ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Histopathological predictive factors for the overall survival rate in patients with urothelial carcinoma of the bladder treated by radical cystectomy: a Romanian cohort study

IOAN ALIN NECHIFOR-BOILĂ[1,2)], BOGDAN CĂLIN CHIBELEAN[1)], ANDRADA LOGHIN[3)], ADELA CORINA NECHIFOR-BOILĂ[3)], TOADER SEPTIMIU VOIDĂZAN[4)], MARIA FLAVIA RĂDULESCU[5)], ANGELA BORDA[3)]

[1)]*Department of Urology, "George Emil Palade" University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania*

[2)]*Department of Anatomy and Embryology, "George Emil Palade" University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania*

[3)]*Department of Histology, "George Emil Palade" University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania*

[4)]*Department of Epidemiology, "George Emil Palade" University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania*

[5)]*V[th] Year Student, Faculty of Medicine, "George Emil Palade" University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania*

Abstract

Introduction: Urothelial carcinoma (UC) variants are considered as having a more aggressive behavior and a more advanced stage at presentation than conventional UC. However, the evidence supporting the role of UC variants on overall survival (OS) is conflicting. We aimed to assess the impact of demographic factors (age at surgery, gender) and tumor characteristics [conventional/variant UC, associated carcinoma *in situ* (CIS), associated papillary component, Tumor, Node, Metastasis (TNM) staging, positive surgical margins] on OS in a series of patients treated for UC in our Department. *Patients, Materials and Methods*: We performed a retrospective, cohort study and included 69 UC patients treated by radical cystectomy (RC) in our Department over an eight-year period, with complete follow-up information. Associations of UC variants as well as demographic and morphological factors with OS were assessed using univariable and multivariable Cox analysis. *Results*: Our data showed that UC variants were statistically significantly associated with the presence of distant metastases (p=0.036) and positive surgical margins (p=0.009), but had no influence on OS (p=0.504). Further on, we demonstrated that age at surgery (p=0.045), tumor stage (p=0.012), lymph node involvement (p=0.009), and presence of positive surgical margins (p=0.002) had a statistically significant influence on OS both by univariable and multivariable Cox analysis. *Conclusions*: Age, tumor stage and lymph node involvement, as well as positive surgical margins represent prognostic factors in RC patients. UC variants were more likely to be associated to metastases and positive surgical margins but had no influence on OS.

Keywords: urothelial carcinoma, demographic factors, radical cystectomy, prognostic, TNM staging.

Introduction

Urothelial carcinoma (UC) is the most common type of bladder cancer with an age-standardized incidence rate of 9.6/100 000 inhabitants in the European Union, in 2018, which is estimated to increase by almost 34.3% by 2040 [1, 2]. It is an aggressive tumor with a well-known propensity for divergent differentiation into UC variants [3, 4]. Reported in variable proportions (10–53%), UC variants can be of several types, starting with the more frequent squamous and glandular and continuing with the rare micropapillary, nested, lymphoepithelioma-like, plasmacytoid and sarcomatoid variants [3, 5–9].

The clinical impact of UC variants is yet controversial. UC variants have been associated in some studies with adverse pathological features, such as higher tumor stages at initial presentation and increased lymphovascular invasion rates. These findings have led to considering UC variants as possible negative prognostic factors for the patient's survival rate. However, more recent studies have shown that UC variants cases had a similar prognosis with conventional UCs, when adjusting for tumor stage and grade [5, 9–11]. Further on, other studies have demonstrated that the presence of certain UC variants (*e.g.*, micropapillary, plasmacytoid) could also predict a poorer response to neoadjuvant chemotherapy when compared to the small cell variant (the latter having a poorer prognosis from the beginning, but an excellent response to chemotherapy) [12–14].

The 2016 update of the *World Health Organization (WHO) Classification of Tumors of the Urinary System* emphasized the importance of reporting UC variants together with their percent proportion, while admitting the controversial influence of UC variants on the patients' outcome [15].

Aim

In the present study, we aimed to assess the impact of demographic factors (age at surgery, gender) and morphological tumor characteristics [conventional UC

versus UC variants, associated carcinoma *in situ* (CIS), associated papillary component, Tumor, Node, Metastasis (TNM) staging, positive surgical margins] on the overall survival (OS) rates in a series of patients treated by radical cystectomy (RC) for UC in our Department.

