Original Article

# CD47 expression in urothelial carcinoma and its correlation with histopathological findings

**Ganime Çoban, Pelin Yıldız, Nurhan Şahin, Cevper Ersöz[1], Ali Toprak[2], Nurcan Ünver[3], Şahande Elagöz**

Departments of Pathology, [1]Urology and [2]Biostatistics, Bezmialem Vakif University, Faculty of Medicine, Istanbul, [3]Department of Pathology, Yedikule Pulmonary Diseases and Thoracic Surgery Education and Research Hospital, Istanbul, Turkey

**Correspondence to:** Ganime Çoban, E-mail: drgcoban@hotmail.com

## Abstract

**Background:** The incidence of bladder cancers (BCs) is increasing day by day in both males and females with increased smoking. New treatment protocols are required due to the long follow-up times and high recurrence rates. CD47 (integrin-associated protein) is a membrane receptor that is effective in normal and pathophysiological events such as apoptosis, proliferation, adhesion, and immunity. Phagocytosis of both normal and tumor cells is prevented, by binding to the ligand signal regulatory protein-1 (SIRP-1)α on macrophages. Anti-CD47 antibodies can eliminate the binding and may be effective in treatment.

**Methods:** The study included 194 cases with urothelial carcinoma (UC) who underwent transurethral resection (TUR) of the bladder and had been followed up for one year. Cytoplasmic and membranous staining was preferred for CD47-positive staining. Staining results were compared with tumor grades, pathologic tumor stage, recurrence, and metastasis grade.

**Results:** The prevalence and intensity of CD47 staining in BCs were evaluated in this study. High-grade tumors were stained more pronouncedly compared with low-grade tumors and normal epithelium. The prevalence of CD47 staining was significant in pT2 (Pathological tumor stage 2, invades of muscularis propria) tumors, and its correlation with pTa (Pathological tumor stage a, noninvasive papillary carcinoma) tumors was significant ($P < 0.001$). In the intensity evaluation, there was a significant difference between pTa and pT2 and between pT1 (Pathological tumor stage 1, invades lamina propria) and pT2 ($P = 0.003$).

**Conclusion:** CD47 is expressed strongly in high-grade tumors. The prevalence and intensity of CD47 staining are high in pathologic tumor stage, recurrence, and distant metastases and are considered poor prognostic markers. We believe that anti-CD47 antibodies can be used as an alternative to the current treatment or in combination with other medications, and the systemic side effects that may occur with intravesical treatment can be prevented.

**Keywords:**
CD47, immunohistochemistry, macrophage, urothelial carcinoma

## Introduction

Bladder cancer (BC), diagnosed 7th for males and 17th for females worldwide, ranks 13th among cancer-related mortalities.[1] With the increase in smoking in the last 3–4 decades, the rate increased further in developed countries and rose to the 4th and 9th places in females and males, respectively.[1-3]

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Seventy-five percent (75%) of BCs are carcinoma *in situ* (CIS), Ta, or T1. When CIS, Ta, and T1 tumors are compared with T2-4 tumors, the cancer-related mortality is lower and has a longer survival rate.[1,4]

Recent studies revealed that the periphery of the tumor is as important as the tumor itself for targeted treatment. Macrophages, which are responsible for providing homeostasis and the elimination of damaged and aged cells, cannot phagocyte healthy cells expressing CD47 due to the "don't eat me" signal, with the presence of signal regulatory protein-1 (SIRP-1) on their surface. This also applies to tumor cells with CD47 on their surface. Normal and tumor cells that do not express CD47 are rapidly eliminated by macrophages.[5] Studies have shown that CD47 is also effective in the growth and progression of tumor cells. It has been determined to be a poor prognostic indicator, particularly in acute myeloid leukemia (AML), non-Hodgkin's lymphoma, bladder, breast, and oral squamous cell carcinoma.[6-10]

Numerous studies have been conducted with the idea of phagocytosis of tumor cells by macrophages by eliminating CD47–SIRP-1 interaction or inhibiting CD47 expression in targeted therapy.

