

# Clinical correlation between serum YKL-40 protein level and recurrence of non-muscle invasive bladder cancer

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**Background:** To explore the clinical correlation between serum YKL-40 protein level and recurrence of non-muscle invasive bladder cancer (NMIBC).

**Methods:** Totally 76 NMIBC patients (including 34 patients with confirmed recurrence and 42 patients without recurrence during the 2-year post-operative follow-up) and 31 healthy volunteers were recruited in this study. Blood samples were collected early in the morning, and serum YKL-40 protein levels of all these patients were analysed by using enzyme-linked immunosorbent assay (ELISA).

**Results:** Serum YKL-40 protein levels were significantly higher in NMIBC patients than in healthy controls ( $P < 0.001$ ). Meanwhile, serum YKL-40 protein levels were found to be significantly higher in patients with recurrent NMIBC than those without tumor recurrence ( $P = 0.001$ ).

**Conclusions:** Serum YKL-40 protein can be a reliable molecular marker for the clinical diagnosis of NMIBC recurrence. In particular, it can inform the post-operative management.

**Keywords:** YKL-40; non-muscle invasive bladder cancer (NMIBC); recurrence; serum

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## Introduction

Bladder cancer is one of the most common malignant tumors of the urinary system. In particular, the non-muscle invasive bladder cancer (NMIBC) accounts for 70% of newly diagnosed bladder tumors, among which the stage Ta accounts for about 70%, T1 20%, and Tis 10% (1). The preferred treatment for NMIBC is transurethral resection of bladder tumor (TURBT); however, it has been reported that tumor recurrence occurs in up to 50-70% of patients within 2 years after surgery (2). The high post-operative recurrence rate of NMIBC remains a challenging issue in the clinical settings.

YKL-40 (also known as human cartilage glycoprotein 39), a highly conserved glycoprotein, belongs to mammalian

chitinase-like protein family (3). YKL-40 can be secreted by macrophages, neutrophils, fetal and embryonic stem cells, and tumor cells and plays a key role in inflammation, angiogenesis, and cell proliferation, differentiation, and anti-apoptosis (4-7). Relevant research has found the overexpression of YKL-40 in many human malignancies including rectal cancer, lung cancer, ovarian cancer, and bladder cancer (8-11). It has been reported that high serum YKL-40 level is often suggestive of poor prognosis in bladder cancer patients (11). However, few studies have explored the clinical relationship between serum YKL-40 level and NMIBC recurrence. In our current study, we detected the serum YKL-40 expression profiles in patients with or without NMIBC recurrence and in healthy controls

**Table 1** Patients' characteristics and serum YKL-40 levels

Parameters	n	Median [range]	P value
Age (years)			0.246
≤65	42	95.5 [44-169]	
>65	34	87.5 [1-292]	
Gender			0.172
Males	59	92 [11-230]	
Females	17	101 [70-292]	
Smoking			0.054
Yes	35	105 [11-292]	
No	41	91 [11-230]	
Recurrence			0.001
Yes	34	105 [11-292]	
No	42	86 [11-252]	
Normal control	31	49 [13-189]	
Pathological grade			0.345
High-grade urothelial carcinoma	64	96 [11-292]	
Low-grade urothelial carcinoma	12	82 [44-144]	
Tumor sites			0.637
Solitary	29	92 [44-292]	
Multiple	47	97 [11-252]	
Tumor size (cm)			0.355
≤3.0	71	93 [11-292]	
>3.0	5	120 [88-230]	
Tumor stage			0.228
Tis	1	137	
T1	19	102 [59-292]	
Ta	56	92 [11-252]	
Tumor group	76	93.5 [11-292]	0.000

by using enzyme-linked immunosorbent assay (ELISA), with an attempt to analyse the relationship between serum YKL-40 expression and NMIBC recurrence and investigate its value in the early prediction and monitoring of NMIBC recurrence, so as to guide the post-operative management of NMIBC.

