# Correlation between *Ki-67* or Profilin-1 Expression Levels, Clinicopathological Characteristics and Postoperative Prognosis in Patients with Bladder Cancer

*Ann. Ital. Chir.*, 2024 95, 2: 246–252
https://doi.org/10.62713/aic.3335

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Background: Bladder cancer is the most common malignancy of the urinary system, and the search for new and reliable biomarkers has important clinical significance for the personalized treatment of bladder cancer. This study aims to explore the correlation between nuclear proliferation antigen (*Ki-67*) or Profilin-1 (*PFN1*) levels, clinicopathological characteristics, and postoperative prognosis in patients with bladder cancer.

Methods: Patients with bladder cancer who underwent transurethral resection of bladder cancer tumor in The Fourth Affiliated Hospital of Soochow University, hospital from January 2019 to January 2021 were selected as the study group (n = 60), and patients with benign lesions of bladder cancer during the same period were selected as the control group (n = 60). The expression of *Ki-67* and *PFN1* in tumor and bladder tissues of the two groups was analyzed. *Ki-67* recorded the patient's pathological parameters and calculated the patient's postoperative prognosis. The correlation between *Ki-67* and *PFN1* expression levels, pathological parameters, and postoperative prognosis was analyzed.

Results: The positive expression rates of *Ki-67* and *PFN1* in the study group were 63.33% and 73.33%, respectively, which were significantly higher than the positive expression rates in the control group ($\chi^2$ = 14.803, 17.757, $p < 0.001$). The positive expression rates of *Ki-67* and *PFN1* were related to histological grade, clinical stage, infiltration, and lymph node metastasis, and the differences were statistically significant ($p < 0.05$). Bladder cancer patients with non muscle-invasive bladder cancer (NMIBC), high-grade histological grade, Ta~T1 clinical stage, invasive, and lymph node metastasis have a higher *Ki-67* positive expression rate than bladder cancer patients with muscle-invasive bladder cancer (MIBC), low-grade histological grade, T2~T4, non-invasive, and no lymph node metastasis. The high expression level of *Ki-67* has little relationship with gender, age, tumor diameter, and vascular invasion ($p > 0.05$). The survival time and three-year survival rate of the *Ki-67* positive expression group were significantly lower than those of the *Ki-67* negative expression group ($p < 0.05$). The survival time and three-year survival rate of the *PFN1* positive expression group were significantly lower than those of the *PFN1* negative expression group ($p < 0.05$).

Conclusion: The positive expression rates of *Ki-67* and *PFN1* in bladder tumor tissue are significantly higher than those in bladder tissue, and pathological pattern, histological grade, clinical stage, infiltration, and lymph node metastasis are related to the positive expression rates of *Ki-67* and *PFN1*, and different genders, ages, tumors diameter and vascular invasion are not related to the positive expression rates of *Ki-67* and *PFN1*. The survival time and three-year survival rates of bladder cancer patients with *Ki-67* positive and *PFN1* positive expression are shorter.

**Keywords:** bladder cancer; nuclear proliferation antigen; Profilin-1; clinicopathological features; prognosis

## Introduction

Bladder cancer ranks as the most prevalent malignant tumor affecting the urinary system, with the highest incidence among all urinary and reproductive system tumors in China [1]. Bladder cancer holds the second position in terms of incidence in the West, following prostate cancer [2]. It is a significant ailment that directly threatens human health and survival [3]. Bladder cancer is the most common urothelial cancer. According to the degree of invasion, it can be divided into non-muscle invasive bladder cancer and muscle-invasive bladder cancer [4]. The two have entirely different infiltration methods and treatment options [5]. At present, total radical cystectomy followed by postoperative chemotherapy and radiotherapy is a standard clinical treatment plan, but the five-year survival rate of patients is only 50% [6]. Bladder cancer is very easy to relapse after surgery and metastasize to intermediate and advanced stages, and relevant biomarkers have become difficult in treating early bladder cancer [7]. Therefore, finding new and reliable biomarkers can help identify early bladder cancer diagnosis, improve prognosis, and guide treatment. It has important clinical significance for personalized treatment of bladder cancer.