This study aimed to evaluate the relationship between CD47 expression and urothelial carcinoma (UC) grading, to compare its correlation with the presence of recurrence at one-year follow-up, and to investigate whether it may be an alternative to the current treatment.

## Methods

A total of 194 patients who had been diagnosed with UC, underwent transurethral resection (TUR) of the bladder, and had one-year follow-ups between January 2016 and October 2019 were included in the study. Demographic and clinical information about the patients was obtained from the hospital information system. A total of 63 cases of low-grade UC and 131 cases of high-grade UC were recorded. As part of the evaluation process, normal epithelium close to 39 low-grade UCs was included as an internal control. To eliminate the differences between the observers, all hematoxylin–eosin-stained preparations of the tumors were removed from the archive and reevaluated by two uropathologists based on the 2016 World Health Organization classification. The cases with a consensus were included in the study. A provision in the consent form obtained before the operation states that the tissues may be utilized for educational purposes. In addition, the ethical board of our institution approved this retrospective study (decision number: 54022451-050.05.04).

### Immunohistochemistry

Slides containing sufficient tumor and normal urothelial epithelium were selected for immunohistochemical (IHC) examination. Four-micron sections were obtained from paraffin blocks, and the CD47 antibody was stained on the Ventana Benchmark Ultra Device. The cytoplasmic and membranous staining was accepted as positive CD47 staining. The IHC staining of CD47 was evaluated by two pathologists who were blind to the clinical data. The H-score was calculated based on the prevalence and intensity of staining. The prevalence of the staining was scored as 1 for less than 25%, 2 for between 25% and 75%, and 3 for more than 75% staining. The intensity of staining was graded as 0 (no), 1 (weak), 2 (moderate), or 3 (severe). The H-scores of staining prevalence and intensity were considered low expression if the score was between 1 and 4 and high if the score was between 5 and 9.[11] The staining results were also compared with tumor grade and pathological tumor stage.

### Statistical analysis of data

The data were analyzed using Statistical Package for the Social Sciences (SPSS) statistics software version 22.0. A Chi-square test was performed for the distribution of categorical variables, the Kruskal–Wallis test was performed for the difference in continuous variables between multiple groups and significant variables, and Dunn's test was performed for pairwise comparisons. The differences between continuous variables were evaluated by the Mann–Whitney U-test. The median, mean, standard deviation, frequency, and percentage values were given as descriptive statistics. A $p < 0.05$ was considered statistically significant.

## Results

Of the 194 subjects included in the study, 29 (14.94%) of the patients were female and 165 (85.05%) were male. The youngest patient was 21 and the oldest was 93, and the mean age was 67.2 years. Of the patients, 63 (27%) were low grade and 131 (56.2%) were high grade. The pathology stage of all low-grade tumors was pTa. Of the high-grade tumors, 45 (34.35%) were pTa, 44 (33.58%) were pT1, and 42 (32.06%) were pT2.

There was a significant difference in CD47 staining intensity and the extent of the high-grade tumors in comparison with the low-grade tumors and normal epithelium. In the evaluation of the H-score, it was observed that high-grade tumors had statistically significantly stronger staining compared with low-grade tumors and normal

## Key Message

*Immunohistochemical CD47 expression is increased in pathological tumor stage, tumor grade, recurrence and distant metastases. It can be considered a poor prognostic marker. Anti-CD47 antibodies can be used as an alternative to current treatment or in combination with other drugs and systemic side effects that may occur with intravesical treatment can be prevented.*

urothelial epithelium ($P < 0.001$) [Figure 1]. No significant difference was found between low-grade tumors and normal urothelial epithelium in terms of H-scores [Table 1].

When comparing the pathological tumor stage with the prevalence and intensity of CD47 staining, a statistically significant difference was found between the extent of CD47 staining, pT2, and pTa tumors ($P < 0.001$) [Figure 2]. There was a significant difference in CD47 staining intensity between pTa and pT2 and between pT1 and pT2 ($P = 0.003$). No significant difference was observed between pTa and pT1 in terms of prevalence and intensity ($P > 0.05$). In the evaluation of the H-score, a statistically significant result was noted between pTa and pT2 and between pT1 and pT2 ($P < 0.001$). No significant results were found between pTa and pT1 ($P > 0.005$) [Table 2].