Patients, Materials and Methods

Case selection

All patients with UC that underwent RC in the Department of Urology, Emergency County Hospital of Târgu Mureş, Romania, between November 2011 and October 2018, have been re-evaluated for inclusion in the study. Ethical approval was obtained from the Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureş (Approval Letter No. 195/24.10.2018). Patients with RC performed for other pathology than UC (*e.g.*, other types of cancer, tuberculosis), with ureteral or renal UC (upper urothelial tract carcinomas), with positive ureteral resection margins, as well as all UC cases lost to follow-up were excluded.

Demographic data (age at surgery and gender), pre-operative diagnosis and tumor staging, as well as details on the surgical procedure (type of urinary diversion, concomitant nephrectomy, etc.), were all retrieved from the patients' files. The standard surgical procedure consisted in RC with lymphadenectomy. However, in some palliative situations (*e.g.*, cystectomy for massive hematuria) lymph-adenectomy was not performed (n=24, 34.3%).

Pathological data

Pathological data were retrieved from original histopathological (HP) reports. The following data were collected: tumor histological type (conventional UC *versus* histological variants of UC), growth pattern (infiltrative, papillary or both), associated CIS, tumor extension (T stage), lymph node involvement (N stage), presence of distant metastases (M stage), presence of positive surgical resection margins and associated pathology (concomitant prostate cancer).

The original HP interpretation had been performed according to both the 2004 (before 2016) and the 2016 (from 2016 onward) versions of *WHO Classification of Tumours of the Urinary System and Male Genital Organs* [3, 16].

In order to avoid misclassification, after inclusion, all slides from the selected cases were reassessed by two experienced uropathologists (AB and AL) and reclassified according to the *2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs* in non-invasive papillary UC and/or infiltrating UC [3].

Follow-up data

Only patients with complete follow-up data were included in the study. The follow-up was defined as the period between the initial surgical treatment and the last clinical evaluation and covered the period between January 2011 and December 2018. Follow-up data were collected from the *Romanian National Insurance System* database, as well as from the databases of the Department of Urology and the Department of Oncology, Emergency County Hospital of Târgu Mureş.

All patients had follow-up visits scheduled according to the *Guidelines* issued by the Romanian Ministry of Health, in accordance to the *2019 EAU Guidelines on muscle-invasive and metastatic bladder cancer* [5, 17].

Thus, all patients were invited for follow-up visits at one month after surgery, then every three months for the first two years, every six months in the third year, then yearly from the fourth year. In the case of patients with cutaneous ureterostomy, regular visits were performed every two or three months for the replacement of the ureterostomy catheters.

However, some patients were lost from view or unwilling to consent to the follow-up schedule. The follow-up information for these patients was obtained by direct inquiry to them or their contact person accordingly.

Statistical analyses

Descriptive statistics were performed on categorical values based on frequencies and proportions. Means, medians and standard deviations were calculated for continuous variables. The Mann–Whitney test was used to assess the statistical significance of differences in means of two independent groups. For the comparison of three and more independent groups, we used the Kruskal–Wallis test. Correlations between the different variables were analyzed using Pearson's (for parametric variables) or Spearman's tests (for non-parametric variables), *e.g.*, between the OS period and the type of UC. Survival analysis was performed both with the Kaplan–Meier and Cox regression methods. The Kaplan–Meier method was used to assess OS in the study group in relation to the presence of the different variants of UC. Univariate and multivariate Cox regression was used to analyze the effect of a series of factors like variant histology on OS after adjusting for all available confounders. Statistical significance was considered at p<0.05. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) Statistics 23.0 (SPSS, IBM Corp, Armonk, NY, USA).

Results

Demographic and HP data

Eighty-three RCs were performed in our Department over the study period. UC was found in 72 (86.7%) cases. The majority (n=69, 95.8%) had complete follow-up data and were further included in the study. Three cases were lost to follow-up and 11 cases were excluded because of the followings: four (4.8%) were non-UCs, six (7.2%) cases had no residual tumor tissue after transurethral resection (TUR) on the RC specimen (pT0) and in one (1.2%) case the cystectomy was performed for a non-tumor cause (sequelae after tuberculous cystitis).

Table 1 summarizes the demographic and pathological data for the study cases.

