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Original article

A preoperative marker panel for the prediction of residual tumor and the decision making for repeat transurethral resection

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Abstract

Objective: To assess the ability of a combined preoperative marker panel to identify patients with residual non–muscle-invasive bladder cancer who might benefit from repeat transurethral resection (reTUR).

Methods: Ki67, p53, vascular endothelial growth factor-C, E-cadherin, and survivin expressions were evaluated by immunohistochemical staining of surgical specimens from 72 patients who underwent reTUR. Related clinical and molecular markers were analyzed by univariate analyses to develop a marker panel. The predictive value of the marker panel was calculated by receiver operating characteristic curves.

Results: Univariate analyses identified tumor size, number of tumors, p53 expression, E-cadherin expression, and the number of altered markers as risk factors for residual tumor ($P = 0.03$, 0.05, 0.06, 0.024, and 0.005, respectively). After adjusting for the effects of tumor stage and grade, multivariate analyses identified the number of altered markers as a risk factor for residual tumor ($P = 0.004$). The addition of tumor size, E-cadherin, and the number of altered markers to the base model (based on tumor stage and tumor grade) increased its discrimination for predicting residual tumor (5%, 6%, and 10%, respectively).

Conclusion: Some clinical and molecular markers could improve the accuracy of residual tumor prediction at reTUR. Such a marker panel may help to identify patients with non–muscle-invasive bladder cancer who have residual tumor after first TUR and who may therefore benefit from reTUR.

Keywords: Marker; Bladder cancer; Residual tumor; Repeat transurethral resection

1. Introduction

Bladder cancer (BC) is the sixth most common cancer in the United States, with 72,570 new cases estimated in 2013 [1]. About 75% to 85% of patients with BC present with non–muscle-invasive BC (NMIBC) [2]. Transurethral resection (TUR) followed by adjuvant intravesical chemotherapy is the mainstay in the treatment of NMIBC [3]. However, more than half of the patients with NMIBC experience recurrence within 5 years, especially those with higher tumor stage (T1) [4]. Residual tumor following TUR has been considered to be partly responsible for this recurrence [5,6], and European Association of Urology (EAU) guidelines [7] accordingly recommend repeat TUR (reTUR) to eradicate residual tumor and to exclude understaging.

reTUR, performed after complete first TUR, has been shown to decrease the recurrence and progression rates significantly in patients with newly diagnosed NMIBC [8]. However, reTUR imposes additional economic and emotional burdens on patients. Furthermore, around one-third of patients with T1 tumors never experience a recurrence [9]. Because residual tumors are found in less than half the patients with NMIBC who undergo reTUR [8], most patients thus undergo a second anesthesia and surgery unnecessarily. Besides pathologic staging and grading, there are some clinical markers and molecular markers that are related to specific and variable clinical behaviors of bladder tumors and a predisposition to a higher tumor stage. A preoperative system able to discriminate between patients with residual tumors who might benefit from reTUR and

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those without residual tumors who would be unlikely to benefit would thus be very valuable.

In the present prospective study, we aimed to evaluate the prognostic values of several clinical and molecular markers for the prediction of residual tumor at reTUR and to assess if a combined preoperative marker panel could identify patients with NMIBC who would be likely to benefit from reTUR.

2. Patients and methods

2.1. Patients

This study was approved by the ethics committee of Central South University. Informed consent was obtained from all the patients. Patients undergoing reTUR at our institution between January 2009 and May 2014 were retrospectively selected for analysis from the prospectively collected database. The following criteria were followed for inclusion of patients: (1) those who met the reTUR indications according to 2013 EAU guidelines [7] and (2) those who underwent both initial TUR and reTUR at our institution; additionally, patients who lacked muscle tissue in the initial TUR specimen were excluded. Further, reTUR was done for all included patients 2 to 6 weeks later after initial TUR. reTUR involves the resection of all visible tumors and areas with a scar or edema caused by the previous resection [10]. The presence of residual tumor was confirmed by histologic review of the reTUR specimens. After the initial TUR, each patient immediately underwent 6 months of postoperative pirarubicin intravesical chemotherapy. The study included 76 consecutive patients who underwent reTUR at our center; none had visible tumor at the end of the initial TUR. Of the 76, 4 patients were excluded because of a lack of muscle tissue in the initial TUR specimen, leaving 72 patients for the analyses.