For the one-year follow-up of the patients, 96 (73.2%) of 131 high-grade tumors were under follow-up, 28 (21.3%) were not followed up, and 7 (5.3%) passed away. Of the 96 patients who were under follow-up, 16 (16.6%) developed a recurrence of the disease within the first year, occurring at 6.8 months on average. In the H-score evaluation of 16 patients who had a recurrence of the disease in one year, 11 (68.75%) had high expression of CD47 and five (31.25%) had low expression of CD47.

The number of low-grade patients was 63, and 47 patients were under follow-up. However, the follow-up data of 14 patients could not be obtained. Of the 47 patients under follow-up, 14 (29.78%) developed a recurrence of the disease, while 33 (70.21%) had no recurrence. Recurrence occurred

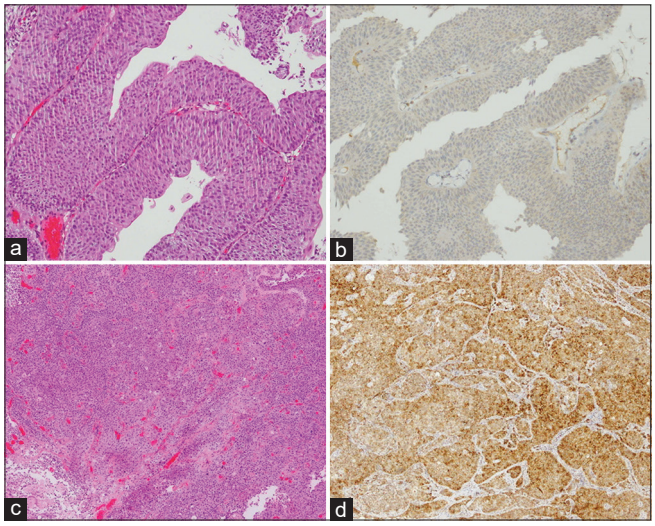


**Figure 1: Low-grade urothelial carcinoma (a, HE×100), weak and focal cytoplasmic and membranous expression of CD47 in low-grade urothelial carcinoma (b, x100), high-grade urothelial carcinoma (c, HE×100), and diffuse and strong cytoplasmic and membranous expression of CD47 in high-grade urothelial carcinoma (d, x100)**

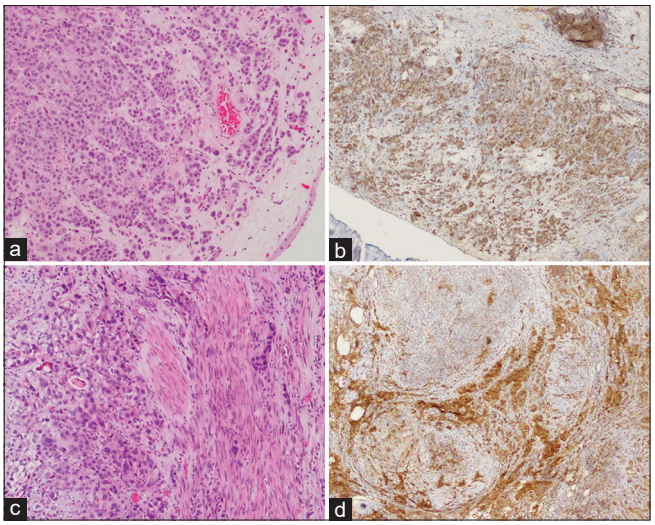


**Figure 2: High-grade pT1 urothelial carcinoma (a, HE×100), diffuse and strong CD47 expression in high-grade urothelial carcinoma with lamina propria invasion (pT1) (b, x100), high-grade pT2 urothelial carcinoma (c, HE×100), diffuse and strong CD47 expression in high-grade urothelial carcinoma with extensive invasion within the detrusor muscle (pT2) (d, x100)**

