrence (P value = 0.633) which was in accordance with Ahmad et al. [9] who reported that there was no significant association between *CD14* immunohistochemical expression and recurrent cases of urothelial bladder carcinoma (Rho=0.162, P-value = 0.220).

In conclusion, the results of this study which aimed to rule out the prognostic value of *CD14* antigen expression conclude that the intensity of *CD14* expression was weak and even lost in high-grade and late-stage urothelial bladder carcinoma cases, while low-grade urothelial carcinoma cases showed *CD14* overexpression. Accordingly, the significance of therapeutic approaches targeting *CD14* in high-grade and late-stage urothelial bladder carcinoma shall be questionable. Wider scale studies using larger sample sizes and longer-term follow-up are further needed to establish the prognostic significance of *CD14* expression. Further molecular studies should be carried out to support the findings of this research.

Author Contribution Statement

All Authors contribute to collecting data, reading the slides & writing the manuscript.

Acknowledgements

We thank our Anatomic Pathology Department staff for their continuous help & support.

Ethical Declaration:

This study protocol was approved and accepted by The Research Ethics Committee (REC) of the Faculty of Medicine, Cairo University conducted according to ICH GCP standards and appropriate local and institutional regulations and guiding principles that govern REC operation (Code: N-405-2023) on 11/11/2023.

Data Availability

Data is available upon request according to the institutional regulations and with official permission.

Conflict of Interest

Authors declare that they have no conflict of interest.

References

1. Bhanvadia SK. Bladder cancer survivorship. Curr Urol Rep. 2018;19(12):1-8. https://doi.org/10.1007/s11934-018-0860-6
2. Izquierdo L, Bolton DM, Lawrentschuk N. Radical cystectomy and orthotopic bladder substitution: surgical tricks and management of complications. Minerva Urol Nefrol. 2013;65(4):225-34.
3. Mari A, Campi R, Tellini R, Gandaglia G, Albisinni S, Abufaraj M, Hatzichristodoulou G, et al. Patterns and predictors of recurrence after open radical cystectomy for bladder cancer: a comprehensive review of the literature. World J Urol. 2018;36(2):157-70. https://doi.org/10.1007/s00345-017-2115-4
4. World Health Organization, International Agency for Research on Cancer. Global cancer observatory (Globocan) [Internet]. Egypt fact sheet. 2020. Available from: https://gco.iarc.fr/today/data/factsheets/populations/818-egypt-fact-sheets.pdf
5. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74. https://doi.org/10.1016/j.cell.2011.02.013
6. Cheah MT, Chen JY, Sahoo D, Contreras-Trujillo H, Volkmer AK, Scheeren FA, et al. *CD14*-expressing cancer cells establish the inflammatory and proliferative tumor microenvironment in bladder cancer. Proc Natl Acad Sci U S A. 2015;112(15):4725-30. https://doi.org/10.1073/pnas.1424795112
7. Yap NY, Ong TA, Pailoor J, Gobe G, Rajandram R. The significance of *CD14* in clear cell renal cell carcinoma progression and survival prognosis. Biomarkers. 2023;28(1):24-31. https://doi.org/10.1080/1354750X.2022.2142292
8. Eruslanov E, Neuberger M, Daurkin I, Perrin GQ, Algood C, Dahm P, et al. Circulating and tumor-infiltrating myeloid cell subsets in patients with bladder cancer. Int J cancer. 2012;130(5):1109-19. https://doi.org/10.1002/ijc.26123
9. Ahmad A, Yusoff SM, Ali RM, Osman M, Kong LS, Hussin H. High *CD14* Antigen Expression in Urothelial Bladder Carcinoma Establishes that the Inflammatory Microenvironment Promotes Tumour Cell Proliferation. Malays J Med Health Sci. 2022;18(s21):30-5. https://doi.org/10.47836/mjmhs.18.s21.6
10. WHO Classification of Tumours Editorial Board. Urinary and Male Genital Tumours, 5th ed.; International Agency for Research on Cancer: Lyon, France, 2022; Volume 8. Available from: https://tumourclassification.iarc.who.int/chapters/36 (accessed on 15 June 2024).
11. World Health Organization, International Agency for Research on Cancer. Global cancer observatory (Globocan) [Internet]. Bladder cancer. 2022. Available from: https://www.iarc.who.int/cancer-type/bladder-cancer/ (accessed on 23 July 2024).
12. Amin HA, Kobaisi MH, Samir RM. Schistosomiasis and bladder cancer in Egypt: truths and myths. Open Access Maced J Med Sci. 2019;7(23):4023. https://doi.org/10.3889/oamjms.2019.857
13. Ragab HH, El-Badry MS, Abdel Ghani MM, Aboelhassan MH. Urinary Bladder Carcinoma Pattern at Urology Minia University Hospital. Minia J Med Res. 2021;32(1):36-43. https://doi.org/10.21608/mjmr.2022.220576
14. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, et al. Epidemiology and risk factors of urothelial bladder cancer. Eur urol. 2013;63(2):234-41. https://doi.org/10.1016/j.eururo.2012.07.033
15. Daugherty SE, Lacey Jr JV, Pfeiffer RM, Park Y, Hoover RN, Silverman DT. Reproductive factors and menopausal hormone therapy and bladder cancer risk in the NIH-AARP Diet and Health Study. Int J Cancer. 2013;133(2):462-72. https://doi.org/10.1002/ijc.28022
16. Morales EE, Grill S, Svatek RS, Kaushik D, Thompson Jr IM, Ankerst DP, Liss MA. Finasteride reduces risk of bladder cancer in a large prospective screening study. Eur urol. 2016;69(3):407-10. https://doi.org/10.1016/j.eururo.2015.08.029.
17. Mori K, Abufaraj M, Mostafaei H, Quhal F, Karakiewicz PI, Briganti A, et al. A systematic review and meta-analysis of variant histology in urothelial carcinoma of the bladder treated with radical cystectomy. J Urol. 2020;204(6):1129-40. https://doi.org/10.1097/JU.0000000000001305
18. Prijovic N, Acimovic M, Santric V, Stankovic B, Nikic P, Vukovic I, et al. The Impact of Variant Histology in Patients with Urothelial Carcinoma Treated with Radical Cystectomy: Can We Predict the Presence of Variant Histology? Curr Oncol. 2023;30(10):8841-52. https://doi.org/10.3390/curroncol30100638
19. Al-Hindi ZS, Al-Hasnawy HH, Alkafaji HA. Immunohistochemical expression of CD 14 in transitional cell carcinoma of the urinary bladder. Int J Health Sci. 2022;1583-92. https://doi.org/10.53730/ijhs.v6nS4.6288
20. Mousa HM, Jassem AN, Hussain MJ. *CD14* expression and microbial infection in bladder tumors. J Biol Sci. 2017;17(8):394-9. https://doi.org/10.3923/jbs.2017.394.399