Among the 69 patients with UC included in the study, the majority were men (n=57, 82.6%) and only 17.4% (n=12) were women. The median age of the patients was 64 years old.

Non-invasive papillary UC category (n=3/69 cases, all three conventional UCs) included cases with a papillary urothelial neoplastic proliferation showing different grades of cytological and architectural disorder, and no invasion beyond the basement membrane. One case (n=1/69) in our study revealed no tumor tissue on the RC specimen, instead it had loco-regional lymph node involvement (micropapillary pattern).

Table 1 – ***Demographic and pathological data for the study cases***

Characteristic	Median value (minimum, maximum) or *N* (%)
Age [years]	64 (40, 77)
Gender	
Males	57 (82.6%)
Females	12 (17.4%)
Hospitalization period [days]	17 (6.37)
UC pattern	
Non-invasive papillary UC	3 (4.3%)
Infiltrating	65 (94.2%)
Both	26 (37.7%)
Other[a]	1 (1.5%)
UC histological subtypes	
Conventional UC	40 (58%)
Poorly-differentiated	8 (11.6%)
Squamous	5 (7.2%)
Sarcomatoid	1 (1.4%)
Micropapillary	7 (10.1%)
Plasmacytoid	3 (4.3%)
Glandular	2 (2.9%)
Other[b]	3 (4.3%)

[a]This particular case had no tumor tissue on the radical cystectomy specimen, instead had lymphatic node metastases in the loco-regional nodes (micropapillary pattern); [b]One case of syncytiotrophoblastic UC, one case of mixed glandular and squamous UC, one case of mixed micropapillary and glandular UC. UC: Urothelial carcinoma.

Infiltrating UCs (*n*=65/69 cases) were generally high grade tumors; the defining histological criterion was tumor invasion beyond the basement membrane, exhibiting a wide range of architectural patterns (variably sized nests with smooth borders, sheets, trabeculae, cords and single cells or often a mixture of patterns) (Figure 1a).

Among infiltrating UCs, more than half of the cases (*n*=37/65, 56.9%) were conventional UCs, whereas the remaining 28 (43.1%) cases contained UC variants.

UCs with divergent differentiation (squamous, glandular and trophoblastic) or specific variants (micropapillary, plasmacytoid, sarcomatoid, poorly-differentiated UCs) were defined according to *2016 WHO Classification* [3].

Squamous differentiation (*n*=5/69, 7.2%) was characterized by the presence of intracellular keratin, intercellular bridges and/or keratinization in a background of conventional UC (Figure 1b). A diagnosis of *UC with glandular differentiation* (*n*=2/69, 2.9%) was made in the presence of glands formation within the tumor, resembling those of usual colonic adenocarcinoma. *Trophoblastic differentiation* (*n*=1/69, 1.4%) was recognized in the presence of individual tumor giant cells resembling syncytiotrophoblastic giant cells.

A diagnosis of *micropapillary UC* (*n*=7/69, 10.1%) was set when the tumor displayed small tumor nests without vascular cores surrounded by lacunae, resembling vascular invasion (Figure 1c), composed of tumor cells with atypically peripherally oriented nuclei. The *plasmacytoid variant* (*n*=3/69, 4.3%) consisted entirely of large discohesive malignant cells resembling plasma cells and/or monocytes in a loose or myxoid stroma (Figure 1d).