**Table 1: Comparison of H-scores by grades**

| Group | Number of cases (*n*) | Median (min–max) | Mean±Std. deviation |
|---|---|---|---|
| Control | 39 | 0.000 (0.00-2.00) | 0.3333±0.5773 |
| Low grade | 63 | 0.000 (0.00-4.00) | 0.4754±0.9765 |
| High grade | 131 | 6.000 (0.00-9.00) | 5.4769±2.7257 |

**Table 2: CD47 staining according to tumor stages**

| Pathological stage | CD47 prevalence Median (min–max)/mean±Std. deviation | CD47 intensity Median (min–max)/mean±Std. deviation | CD47 H-score Median (min–max)/mean±Std. deviation |
|---|---|---|---|
| Ta (*n*=45) | 2.000 (0.00-3.00)/1.888±0.7752 | 2.00 (0.00-3.00)/2.1333±0.8944 | 4.00 (0.00-9.00)/4.5111±2.2825 |
| T1 (*n*=44) | 2.000 (0.00-3.00)/2.227±0.8314 | 2.00 (0.00-3.00)/2.1136±0.8948 | 6.00 (0.00-9.00)/5.1628±2.9514 |
| T2 (*n*=42) | 3.000 (1.00-3.00)/2.5476±0.6176 | 3.00 (1.00-3.00)/2.6429±0.6176 | 6.00 (1.00-9.00)/6.8333±2.4185 |

within 6.2 months on average. All the patients who had a recurrence of the disease had an H-score of less than 4 and had low expression of CD47.

Distant metastasis was noted in six high-grade BC patients, and the H-score of all these patients was 9. Sixteen patients underwent cystectomy and lymph node dissection, and one patient underwent surgery in another medical center. Of 15 patients, five had lymph node metastases, and three of the five had a high H-score, while two had a low H-score.

## Discussion

CD47, known as integrin-related protein, is a transmembrane protein from the immunoglobulin superfamily, weighing 50 kDa, and is effective in physiological and pathophysiological processes, such as apoptosis, proliferation, adhesion, immunity, and cardiovascular effect.[12] CD47 also acts as the receptor for thrombospondin-1 (TSP-1), known as the extracellular matrix glycoprotein.[13] CD47 acts as the ligand for SIRP-1. It is a transmembrane receptor of SIRP-1 macrophage and granulocytes, as well as dendritic cells, endothelial cells, and neurons on the surface of the immunoglobulin superfamily.[12] SIRP-1 interacts with CD47 and triggers phosphorylation of the tyrosine-based inhibition motif (ITIM).[14] Macrophages cannot phagocytize healthy cells that send a "don't eat me" signal due to the binding of CD47–SIRP-1 α, nor can they phagocytize tumor cells expressing CD47.[5]

Bladder carcinomas may be of different grades and stages, but the primary treatment is TUR, followed by intravesical BCG (Bacillus Calmette-Guérin), adjuvant chemotherapy, and cystectomy that may be decided according to risk groups.[15] High-risk patients should be under follow-up and treatment for long periods since the recurrence rate is high, particularly in high-grade tumors. It is suggested that there may be alternative treatment options for bladder carcinoma, and also, treating the periphery of the tumor may be effective in treatment.

The binding of CD47 and SIRP-1α is important for the protection of tumor cells from phagocytosis and tumor progression.[16] Treatments targeting CD47 facilitate the phagocytosis of malignant cells and help preventing tumor growth and metastasis.[5]

In this study, the prevalence and intensity of CD47 staining in bladder tumors were evaluated. High-grade tumors had strong staining in comparison with low-grade tumors and normal epithelium.

In the literature, there are studies on CD47 staining in many organs, including the prostate, kidney, liver, lung, brain, ovary, stomach, and bladder, and were evaluated as a poor prognostic factor.[5,17-19] CD47 stimulation is considered to be associated with the PI3K-Akt (phosphatidylinositol 3-kinase-protein kinase B) pathway in the proliferation of cancer cells in astrocytoma and with vascular endothelial growth factor in angiogenesis.[20,21] In their study, Chan *et al*.[22] have found that CD47 is expressed intensively and is a poor prognostic factor in BC. In a study conducted by Wang *et al*.,[23] it has been reported that the expression of integrin β4, CD44v6 (Cluster of differentiation 44 exon v6), and CD47 was high in tumors of the posterior wall of the bladder and concluded that this expression is a poor prognostic factor.

