

## The Role of Squamous Differentiation in Patients with Transitional Cell Carcinoma of the Bladder Treated with Radical Cystectomy

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### ABSTRACT

*Objective:* We aim at determining the prognostic value of squamous differentiation in patients with transitional cell carcinoma (TCC) of the bladder that were treated with radical cystectomy.

*Materials and Methods:* From January 1993 to January 2005, we retrospectively selected 113 patients. Correlations among squamous differentiation with other clinical and pathological features were assessed by both chi-square and Fisher tests. The Kaplan-Meier method was used to evaluate survival curves and statistical significance was determined by the log-rank test. Multivariate analysis was performed through a Cox proportional hazards regression model.

*Results:* Squamous differentiation was observed in 25 (22.1%) of the 113 patients. This finding was significantly related only to the pathological stage. Mean follow-up after cystectomy was  $31.7 \pm 28.5$  months. Disease recurrence occurred in 16 (64%) and 30 (34%) patients with and without squamous differentiation (log-rank test,  $p = 0.001$ ), and mortality occurred in 10 (40%) and 14 (16%) of the patients with and without squamous differentiation respectively. Univariate analysis revealed that pathological stage, squamous differentiation, tumor size and lymph node involvement were significant predictors of cancer-specific survival. However, only squamous differentiation and tumor size were independent prognostic variables on multivariate analysis.

*Conclusions:* Squamous differentiation was an independent prognostic factor for cancer specific survival in patients with bladder cancer treated with radical cystectomy. Further studies with a larger number of patients are necessary to confirm these results.

*Key words:* carcinoma, transitional cell; squamous cell neoplasms; cystectomy; prognosis

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### INTRODUCTION

Bladder cancer is the fourth leading cause of cancer in American men. It is estimated that 61,420 new cases will be diagnosed in 2006 and approximately

20% to 30% of these patients will present muscle infiltrative tumors (1). The high mortality rates of 50% in 5 years (2) even in patients who undergo aggressive treatment with radical cystectomy has motivated the finding of new clinical or pathologic prognostic factors

that could help selecting patients for adjuvant treatment.

Although pure squamous cell carcinoma of the bladder is found in only 5% of all bladder cancers (3,4), the incidence of squamous differentiation in transitional cell carcinoma (TCC) of the bladder is higher and ranges from 11% to 60% of the cases (5,6). The prognostic value of squamous differentiation in patients with bladder transitional cell carcinoma is controversial. While some authors have shown relation of this finding to adverse pathologic features (7), to a higher resistance to radiotherapy (8), or to a disease-free period shorter than pure TCC (4,9), most studies could not show the independent statistical value of this variable on multivariate analysis (5,6,10).

In the present study, we sought to determine the prognostic relevance of squamous differentiation in patients with TCC of the bladder treated with radical cystectomy.

## MATERIALS AND METHODS

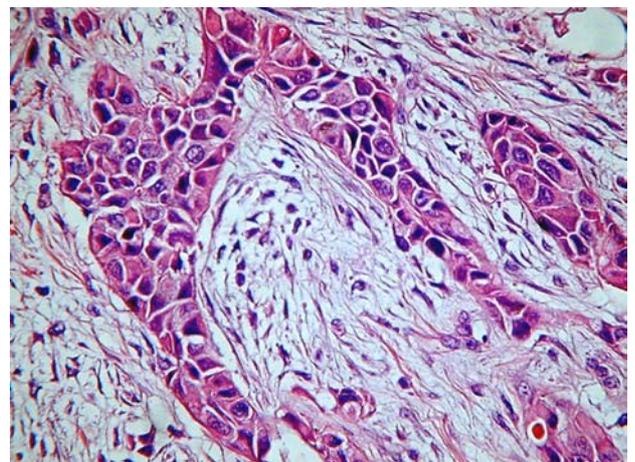
The study comprised a retrospective analysis of 113 patients who underwent radical cystectomy and urinary diversion for bladder cancer during the period of January 1993 to January 2005. All patients who had been treated with neoadjuvant chemotherapy, who had incompletely documented procedures, who had presented other malignant bladder tumors, and who had no available follow-up were excluded from the study. This represented 37 cases. Among the excluded cases, there were 3 squamous cell carcinomas and 3 adenocarcinomas of the bladder. All the others presented TCC of the bladder and were included in the study.