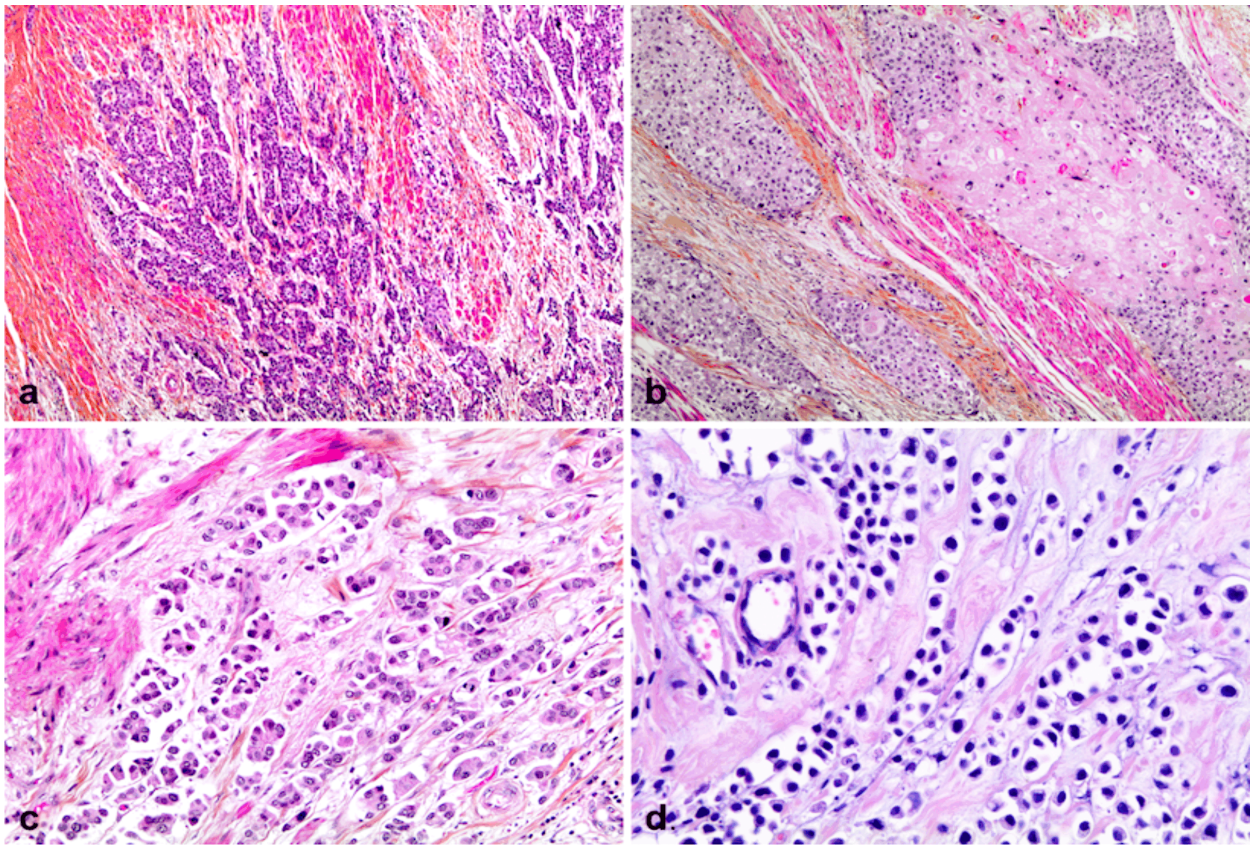

Figure 1 – ***Various pathological features for the study cases: (a) Infiltrating UC exhibiting as tumor sheets, trabeculae, cords and single cells that infiltrate the muscle; (b) UC with squamous differentiation characterized by the presence of intracellular keratin and intercellular bridges in a background of conventional UC; (c) Micropapillary UC presenting as small tumor nests without vascular cores surrounded by lacunae, resembling vascular invasion; (d) Plasmacytoid variant of UC consisting entirely of large discohesive malignant cells resembling plasma cells and/or monocytes. Hematoxylin–Eosin (HE) staining: (a and b) ×100; (c and d) ×200.***

Sarcomatoid variant (*n*=1/69, 1.4%) was defined by the presence of histological features that were morphologically indistinguishable from those of a sarcoma (high-grade spindle or pleomorphic cells, associated heterologous components).

Some cases (*n*=2/69, 2.9%) displayed more than one type of differentiation and were thus classified as *mixed UCs* [3, 18].

Poorly-differentiated UC cases (*n*=8/69, 11.6%) covered a spectrum that included tumors with mixed morphologies, such as small cell carcinoma, undifferentiated carcinoma not otherwise specified (NOS) or osteoclast-rich undifferentiated carcinoma.

Associated *urothelial CIS* was defined in the presence of a flat urothelial lesion of variable thickness, devoid of papillary structures with urothelial cells exhibiting cytological and architectural disorder. Less than half of all cases (*n*=33, 47.8%) had associated CIS.