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Nuclear proliferation antigen (*Ki-67*) is a cell proliferation protein that participates in cell cycle regulation and can be used to evaluate tumor cell division status in terms of tumor proliferation [8]. Its expression level is closely related to clinical pathological parameters, which can reflect the malignancy of the tumor from the side, and plays an auxiliary observation role in the diagnosis and prognosis of bladder cancer [9]. Profilin-1 (*PFN1*) is an actin-binding protein and one of the key proteins involved in fiber actin dynamics [10]. *PFN1* interacts with various proteins and participates in pivotal biological processes, including cell proliferation, cell survival, and cell division [11]. *Ki-67* and *PFN1* play significant roles in the onset and progression of various malignant tumors and are poised to emerge as novel biomarkers [12, 13]. Research shows that *PFN1* is closely related to lung cancer metastasis and invasion and plays an important role in tumor development. Currently, research both domestically and internationally primarily centers on *Ki-67*, *PFN1*, and tumor clinicopathological characteristics are mostly focused on malignant tumors such as lung cancer and gastric cancer, with limited focus on bladder cancer. Drawing from prior studies, this research analyzed patients' clinical pathological characteristics and postoperative prognosis by detecting *Ki-67* and *PFN1* levels in tumors and bladder tissues. This exploration aims to elucidate the relationship between these biomarkers and the clinicopathological features and prognosis of bladder cancer, thus aiding in early diagnosis, treatment evaluation, and prognostic assessment for individuals affected by this disease.

## Materials and Methods

*Research Subjects*

Patients with bladder cancer who underwent transurethral resection of bladder cancer tumors in our hospital from January 2019 to January 2021 were retrospectively selected as the study group (n = 60), and patients with benign lesions of bladder cancer during the same period were selected as the control group (n = 60). Among them, there were 84 males and 36 females, aged 53 to 77 years old, with an average age of (66.50 $\pm$ 2.45) years; body mass index of 20 to 29 kg/m$^2$, with an average of (25.73 $\pm$ 3.12) kg/m$^2$. There was no statistically significant difference in general data such as gender, age, and body mass index between the two groups of patients ($p > 0.05$). This study was approved by the Medical Ethics Committee of the The Fourth Affiliated Hospital of Soochow University (2022-LS-KY027). The entire experimental procedure adhered to the principles of informed consent, with patients or their family members being provided with information about the study. The study was carried out in compliance with the Declaration of Helsinki.

Inclusion criteria: ① Meet the diagnostic criteria for bladder cancer in "Clinical Oncology". ② Bladder cancer was diagnosed through cystoscopy and histopathological examination. ③ Transurethral bladder tumor resection in our hospital. ④ Clinical and imaging data records are complete.

Exclusion criteria: ① Those with serious lesions in other vital organs such as heart, liver, kidney, etc. ② Those with severe cerebrovascular disease. ③ Those with other malignant tumors. ④ Those with autoimmune diseases and infectious diseases. ⑤ Those with various mental disorders.

*Research Methods*

Observation and Evaluation Criteria of *Ki-67* and *PFN1*

Samples of bladder cancer tissue and adjacent normal tissue from the patient (>5 cm away from the lesion; no cancer cells were found by microscopy) were collected, repeatedly rinsed with normal saline, and frozen in liquid nitrogen for storage until testing. An RNA extraction kit (12183555, Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from the bladder and adjacent normal tissue per the manufacturer's instruction. Following the manufacturer's instruction, a reverse transcription kit (4366597, Applied Biosystems, Foster City, CA, USA) was used to synthesize cDNA from the extracted RNA.