Preoperative diagnosis was made by transurethral resection. All patients underwent physical examination, chest radiography and computed tomography of the abdomen and pelvis. The majority of patients underwent surgery for muscle infiltrative tumors (T2). Other indications included superficial bladder cancer refractory to intravesical therapy and multifocal stage T1, grade 3, disease. Fifteen patients with extravesical disease (T3b or T4) and / or positive lymph nodes were submitted to adjuvant

chemotherapy. The methotrexate, vinblastine, doxorubicin and cisplatin protocol was used for at least three cycles.

The variables evaluated were patient age, gender, pathological stage, tumor grade, tumor size, presence of carcinoma in situ (CIS), lymph node involvement and the presence of squamous differentiation. Staging and grading were performed according to the TNM classification and to the World Health Organization System respectively (11,12). Tumor size was considered as the greater diameter on macroscopic analysis of the surgical specimen. For analysis of pathological stage patients were grouped as follows: T0, T1 (T1 + Tis), T2 (T2a + T2b), T3 (T3a + T3b) and T4. A tumor component was considered to be squamous when intercellular bridges and/or keratinization were evident (Figure-1). Follow-up consisted of a visit two months after surgery, then another visit every 4 months for 1 year. After this period they were seen every 6 months until disease progression or death.

Main postoperative end-points were recurrence-free survival and cancer-specific survival. The recurrence-free survival period was estimated from the time of surgery to the date of the first documented clinical recurrence or until the last follow-up if the disease had not recurred. Bladder cancer recurrences in the pelvis were considered as local



**Figure 1** – Transitional cell carcinoma of the bladder with areas of squamous differentiation (HE).

recurrences, outside the pelvis as distant metastases and in the urinary tract as urothelial recurrences. Patients who died from other causes were censored at the time of death. Correlations among squamous differentiation with other pathologic features were assessed by the Chi-square or Fisher's exact tests. The Kaplan-Meier method was used to evaluate survival curves and statistical significance was determined by the log-rank test. Statistical significance was set as a p value of 0.05 or less. Statistical analysis was performed using the SPSS 12.0 for Windows software.

## RESULTS

Patient characteristics are shown in Table-1. Mean patient age was 65.9 years (range 42 to 90 years) and most patients (85.8%) were male. Forty nine percent presented organ-confined disease (Tis, T1 and T2) and the great majority (86.9%) were high graded. Concomitant CIS was observed in 40.7% of patients and lymph nodes were involved in 15.7% of cases. Twenty nine patients had no information regarding tumor grade and 43 regarding lymph node status. These last patients were treated in the beginning of the study period when routine pelvic lymphadenectomy was not performed. The remaining 70 patients underwent systematic lymphadenectomy that consisted in the removal of lymph nodes from the obturator fossa to common iliac arteries. As many patients had several transurethral resections before cystectomy, in 20 reports tumor size could not be precisely described. Forty six (49%) presented tumors measuring less than 3 cm and 47 (51%) tumors measuring 3 cm or more. This cut-off point was based on the median size.

Squamous differentiation was observed in 25 (22.1%) of the 113 patients. This finding was significantly related only to pathologic stage (Table-2). Patients with squamous differentiation presented stage T0, T1, T2 and T3 in 0%, 16%, 20% and 52% of the cases respectively ( $p = 0.031$ ).

Mean follow up after cystectomy was  $31.7 \pm 28.5$  months (median 24.0 months). By the end of follow-up, 46 (40.7%) tumors had recurred. Twenty

four (21.2%) patients died of bladder cancer. We noted important differences in recurrence-free and cancer-specific survival rates between patients with squamous differentiation and without squamous differentiation (Figures-2 and 3). Disease recurrence occurred in 16 (64%) and in 30 (34%) patients with and without squamous differentiation respectively (Log-Rank test;  $p = 0.001$ ), and mortality occurred in 10 (40%) and 14 (16%) of patients with and without squamous differentiation respectively (Log-Rank test;  $p = 0.002$ ). On univariate Cox regression analysis, an advanced pathologic stage, lymph node involvement, higher tumor size and the presence of squamous differentiation were significantly associated to cancer-specific survival (Table-3). The Hazards ratios for tumor stage was not determined since no patient with T0 disease died