TNM staging was performed in accordance to the *American Joint Committee on Cancer (AJCC) Cancer Staging Manual, Eighth Edition* (2017) [19]. Sixty-five (94.2%) cases were infiltrating UCs, among which 26 (37.7%) had also an associated papillary component. Three (4.3%) cases had an exclusively exophytic growth pattern (pTa). One case (1.5%) had no residual tumor tissue on the RC specimen but had lymph node metastasis of micropapillary UC (pT0N1). Concerning the staging, the majority of cases were pT3 (*n*=22 cases, 31.9%). Eighteen (26.1%) cases were pT2, 15 (21.7%) cases were pT4 and 10 (14.5%) cases were pT1. Four (5.8%) cases were pT0-pTa-pTis.

Table 2 documents the demographic and pathological characteristics of the study cases stratified according to pathological variants of UC. No statistically significant differences were found between conventional UC and variants of UC cases regarding the staging or lymph node involvement (N1, N2, N3) status (*p*=0.845 and *p*=0.142, respectively).

Table 2 – ***Association of conventional UC versus variants of UC with demographic and pathological characteristics****

Parameter	Pure UC	Poorly differentiated	Squamous	Sarcomatoid	Micropapillary	Plasmacytoid	Glandular	*p* (<0.05)
Age (median) [years]	63 (40–77)	60 (52–72)	65 (58–77)	68 N/A	60 (55–68)	63 (58–66)	65.5 (64–67)	0.667[a]
Gender								
Males	35 (50.7%)	4 (5.8%)	5 (7.2%)	1 (1.4%)	6 (8.7%)	3 (4.3%)	1 (1.4%)	0.092[b]
Females	5 (7.2%)	4 (5.8%)	0	0	1 (1.4%)	0	1 (1.4%)	
T stage								
T0-Ta-Tis	3 (4.3%)	0	0	0	1 (1.4%)	0	0	0.845[b]
T1	9 (13%)	0	0	0	0	0	1 (1.4%)	
T2	8 (11.6%)	4 (5.8%)	2 (2.9%)	0	1 (1.4%)	2 (2.9%)	0	
T3	11 (15.9%)	4 (5.8%)	1 (1.4%)	0	3 (4.3%)	0	1 (1.4%)	
T4	9 (13%)	0	2 (2.9%)	1 (1.4%)	2 (2.9%)	1 (1.4%)	0	
N stage								
Nx	15 (21.7%)	3 (4.3%)	1	0	1 (1.4%)	2 (2.9%)	0	0.142[b]
N0	20 (29%)	2 (2.9%)	2 (2.9%)	1 (1.4%)	0	1 (1.4%)	2 (2.9%)	
N1	3 (4.3%)	0	0	0	0	0	0	
N2	2 (2.9%)	2 (2.9%)	2 (2.9%)	0	5 (7.2%)	0	0	
N3	0	1 (1.4%)	0	0	1 (1.4%)	0	0	
M stage								
M0	36 (52.2%)	8 (11.6%)	3 (4.3%)	0	6 (8.7%)	1 (1.4%)	2 (2.9%)	0.036[b]
M1	4 (5.8%)	0	2 (2.9%)	1 (1.4%)	1 (1.4%)	2 (2.9%)	0	
CIS								
Absent	20 (29%)	4 (5.8%)	3 (4.3%)	1 (1.4%)	3 (4.3%)	2 (2.9%)	0	0.082[b]
Present	20 (29%)	4 (5.8%)	2 (2.9%)	0	4 (5.8%)	1 (1.4%)	2 (2.9%)	
Positive margins								
Absent	41 (59.4%)	5 (7.2%)	5 (7.2%)	2 (2.9%)	5 (7.2%)	1 (1.4%)	2 (2.9%)	0.009[b]
Present	2 (2.9%)	2 (2.9%)	0	0	0	1 (1.4%)	0	

*One case of syncytiotrophoblastic UC, one case of mixed glandular and squamous UC, one case of mixed micropapillary and glandular are not displayed in the table; [a]Kruskall–Wallis test; [b]Chi-square test; UC: Urothelial carcinoma; N/A: Not applicable; CIS: Carcinoma *in situ*.

Distant metastases (lung, liver, ovarian, brain or peritoneal) were documented in 10 (14.5%) patients (three had distant metastasis at the time of surgery and seven patients developed distant metastasis during the follow-up period). Distant metastases were more prevalent among UC variants compared to conventional UC (*p*=0.036): two (2.9%) cases of squamous UC, two (2.9%) cases of plasmacytoid UC, one case of sarcomatoid UC and one case of micropapillary UC.