For amplification, real-time fluorescent quantitative PCR (qRT-PCR) was performed using the instrument (CFX384 real-time system, Bio-Rad, Hercules, CA, USA). The protocol for the PCR amplification system included 10 μL of Power SYBR Green PCR Master Mix, 2 μL of upstream and downstream primers of the target gene, 4 μL of cDNA template, and 4 μL of nuclease-free water (AM9914G, Thermo Fisher Scientific, Waltham, MA, USA). Amplification conditions were: pre-denaturation at 95 °C for 10 minutes, denaturation at 95 °C for 15 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds for 40 cycles. The CT values of the sample were read and compared to internal reference genes to calculate the relative expression of each sample gene using $2^{-\Delta\Delta CT}$. The percentage of positive cells in the cell, <10% is negative, ≥10% is positive. The primer sequence are as follows: *Ki-67*: upstream (5′-AACCGGAAAGAAGTGTTGCG-3′), downstream (5′-CCCTGGAGTCACAAACTCATAC-3′). *PFN1*: upstream (5′-TCCAGTTGATCCGCATAAGGT-3′), downstream (5′-CTTCCCTATTTTCCGTGGCTG-3 ′). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*): upstream (5′-TGACCTCAACTACATGGTCTACA-3′), downstream (5′-CTTCCCATTCTCGGCCTTG-3′).

Recording of the Prognostic Status of Clinicopathological Parameters

The clinicopathological parameters of patients with bladder cancer were recorded in detail, including gender, age (<60 years old, ≥60 years old), tumor diameter (<3 cm, ≥3 cm), vascular invasion (yes, no), and pathological pattern [non muscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC)], histological grade (high grade, low grade), clinical stage (Ta~T1, T2~T4), invasiveness (yes, no), lymph node metastasis (yes, no) and other general clinicopathological data.

**Table 1. Comparison of *Ki-67* and *PFN1* expression between two groups [n (%)].**

| Group | Number of cases (*n*) | *Ki-67* | *PFN1* |
|---|---|---|---|
| Study group | 60 | 38 (63.33) | 44 (73.33) |
| Control group | 60 | 17 (28.33) | 21 (35.00) |
| $\chi^2$ value | | 14.803 | 17.757 |
| *p* value | | <0.001 | <0.001 |

*Ki-67*, nuclear proliferation antigen; *PFN1*, Profilin-1.

*Statistical Analysis*

The statistical analysis of the data was conducted using SPSS23.0 software (IBM, Armonk, NY, USA). Counting data such as *Ki-67* and *PFN1* positive rate is presented as [n (%)], and comparisons were analyzed using the $\chi^2$ test; measurement data such as average survival time have been tested for normality and are normally distributed as mean $\pm$ standard deviation ($\bar{x} \pm s$), with comparisons between groups analyzed using the independent sample *t*-test. $p < 0.05$ was considered as statistical significance.

## Results

*Comparison of Ki-67 and PFN1 Expression between Two Groups*

The results showed that the positive expression rates of *Ki-67* and *PFN1* in the research group were 63.33% and 73.33%, respectively, which were significantly higher than the positive expression rates in the control group ($\chi^2$ = 14.803, 17.757, $p < 0.001$) (Table 1).

*The Relationship between Ki-67 Expression and Clinicopathological Characteristics*

The positive expression rate of *Ki-67* was related to pathological pattern, histological grade, clinical stage, infiltration, and lymph node metastasis ($p < 0.05$). Bladder cancer patients with NMIBC, high-grade histological grade, $T_a$~$T_1$ clinical stage, invasive, and lymph node metastasis have a higher *Ki-67* positive expression rate than bladder cancer patients with MIBC, low-grade histological grade, $T_2$~$T_4$, non-invasive, and no lymph node metastasis. The high expression level of *Ki-67* has little relationship with gender, age, tumor diameter, and vascular invasion ($p > 0.05$) (Table 2).

*The Relationship between PFN1 Expression and Clinicopathological Characteristics*

The results showed that the positive expression rate of PEN-1 was related to pathological pattern, histological grade, clinical stage, infiltration, and lymph node metastasis ($p < 0.05$). Bladder cancer patients with NMIBC, high-grade histological grade, $T_a$~$T_1$ clinical stage, invasive, and lymph node metastasis have a higher PEN-1 positive expression rate than bladder cancer patients with MIBC, low-grade histological grade, $T_2$~$T_4$, non-invasive, and no lymph node metastasis. The high expression level of *Ki-67* has little relationship with gender, age, tumor diameter, and vascular invasion ($p > 0.05$) (Table 3).

*Correlation between Ki-67 Expression and Postoperative Prognosis*

Analysis showed that the survival time and three-year survival rate of the *Ki-67* positive expression group were significantly lower than those of the *Ki-67* negative expression group ($p < 0.05$) (Table 4).