**Table 1 – Patients characteristics.**

	N (%)
Age	
Mean $\pm$ SD / median	65.9 $\pm$ 9.7 / 67.0
Range	42 to 90
Gender	
Men	97 (85.8)
Women	16 (14.2)
Tumor stage	
T0	13 (11.5)
Tis	9 (8.0)
T1	18 (15.7)
T2a	18 (15.7)
T2b	11 (9.7)
T3a	12 (10.6)
T3b	23 (20.4)
T4	9 (8.0)
Grade	
Low	11 (13.1)
High	73 (86.9)
Carcinoma in situ	
Yes	46 (40.7)
No	67 (59.3)
Tumor size (cm)	
Mean $\pm$ SD / median	3.2 $\pm$ 2.2 / 3.0
Range	0.8 to 10.0
Lymph node involvement	
Yes	11 (15.7%)
No	59 (84.3%)

## Squamous Differentiation in Bladder Cancer

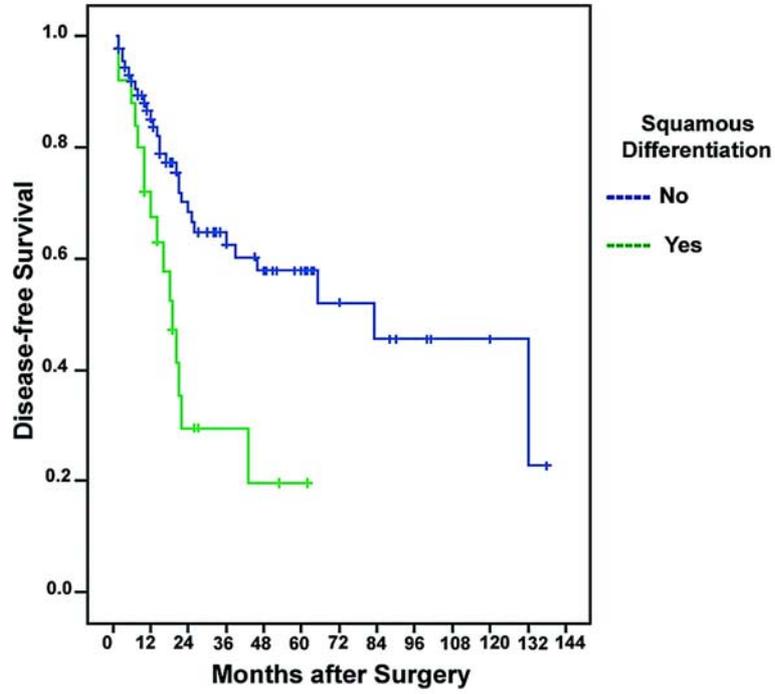


Figure 2 – Disease-free survival among patients with and without squamous differentiation (log-rank test;  $p = 0.001$ ).