Five (7.2%) cases had positive surgical resection margins; two were poorly-differentiated UC (pT2b and pT3b stage, respectively), one plasmacytoid UC (pT4a) and two conventional UC (pT4a) (Table 2) (*p*=0.009). One (1.4%) patient with poorly-differentiated UC had local tumor recurrence during the follow-up period.

Eight patients had also concomitant prostate cancer, five cases were Gleason 3+3=6 and three cases had a Gleason score of 3+4=7. All prostate cancer cases corresponded to small, incidentally discovered tumor foci and thus considered as insignificant for the patient outcome.

Follow-up data

The patients' OS ranged from one to 85.1 months, with a median survival rate of 20 months and a mean survival rate of 25.96 months, 95% confidence interval (CI) [20.29, 31.63].

The following clinico-pathological factors revealed a statistically significant correlation with the survival period: age at surgery (p=0.05), T (p=0.002) and N stage (p=0.025). By contrast, the patients' gender (p=0.506), metastasis (p=0.13), associated CIS (p=0.597) or positive surgical margins (p=0.2) did not reveal a statistically significant association with the OS rate.

A separate analysis was performed for the main variant UC types and found no statistically significant correlation to the survival period: squamous UC (p=0.629), micropapillary UC (p=0.393), poorly-differentiated UC (p=0.567), plasmacytoid (p=0.341) and sarcomatoid (p=0.183).

In patients with UC with squamous differentiation, the median survival was 28 months [95% CI: 0, 62.9], inferior to the patients without this variant: 46 months, [95% CI: 17, 74.9], although not statistically significant (p=0.95). Similarly, in poorly differentiated UC, the median survival period was also inferior when compared to the patients without this variant: 20.13 months [95% CI: 0, 43] *versus* 46 months [95% CI: 18.5, 73.4] (p=0.933). The same tendency was found in micropapillary (11 months [95% CI: 0, 31.5] *versus* 46 months [95% CI: 17.6, 74.3], p=0.3) and plasmacytoid UC patients (0.26 months [95% CI: 0, 0.676] *versus* 46 months [95% CI: 21.3, 70.6], p=0.094).

Figure 2 displays the individual survival charts for conventional UC as well as for UC variants cases.