## Subjects and methods

### General data

Totally 76 NMIBC patients [59 men and 17 women, aged

45-83 years (mean: 63.3 years)] who were diagnosed, treated and followed in our center from January 2010 to December 2011 were enrolled in this study. All these patients underwent transurethral resection of bladder tumor (TURBT). NMIBC was confirmed by postoperative pathology. Intravesical chemotherapy was performed after the surgery. During the 2-year follow-up, recurrence was detected by cystoscopy and confirmed by pathology in 34 patients (the recurrence group). No tumor recurrence was detected by cystoscopy in the remaining 42 patients (the non-recurrence group). In addition, 31 healthy subjects who received health check-ups in our center during the same period were used as the normal control (NC) group. Patients with diseases (e.g., arthritis and other types of tumors) that may affect the serum YKL-40 level were ruled out from this study. All the subjects had no major underlying diseases (without disorders in vital organs including heart, liver, lung, and kidney, and with normal liver and kidney functions) before surgery. All the subjects signed the informed consent forms. This study was approved by the ethics committee of our hospital.

### Determination of serum YKL-40 level

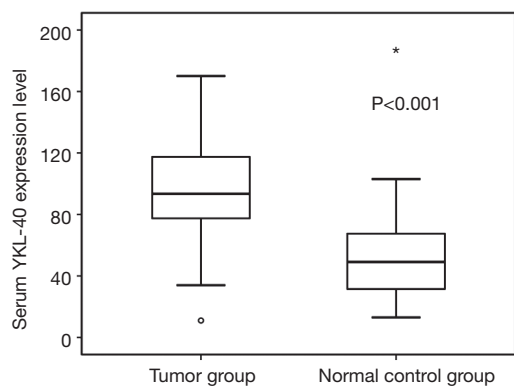
Fasting blood specimens were collected early in the morning and then centrifuged at 1,500 rpm at 4 °C for 40 min. The serum was pipetted into an Eppel tube and stored at -20 °C. Serum YKL-40 level was determined using human cartilage glycoprotein-39 ELISA kit (Quidel, USA) in strict accordance with the manufacturer's instructions.

### Statistical analysis

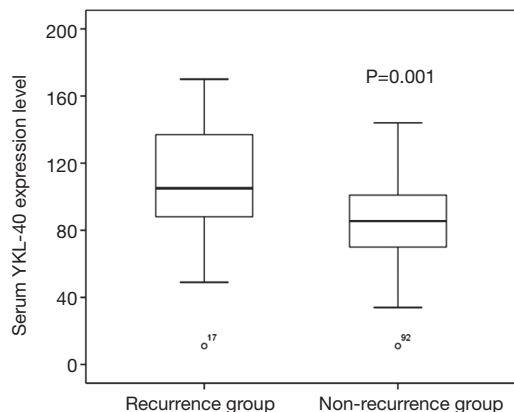
The statistical analysis was performed using the SPSS 19.0 software package. Means were compared using Mann-Whitney U test. Receiver operating characteristic (ROC) curves and the areas under the ROC curves were used for diagnosis/prediction performance analysis. A P value of <0.05 was regarded as statistically significant.

## Results

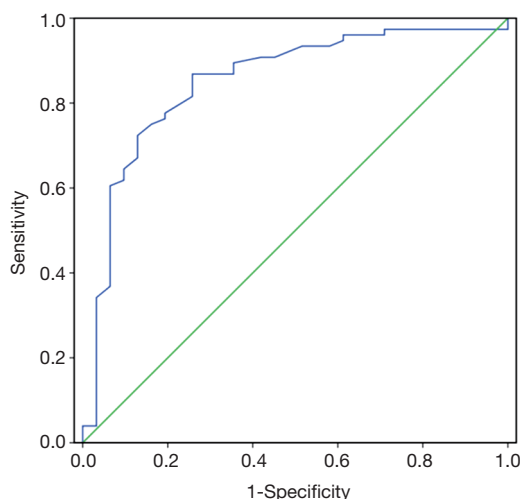
The general data of the subjects are shown in *Table 1*. The YKL-40 protein expression was significantly higher in NMIBC patients than subjects in NC group (P=0.001) (*Figure 1*); meanwhile, it was significantly higher in recurrence group than in non-recurrence group (P<0.001) (*Figure 2*). These findings were independent from tumor



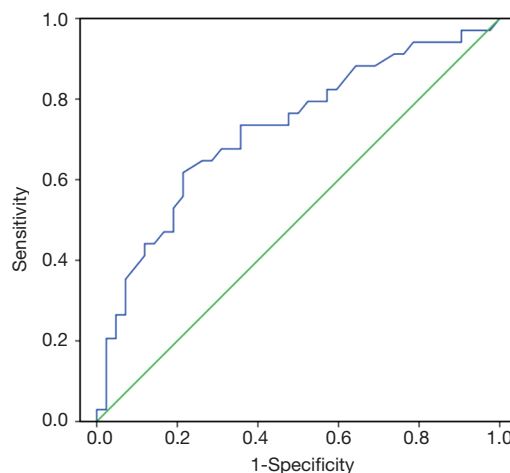
**Figure 1** Serum YKL-40 expression level of tumor patients and the controls.