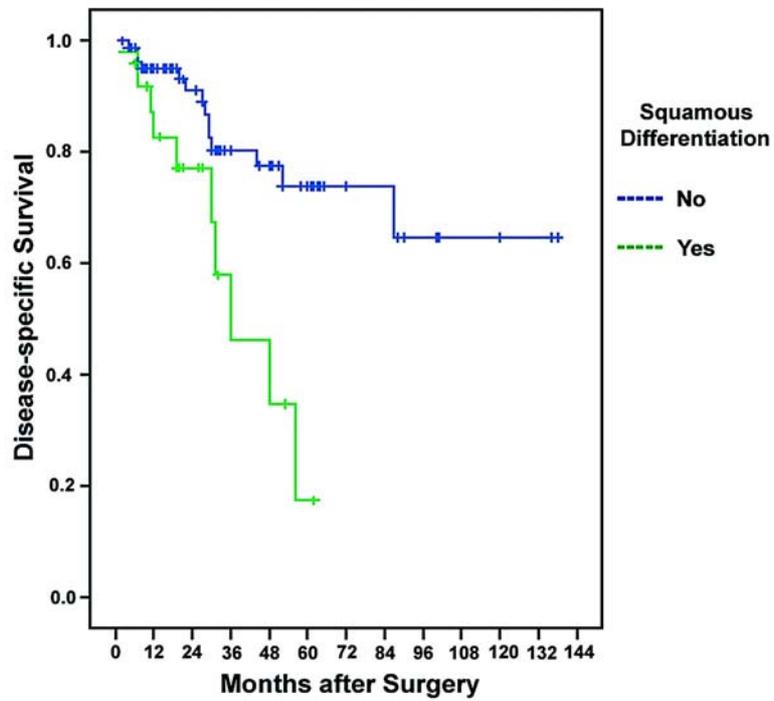


Figure 3 – Cancer-specific survival among patients with and without squamous differentiation (log-rank test;  $p = 0.001$ ).

during the follow-up period. However, on multivariate analysis, only tumor size and the presence of squamous differentiation remained as independent significant variables (Table-4).

### COMMENTS

In the present study we found a 22.1% rate of squamous differentiation in patients with TTC of the bladder. This feature was significantly related to the pathological stage. Among patients with squamous differentiation 16%, 20% and 52% of the patients had pT1, pT2 and pT3 stage respectively. The fact that only 12% of patients had pT4 disease can be explained by the small number of patients with this stage. Disease recurrence occurred in 64% and in 34% patients with and without squamous differentiation respectively (Log-Rank test;  $p = 0.001$ ), and mortality occurred in 40% and 14 16% of patients with and without squamous differentiation respectively (Log-Rank test;  $p = 0.002$ ). The presence of squamous differentiation was a significant prognostic variable for cancer-specific survival in either uni or multivariate analysis.

Billis et al. (7), reviewed specimens of bladder TCC transurethral resections (TUR) from 165 patients and found 12 (7.27%) cases with squamous and/or glandular differentiation. As in the present series, they showed that tumors with squamous and/or glandular differentiation had a significant statistical correlation with higher stage at clinical presentation, suggesting that tumors with this features are more aggressive neoplasms. Martin et al. (8), also analyzed TUR specimens from 100 patients with bladder tumor and reported that while 78% of patients with squamous metaplasia failed to respond to radiotherapy while a 90% response rate was seen among patients without this finding. The authors concluded that alternative methods should be sought to the former group since they may have radiotherapy resistant tumors. Vecchioli et al. (9), reported that the presence of extensive areas of squamous metaplasia (metaplastic areas = or greater than 50% of neoplastic surface) was always found in association with high grade neoplasms and with a disease-free period shorter than pure TCC.

**Table 2** – Distribution of variables according to the presence of squamous differentiation.

	Squamous Differentiation		p Value
	No	Yes	
Age			0.939 <sup>+</sup>
< 67 years	43 (48.9%)	12 (48.0%)	
≥ 67 years	45 (51.1%)	13 (52.0%)	
Gender			0.751 <sup>++</sup>
Female	12 (13.6%)	4 (16.0%)	
Male	76 (86.4%)	21 (84.0%)	
Grade			0.277 <sup>++</sup>
Low	10 (15.9%)	1 (4.8%)	
High	53 (84.1%)	20 (95.2%)	
Tumor size			0.959 <sup>+</sup>
< 3.0 cm	37 (49.3%)	9 (50.0%)	
≥ 3.0 cm	38 (50.7%)	9 (50.0%)	
Ca in situ			0.400 <sup>+</sup>
No	54 (61.4%)	13 (52.0%)	
Yes	34 (38.6%)	12 (48.0%)	
Lymph node involvement			0.135 <sup>++</sup>
No	46 (88.5%)	13 (72.2%)	
Yes	6 (11.5%)	5 (27.8%)	
Stage			0.031 <sup>++</sup>
T0	13 (14.8%)	-	
T1	23 (26.1%)	4 (16.0%)	
T2	24 (27.3%)	5 (20.0%)	
T3	22 (25.0%)	13 (52.0%)	
T4	6 (6.8%)	3 (12.0%)	

<sup>+</sup> = Pearson's qui-squared test, <sup>++</sup> = Fisher's exact test

Conversely, other authors could not demonstrate the independent prognostic significance of squamous differentiation. Mazzucchelli et al. (5), found in a prospective analysis of 70 patients treated according to a program of radical cystectomy alone or followed by adjuvant chemotherapy, that the presence of squamous differentiation along with the depth of invasion and the absence of CIS were the only variables related to a poor survival outcome. However, on multivariate analysis, only the last two variables were independent prognostic factors. Likewise, Frazier et al. (10), in a study of 531 patients