*Correlation between PFN1 Expression and Postoperative Prognosis*

Analysis showed that the survival time and three-year survival rate of the *PFN1* positive expression group were significantly lower than those of the *PFN1* negative expression group ($p < 0.05$) (Table 5).

## Discussion

In this study, *Ki-67* and *PFN1* levels in tumor and bladder tissues were detected to explore the correlation between *Ki-67* and *PFN1* and the clinicopathological features and postoperative prognosis of bladder cancer. The study found that by analyzing the differences in the expression of *Ki-67* and *PFN1* in tumor and bladder tissues, the diagnosis and differentiation of bladder cancer patients can be improved. Bladder cancer originates from the bladder mucosal epithelium. Its main symptoms are hematuria [14]. Research has indicated that some patients with bladder cancer may relapse within a short period, even after undergoing resection, or even progress to muscle-invasive bladder cancer [15]. When diagnosing and evaluating the efficacy, cystoscopy and tissue biopsy are still the gold standards for diagnosis. However, cystoscopy is the most invasive examination, and frequent reexaminations will seriously affect the patient's quality of life [16]. Pathological features such as tumor stage, histological grade, and lymph node metastasis are auxiliary methods [17]. However, there are still limitations in the auxiliary diagnosis of clinical and pathological features, which cannot accurately evaluate the biological behavior of bladder cancer [18].

*Ki-67*, an antigen of cell proliferation, is expressed in the nucleus. It is expressed in other cycles of cell proliferation except the G0 phase [19]. Lashen A *et al.* [20] have shown that *Ki-67* is widely expressed in various malignant tumor cells and closely related to the proliferation of malignant tumors. Other studies have shown that abnormal expression or deletion of *PFN1* can affect the normal physiological activities of cells and lead to the occurrence of diseases [21]. The dysregulated expression of *Ki-67* and *PFN1* is associated with various tumors, making it possible to use them as biological markers for diagnosis and prognosis [22]. In this study, patients with bladder cancer undergoing transurethral resection of bladder tumor were selected as the study subjects, the expression of *Ki-67* and *PFN1* in tumors and bladders was detected, the relation-

**Table 2. Relationship between *Ki-67* expression level and clinicopathological parameters [n (%)].**

| Group | Number of cases (*n*) | Positive rate | $\chi^2$ value | *p* value |
|---|---|---|---|---|
| Gender | | | | |
| Male | 34 | 19 (55.89) | 0.558 | 0.455 |
| Female | 26 | 12 (46.15) | | |
| Age | | | | |
| <60 years | 28 | 18 (64.29) | 0.765 | 0.382 |
| ≥60 years | 32 | 17 (53.13) | | |
| Tumor diameter | | | | |
| <3 cm | 41 | 24 (58.54) | 0.537 | 0.464 |
| ≥3 cm | 19 | 13 (68.42) | | |
| Vascular invasion | | | | |
| With | 27 | 12 (44.44) | 0.606 | 0.436 |
| Without | 33 | 18 (54.55) | | |
| Pathological pattern | | | | |
| NMIBC | 45 | 37 (82.22) | 5.007 | 0.025 |
| MIBC | 15 | 8 (53.33) | | |
| Histological grading | | | | |
| High level | 26 | 22 (84.62) | 10.222 | 0.001 |
| Low level | 34 | 15 (44.12) | | |
| Clinical stage | | | | |
| $T_a$~$T_1$ | 36 | 27 (75.00) | 10.286 | 0.001 |
| $T_2$~$T_4$ | 24 | 8 (33.33) | | |
| Infiltration | | | | |
| With | 23 | 18 (78.26) | 10.563 | 0.001 |
| Without | 37 | 13 (35.14) | | |
| Lymph node metastasis | | | | |
| With | 15 | 13 (86.67) | 10.756 | 0.001 |
| Without | 45 | 17 (37.78) | | |

NMIBC, non muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer.