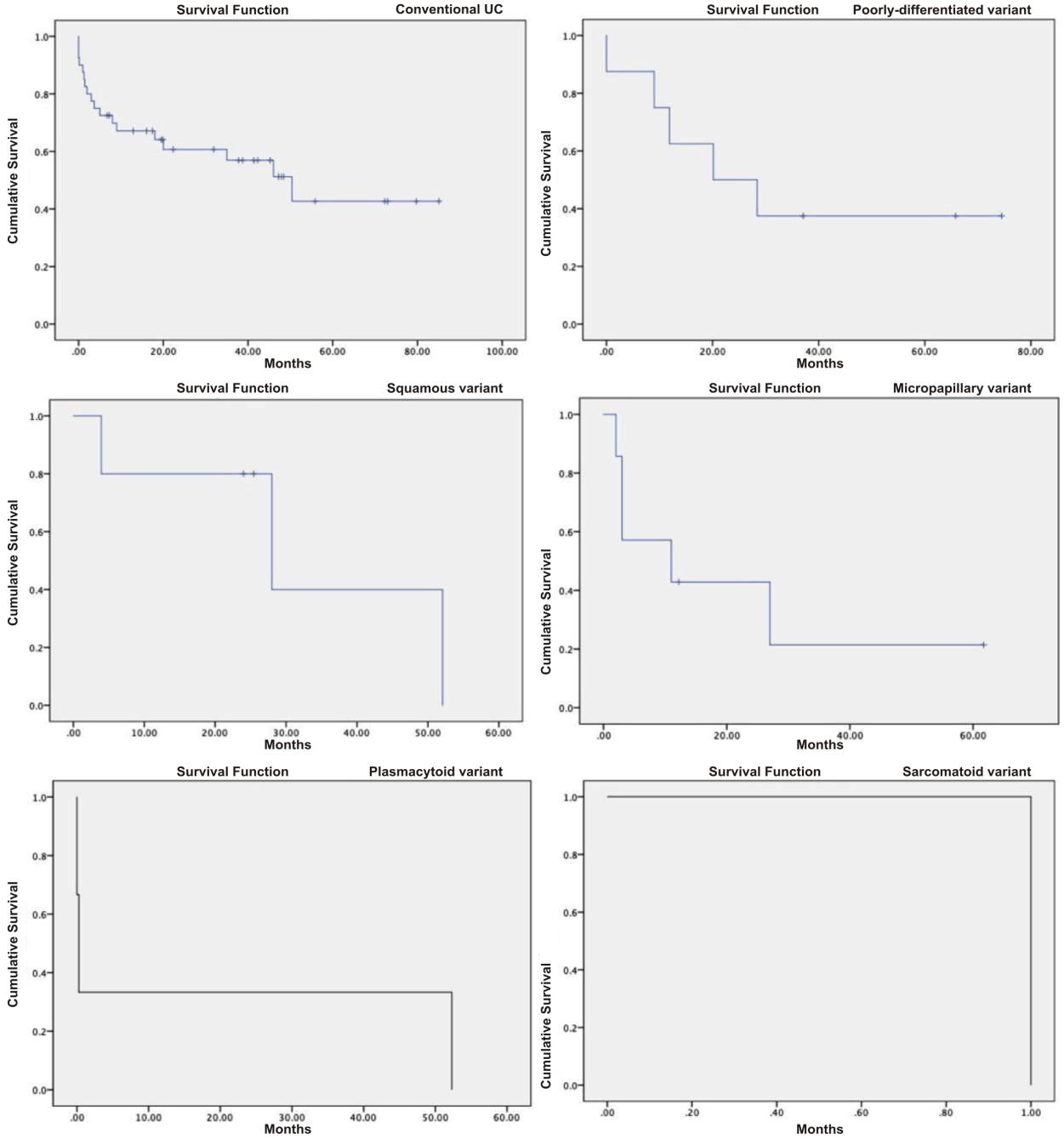

Figure 2 – ***Comparative display of individual survival curves for conventional UC, as well as the most representative UC variants in our study: poorly-differentiated, squamous, micropapillary, plasmacytoid, and sarcomatoid variants. UC: Urothelial carcinoma.***

On univariable Cox regression analysis, the following clinico-pathological factors were associated to the survival rates: age at surgery (p=0.045), tumor stage (p=0.012), lymph node involvement (p=0.009) and surgical resection margins status (p=0.002). The presence of UC variants was not found as risk factor for survival in RC patients (Table 3). These results were further confirmed by multivariable analysis (Table 4). Thus, age at surgery (p=0.032), tumor stage (p=0.022), lymph node involvement (p=0.031), as well as positive surgical margins status (p=0.009) were found to be statistically significant risk factors for the OS in patients with UC in our study group.

Discussions

The aim of the present study was to assess the impact of a series of demographic (age, gender) and pathological factors (UC variants, tumor stage, lymph node involvement, distant metastases, positive surgical margins and CIS) on the OS rates in 69 patients treated for UC by RC in our Department between November 2011 and October 2018.

Our analysis found that age, tumor stage, lymph node involvement and positive surgical margins had a statistically significant influence on OS rates, both at univariable and multivariable Cox analysis. Similarly, Cheng *et al.* investigated potential survival predicting factors in RC patients and found that age was predictive for the OS rate in univariable Cox analysis (p<0.01), but not in multivariable analysis. Instead, age was proven as a valid predictive factor for loco-regional recurrence rate [20, 21]. However, Moschini *et al.* showed that age was a predictive factor for cancer-specific mortality (p=0.009), as well as overall mortality (p<0.001) in RC patients, in their broad single-center study [8].

Tumor stage and lymph node status were further confirmed as risk factors by the study of Madersbacher *et al.* who reported an association between these factors and recurrence-free and OS. Thus, OS of patients with negative lymph nodes was identical in those with pT1 and pT2 tumors (p>0.05) and significantly different between patients with pT2 and pT3 (p<0.01, p<0.001). Further analysis found no statistically significant difference in survival for the pT3N0 and pT4 N0 groups (p=0.42) [22].

Our results are also supported by the study performed by Marks *et al.*, who determined the impact of UC variants on extra-nodal tumor extension and OS in a cohort of 517 patients and found that age (p<0.001), tumor stage (p<0.001), lymph node status (p<0.001) and positive surgical margins (p=0.02) were independent predictors for survival [11].