Tumor stage, tumor grade, tumor size, and the number of tumors in initial TUR were confirmed by experienced pathologists and urologists. Pathologic staging was determined according to the 2002 TNM classification, and pathologic grading was determined according to the 1973 World Health Organization classification (classified as G1, G2 and G3).

2.2. Immunohistochemistry and scoring

Initial TUR specimens were collected prospectively and embedded in paraffin for subsequent use. Expression levels of 5 biomarkers (Ki67, p53, vascular endothelial growth factor [VEGF]-C, E-cadherin, and survivin) were analyzed by immunohistochemistry, as described elsewhere [11–15]. All the markers were classified as either normal or altered by the same author (W.L.), who was blinded to the clinical outcomes. The Ki67 labeling index was considered to be altered when samples demonstrated 20% or greater reactivity [12]. Nuclear p53 immunoreactivity was considered altered when samples had $\geq 10\%$ nuclear reactivity [14]. Positive staining for VEGF-C in $\geq 25\%$ of tumor cells was considered as altered expression [11]. E-cadherin immunoreactivity was considered altered when samples had $< 90\%$ staining [13]. Survivin was considered altered when samples had $> 10\%$ staining [15].

2.3. Statistical analyses

We used 2-tailed χ^2 tests to determine the significance of differences between proportions. The Mann-Whitney U test or the Wilcoxon signed-rank test was used to compare continuous variables. The clinical and molecular markers likely to be associated with residual tumor ($P < 0.1$) were selected to develop a preoperative marker panel. The predictive significance of certain markers was assessed by univariate and multivariate logistic regression analyses. The candidate variables ($P < 0.1$ in the univariate model) were included in the multivariate model for further analysis. The value of each model for predicting residual tumor was evaluated by calculating the area under the receiver operating characteristic curve. $P < 0.05$ was considered to indicate a significant difference. Statistical analysis was performed using SPSS for Windows v.13.0 and MedCalc statistical software 11.5.0.

3. Results

3.1. Baseline characteristics of patients

Table 1 lists the baseline characteristics of the 72 included patients. There were 64 men and 18 women. The pathologic stage and grade distributions at the initial TUR were as follows: 17 patients with pTa (23.6%, all those with pTa stage had G3 grade owing to the inclusion criteria) and 55 with pT1 (76.4%), and 41 with G1/G2 (56.9%) and 31 with G3 (43.1%), respectively. Concomitant carcinoma in situ (CIS) was seen in 4 patients. Further, 46 patients presented with small lesions (diameter < 3 cm) and 26 larger tumors (diameter ≥ 3 cm), and 36 patients had single lesions, whereas the remaining 36 presented had multiple tumors. Representative immunohistochemical results for the 5 biomarkers are presented in the Fig. Ki67, P53, VEGF-C, E-cadherin and survivin expression were altered in 24 (33.3%), 27 (37.5%), 32 (44.4%), 22 (30.5%), and 46 (63.9%) patients, respectively. Overall, 32 (44.4%) patients had residual tumors at reTUR and 3 (4.1%) patients were upstaged at reTUR (all 3 were T1–T2). As shown in Table 1, patients who presented with multiple tumors or larger tumors might be more likely to have residual tumors ($P = 0.06$ and 0.03, respectively). Patients with altered p53 or E-cadherin status might also be more likely to have residual tumors ($P = 0.08$ and 0.03, respectively). These 4 markers (tumor size, number of tumors, p53, and E-cadherin) were therefore selected to develop the