ship between the two and clinicopathological characteristics was analyzed, and the characteristics of bladder cancer patients were observed. Prognosis explores the correlation between *Ki-67* and *PFN1* expression levels and postoperative prognosis. The findings revealed that the rates of positive expression for both *Ki-67* and *PFN1* were elevated in the study group compared to the control group, indicating aberrant expression of both biomarkers in bladder cancer. Previous investigations similarly revealed significantly elevated levels of *Ki-67* expression in both primary and metastatic renal cell carcinoma tissues. Moreover, the expression levels were notably higher in metastatic renal cell carcinoma tissues compared to primary ones. Additionally, it has been noted that higher levels of *Ki-67* expression correlate with poorer prognoses for patients [23]. *PFN1* binds to various proteins in normal physiological activities and can connect extracellular signaling with the intracellular actin skeleton, including important physiological activities such as membrane transport, cell survival and proliferation, and transcription [24]. This indicates that the expression levels of *Ki-67* and *PFN1* can be used as prognostic predictive biomarkers for bladder cancer.

Studies have reported that *PFN1* expression levels are significantly related to higher tumor grades and stages [25]. The higher the *PFN1* expression level, the shorter the disease-free survival and the shorter the overall survival time, suggesting that the expression level of *PFN1* is related to prognosis [26]. Another investigation discovered that *Ki-67* expression levels were markedly elevated in gastric cancer tissues compared to adjacent tissues. Moreover, a significant positive correlation was observed between *Ki-67* expression and tumor infiltration, lymph node metastasis, and staging in gastric cancer cases [27]. In this study, pathological pattern, histological grade, clinical stage, invasiveness, and lymph node metastasis were related to the positive expression rate of *Ki-67* and *PFN1*. At the same time, different genders, ages, tumor diameter, and vascular invasion were not associated with the positive expression rate of *Ki-67* and *PFN1*. Based on the research results of this article, *Ki-67*, and *PFN1* expression showed higher levels in patients with high infiltration and lymph node metastasis. The infiltration and metastasis of cancer cells can improve their adaptability to harsh environments. The high expression level of *PFN1* can help can-

**Table 3. Relationship between *PFN1* expression and clinicopathological characteristics [n (%)].**

| Group | Number of cases (*n*) | Positive rate | $\chi^2$ value | *p* value |
|---|---|---|---|---|
| Gender | | | | |
| Male | 34 | 20 (58.82) | 0.149 | 0.700 |
| Female | 26 | 14 (53.85) | | |
| Age | | | | |
| <60 years | 28 | 17 (60.71) | 0.350 | 0.554 |
| ≥60 years | 32 | 17 (53.13) | | |
| Tumor diameter | | | | |
| <3 cm | 41 | 17 (41.46) | 1.408 | 0.235 |
| ≥3 cm | 19 | 11 (57.89) | | |
| Vascular invasion | | | | |
| With | 27 | 13 (48.15) | 0.243 | 0.622 |
| Without | 33 | 18 (54.55) | | |
| Pathological pattern | | | | |
| NMIBC | 45 | 36 (80.00) | 6.156 | 0.013 |
| MIBC | 15 | 7 (46.67) | | |
| Histological grading | | | | |
| High level | 26 | 21 (80.77) | 12.310 | <0.001 |
| Low level | 34 | 12 (35.29) | | |
| Clinical stage | | | | |
| $T_a$~$T_1$ | 36 | 29 (80.56) | 11.495 | 0.001 |
| $T_2$~$T_4$ | 24 | 9 (37.50) | | |
| Infiltration | | | | |
| With | 23 | 18 (78.26) | 14.958 | <0.001 |
| Without | 37 | 10 (27.03) | | |
| Lymph node metastasis | | | | |
| With | 15 | 13 (86.67) | 11.769 | 0.001 |
| Without | 45 | 16 (35.56) | | |

**Table 4. Correlation between *Ki-67* expression and postoperative prognosis [$\bar{x} \pm$ s, n (%)].**

| Group | Number of cases (*n*) | Survival time (months) | Three-year survival rate (%) |
|---|---|---|---|
| *Ki-67* positive expression | 38 | 31.46 ± 6.51 | 20 (52.63) |
| *Ki-67* negative expression | 22 | 40.57 ± 8.16 | 18 (81.82) |
| $t/\chi^2$ value | | 4.755 | 5.111 |
| *p* value | | <0.001 | 0.024 |