In their study, Olcucuoglu *et al*.[24] indicated that there was a significant staining difference between Ta and T1 tumors; however, there was no significant difference between tumors with (T2, T3, and T4) and without muscle invasion (Ta, T1). Moreover, they have shown that it was not associated with invasion and lymph node metastasis.

In this study, TUR materials were utilized and T3 and T4 tumors were excluded from the study. However, the expression of CD47 between T2–Ta and T2–T1 tumors was found to be statistically significant. This may suggest that the CD47 expression is related to invasion.

In the treatment of many solid organ tumors, anti-CD47 antibodies were implanted in patient-derived xenograft (PDX), and treatment was administered targeting the CD47–SIRP-α pathway.[5,25,26] CD47 is expressed more in AML cells than in peripheral blood cells and healthy cells in the bone marrow, and the phagocytosis of *in vitro* AML cells was treated by blocking the CD47–SIRP-α pathway.[17] In the study conducted by Chao *et al*.,[7] human B-cell lymphoma (non-Hodgkin lymphoma) was transplanted into mice and they were treated with the anti-CD47

treatment administered simultaneously with anti-CD20 treatment. Similarly, Ye *et al*.[10] have reported that CD47 has stronger staining in leukoplakia and squamous cell carcinoma in the oral mucosa than normal epithelium and that it was an important prognostic marker and anti-CD47 agents may be effective in target treatment.

The expression of CD47 in cancer stem cells of the bladder was increased and evaluated as a prognostic factor and was shown to be a candidate for target treatment.[8]

In this study, the H-score of CD47 in high-grade tumors was significantly higher than the low-grade tumors and healthy epithelial cells. Intravesical anti-CD47 treatment may be included in the target treatment, especially for the treatment of high-grade tumors of the bladder. In addition, there is a significant difference in staining between T2 tumors and Ta and T1 tumors. Of the high-grade tumors with recurrence, 68.75% had a high H-score, suggesting that CD47 expression may be a poor prognostic parameter. Furthermore, patients with distant metastasis had a high expression rate and we believe that it may also be associated with metastasis. However, there are few metastatic cases in our study, and further studies with a high number of cases are required.

CD47 is found in hematopoietic cells, in many healthy cells, and also in tumor cells. Therefore, the most common side effects during treatment are neutropenia and anemia.[27] These side effects may be reduced by intravesical administration of anti-CD47 directly into the bladder. The studies show that CD47-blocking agents and simultaneous immunotherapy may be administered in most malignancies.[10,17,26,28]

There is a high expression of CD47 in bladder tumors and high-grade tumors, and this finding is a poor prognostic marker for the recurrence rate and distant metastases of high-grade tumors. Although T3 and T4 tumors were not included in the study, we consider that the differences in CD47 expression between Ta, T1, and T2 tumors should be determined, and further studies should be conducted with the high number of patients for T3 and T4 tumors. We believe that anti-CD47 antibodies may be used in combination with other medications, and therefore, side effects related to intravesical treatment may be prevented.

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**Conflicts of interest**
There are no conflicts of interest.

**ORCID iDs**
**Ganime Çoban:** https://orcid.org/0000-0002-5779-6797
**Pelin Yıldız:** https://orcid.org/0000-0002-7709-7264
**Nurhan Şahin:** https://orcid.org/0000-0002-5039-1164
**Cevper Ersöz:** https://orcid.org/0000-0001-5508-9370
**Ali Toprak:** https://orcid.org/0000-0003-4471-2790
**Nurcan Ünver:** https://orcid.org/0000-0001-7392-6099
**Şahande Elagöz:** https://orcid.org/0000-0001-5897-5642

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