**Figure 2** Serum YKL-40 expression level of recurrence group and non-recurrence group.



**Figure 3** ROC curves of serum YKL-40 level for diagnosis of NMIBC. ROC, receiver operating characteristic; NMIBC, non-muscle invasive bladder cancer.



**Figure 4** ROC curves of serum YKL-40 level for prediction of recurrence of NMIBC. ROC, receiver operating characteristic; NMIBC, non-muscle invasive bladder cancer.

pathological grade ( $P=0.345$ ), tumor sites ( $P=0.637$ ), tumor size ( $P=0.355$ ), tumor stage ( $P=0.228$ ), age ( $P=0.246$ ), gender ( $P=0.172$ ), and smoking status ( $P=0.054$ ). In addition, the serum YKL-40 level was significantly higher in NMIBC patients than in subjects in NC group ( $P < 0.001$ ). ROC curve showed that, with 63.5 as the cutoff value, serum YKL-40 level had a sensitivity and specificity of 86.8% and 74.2% in determining the occurrence of NMIBC (Figure 3). Meanwhile, the recurrence group had significantly higher serum YKL-40 level than non-recurrence group ( $P=0.001$ ). The ROC curves were fitted and the areas under the ROC curves were calculated, which showed that, with 101.5 as a cutoff value, serum YKL-40 level had a sensitivity and specificity of 61.8% and 78.6% in determining NMIBC recurrence (Figure 4).

### Discussion

Serum YKL-40 remains a potential tumor marker. Up to now many in vitro experiments have demonstrated that YKL-40 is highly expressed in osteosarcoma cell line MG63 (12), glioma TMZ-R U87 cell line (13), colon cancer SW480 cell line (14), and prostate cancer DU-145 and PC-3 cell lines (15). High expression of YKL-40 has also been detected in the culture supernatant. Research (16) has also indicated that YKL-40 may play key roles in the proliferation, differentiation, and anti-apoptosis of tumor cells as well as in angiogenesis. Knock-out of YKL-40 or its receptor can remarkably suppress angiogenesis and tumor progression (17). A recent study (11) showed that serum

YKL-40 level was closely correlated with the occurrence and progression of bladder cancer. However, few studies have explored its relationship with the postoperative recurrence of NMIBC and its clinical significance.

Our current study found that serum YKL-40 level was significantly higher in patients with NMIBC than in the NCs, which was consistent with previous findings (11). We also found serum YKL-40 had relatively high sensitivity and specificity in diagnosing NMIBC. In the future, serum YKL-40 may become a new tumor marker in the diagnosis of bladder tumor.

We further found that serum YKL-40 expression was significantly higher in patients with NMIBC recurrence than those without NMIBC recurrence, indicating that serum YKL-40 level has a high correlation with NMIBC recurrence and plays an important role in this process. Analysis of the area under ROC curve showed that, with 101.5 as a cutoff value, serum YKL-40 is valuable in predicting NMIBC recurrence. Thus, serum YKL-40 level can be a useful diagnostic indicator for the early prediction and monitoring of NMIBC and for assessing the risk of postoperative recurrence.

In summary, serum YKL-40 level is closely correlated with the occurrence and recurrence of NMIBC. High serum YKL-40 level often means higher risk of NMIBC occurrence or recurrence. Serum YKL-40 level can be a useful diagnostic indicator for the early prediction and monitoring of NMIBC and and thus guide the postoperative management. A dynamic measurement and monitoring of serum YKL-40 level during the evaluation and follow-up of an NMIBC patient can remarkably benefit the patient. However, the specific mechanism governing the correlation between YKL-40 and postoperative recurrence of NMIBC requires further investigations.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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