**Table 5. Correlation between *PFN1* expression and postoperative prognosis [$\bar{x} \pm$ s, n (%)].**

| Group | Number of cases (*n*) | Survival time (months) | Three-year survival rate (%) |
|---|---|---|---|
| *PFN1* positive expression | 44 | 32.82 ± 7.02 | 25 (56.82) |
| *PFN1* negative expression | 16 | 43.43 ± 6.87 | 15 (93.75) |
| $t/\chi^2$ value | | 5.206 | 7.202 |
| *p* value | | <0.001 | 0.007 |

cer cells adhere to each other, and increase their metastasis and cell survival ability. The high expression of *Ki-67* indicates the presence of high tumor cell proliferation activity in this tissue, and the higher the expression level of *Ki-67*, the higher the degree of malignancy of the cells; and the higher the degree of infiltration and metastasis. Experimental studies have demonstrated that suppressing *PFN1* expression notably restrains the invasion and metastasis of gastric cancer cell lines [28]. Furthermore, elevated *Ki-67* expression was observed in non-small cell lung cancer, with patients exhibiting lower *Ki-67* expression associated with improved prognoses and higher survival rates [29]. It is further suggested that *Ki-67* and *PFN1* expression levels can be used to diagnose bladder cancer. In addition to abnormal expression in tumor tissues, *Ki-67* and *PFN1* were also found to be differentially expressed in plasma, urine, and extracellular vesicles, making *Ki-67* and *PFN1* potentially useful as liquid biopsy markers for diagnosis or prognosis [30]. Compared with tissue biopsy, liquid biopsy is more practical and can reflect the condition of tumor patients in

real-time. Compared with normal controls, patients with liver cancer exhibited markedly elevated expression levels of the *PFN1* gene in peripheral blood cells. The findings of plasma proteomic analysis in this study found that the expression of *PFN1* in patients with bladder cancer was significantly higher than that of normal people, with a difference of more than two times. Studies have found that overexpression of *Ki-67* causes cells to restore an epithelial-like phenotype and repair their intercellular adhesion [31]. *Ki-67* can promote AMPK activation and p27 phosphorylation, thereby repairing adherens junctions and inducing epithelial morphological reversal in mesenchymal breast cancer [32]. These studies can well demonstrate the role of *Ki-67* and *PFN1* in cell proliferation and differentiation. In this study, the survival time and three-year survival rate of the *Ki-67* and *PFN1* positive expression group were significantly lower than those of the *Ki-67* and *PFN1* negative expression group.

## Conclusion

Analyzing the variance in *Ki-67* and *PFN1* expression between tumor and bladder tissue could potentially enhance the diagnosis and differentiation of bladder cancer patients. Nevertheless, it's essential to acknowledge the limitations of this study, including the restricted sample size and the absence of testing during treatment stages for bladder cancer patients. Future research should aim to expand the sample size for further validation. The positive expression rates of *Ki-67* and *PFN1* in bladder tumor tissue are significantly higher than those in bladder tissue. Pathological pattern, histological grade, clinical stage, infiltration, and lymph node metastasis are related to the positive expression rates of *Ki-67* and *PFN1*, different genders, ages, tumors diameter and vascular invasion were not associated with the positive expression rates of *Ki-67* and *PFN1*. Patients with positive expression of *Ki-67* and *PFN1* experienced shorter survival times and lower three-year survival rates. The diagnosis and differentiation of bladder cancer patients can be improved by analyzing the difference between *Ki-67* and *PFN1* expression in tumor and bladder tissue.

## Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

## Author Contributions

JWL designed the research study. JWL performed the research. JWL analyzed the data. JWL drafted the manuscript. The author read and approved the final manuscript. The author has participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

This study was approved by the Medical Ethics Committee of the The Fourth Affiliated Hospital of Soochow University (2022-LS-KY027). The entire experimental procedure adhered to the principles of informed consent, with patients or their family members being provided with information about the study. The study was carried out in compliance with the Declaration of Helsinki.

## Acknowledgment

Not applicable.

## Funding

This research received no external funding.

## Conflict of Interest

The author declares no conflict of interest.

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