**Table 3** – Univariate Cox regression analysis for cancer-specific survival.

	<b>Hazards Ratio</b>	<b>95% CI</b>	<b>p Value</b>
Age	1.42	[0.62 - 3.25]	0.405
≥ 67 years / < 67 years			
Gender	2.41	[0.57 - 10.25]	0.235
Male / Female			
Grade	3.02	[0.40 - 22.77]	0.283
High / Low			
Carcinoma in situ	1.33	[0.59 - 2.99]	0.486
Yes / No			
Tumor size	4.63	[1.50 - 14.30]	0.008
≥ 3 cm / < 3 cm			
Squamous differentiation	3.51	[1.53 - 8.08]	0.003
Yes / No			
Lymph node involvement	3.17	[1.19 - 8.46]	0.022
Yes / No			
Stage			0.030
T1 / T0			
T2 / T0			
T3 / T0			
T4 / T0			
		Not possible to calculate	

treated with radical cystectomy and followed for a long period at Duke University Medical Center, found that squamous differentiation in the specimen was a predictive factor for poor cancer-specific survival only in univariate analysis. In multivariate analysis only pathological stage, nodal involvement, positive surgical margins, patient age and loss of histologic differentiation achieved independent statistical significance.

In the present series, the finding of squamous differentiation in TCC of the bladder was an independent prognostic factor for disease-specific survival. This finding was associated to a 5.2 times higher risk of dying from disease when compared to patients without squamous differentiation. However, these results must be analyzed carefully. First, the study comprised a retrospective analysis of a relative small number of patients. Second, the lack of information regarding important variables such as lymph node status and tumor grade in 43 and 29 patients respectively may have influenced the

results of final multivariate Cox regression analysis. Third, tumor size, which was an independent prognostic factor along with squamous differentiation, may not be a reliable variable since TUR prior to cystectomy may have precluded an adequate measurement of the tumor. Furthermore, the independent prognostic significance of pathologic stage, which is considered one of the most powerful variables of poor outcome (6), could not be

**Table 4** – Multivariate Cox regression analysis for cancer-specific survival.

	<b>Hazards Ratio</b>	<b>95% CI</b>	<b>p Value</b>
Tumor size	5.49	[1.74 - 17.31]	0.004
≥ 3 cm / < 3 cm			
Squamous differentiation	5.21	[1.87 - 14.54]	0.002
Yes / No			

reproduced in the present series. We believe that two factors may have contributed to this. First, the small number of patients included in the analysis, and second the lack of information regarding lymph node status in some patients, since most tumors with advanced stages have also positive lymph nodes.

Finally, the present results suggest that the presence of a squamous component in patients with bladder TCC may be associated with an ominous outcome. Further studies with larger series and multivariate analysis are necessary to confirm these results.

## CONFLICT OF INTEREST

None declared.

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## EDITORIAL COMMENT

Divergent differentiation is a very peculiar capacity of urothelial tumors (1). Squamous differentiation, defined by the presence of intercellular bridges or keratinization, occurs in up to 20% of urothelial carcinomas (2,3). Glandular differentiation is less common than squamous differentiation (4,5). Several studies have shown that squamous and/or glandular differentiation is an unfavorable feature in patients undergoing radical cystectomy.

In a study from our Institution, we found that 153/165 (92.72%) transurethral resections of the bladder showed conventional urothelial carcinoma and 12 (7.27%) showed squamous and/or glandular differentiation (6). The striking finding was the stage distribution of the tumors with differentiation. All 12 cases were infiltrative (pT1 or pT2) at clinical presentation and none superficial (pTa) ( $p < 0.0001$ ).

Antunes et al., in a very well conducted study, showed that on univariate analysis pathologic stage, squamous differentiation, tumor size and lymph node involvement were significant predictors of bladder cancer-specific survival. However, only squamous differentiation and tumor size were independent prognostic variables on multivariate analysis. The findings emphasize the prognostic importance of squamous differentiation which must be described and detailed in the pathology report.

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