Data concerning the impact of certain UC variants on OS are controversial. First, the 2016 edition of the *WHO Classification of Tumors of the Urinary System and Male Genital Organs* recommends both qualitative (identification of the type of UC variant in the specimen) and quantitative (percentage of UC variant in the tumor) assessment of UC variants. Second, the impact of UC variants on OS remains unclear: on one hand, certain variants are known to harbor a more aggressive behavior (*e.g.*, micropapillary and plasmacytoid variants) but on the other hand, no clear statement is issued concerning the factors determining this particular outcome: tumor morphology itself or tumor stage at presentation [15].

Table 3 – *Univariable analysis in Cox regression examining the influence of several factors on overall survival*

Clinical factors	*p*-value	HR	95% CI for HR	
			Lower	*Upper*
Gender	0.478	1.353	0.587	3.118
Hospitalization period	0.502	0.983	0.934	1.034
Age	0.045	1.049	1.001	1.099
Presence of a variant	0.508	0.802	0.416	1.543
UC variants	0.421			
Conventional UC	Ref			
Poorly differentiated	0.097	0.174	0.022	1.376
Sarcomatoid	0.162	0.208	0.023	1.882
Squamous	0.709	1.707	0.103	28.358
Micropapillary	0.182	0.207	0.021	2.089
Plasmacytoid	0.302	0.317	0.036	2.812
Syncytiotrophoblastic	0.573	0.513	0.050	5.250
T stage	0.012			
Ta-Tis	Ref			
T1	0.100	0.178	0.023	1.389
T2	0.008	0.061	0.008	0.477
T3	0.008	0.293	0.118	0.730
T4	0.140	0.545	0.243	1.220
N stage	0.009			
N0	Ref			
N1	0.895	0.945	0.408	2.186
N2	0.118	2.817	0.768	10.324
N3	0.469	1.393	0.568	3.421
Nx	0.001	22.335	3.491	142.880
M stage	0.095	0.524	0.245	1.118
CIS	0.586	1.200	0.622	2.314
Positive surgical margins	0.002	0.206	0.077	0.554

HR: Hazard ratio; CI: Confidence interval; UC: Urothelial carcinoma; CIS: Carcinoma *in situ*; Ref: Reference.

Table 4 – *Multivariable Cox regression predicting overall survival*

Factor	*p*-value	HR	95% CI for HR	
			Lower	*Upper*
Age	0.032	1.059	1.005	1.117
T stage	0.022			
Ta-Tis	Ref			
T1	0.164	0.225	0.028	1.836
T2	0.017	0.077	0.009	0.636
T3	0.006	0.260	0.099	0.681
T4	0.111	0.474	0.189	1.187
N stage	0.031			
N0	Ref			
N1	0.398	0.629	0.214	1.845
N2	0.014	6.266	1.451	27.068
N3	0.441	1.514	0.528	4.341
Nx	0.227	2.866	0.520	15.811
Positive surgical margins	0.009	0.198	0.059	0.664

HR: Hazard ratio; CI: Confidence interval; Ref: Reference.

A new molecular classification of UCs has recently been proposed by genetic studies. The molecular signatures identify clinically distinct subtypes of UCs, which may help to better define subsets of patients who will respond and achieve higher survival rates [3]. This classification and its prognostic impact still have to be validated by larger studies.

Our data revealed that two out of five patients were diagnosed with an UC variant at RC, making UC variants relatively common in our cohort. The most frequent types were poorly differentiated followed by micropapillary and squamous variants. Similar occurrence rates for UC variants were published by Moschini *et al.* who reported a proportion of one out of three patients and a majority of squamous variant, followed by the micropapillary variant [8].

We also demonstrated that UC variants were more likely to be associated to the presence of distant metastases (p=0.036) and positive surgical margins (p=0.009), when compared to conventional UC cases. These results are in accordance to the findings of Xylinas *et al.*, who reported a larger propensity for advanced tumor stages, lymph node metastasis, lymphovascular invasion and positive surgical margins in UC variants, when compared to conventional UC patients [9].

Our analysis found no influence of UC variants on the OS rates in our series of patients. Similar results were obtained by Marks *et al.*, who found no association between presence of UC variants and survival after adjustment for standard clinico-pathological factors, including lymph node status (recurrence-free survival, cancer-specific survival and OS) [11]. Our findings are also in accordance with those of Xylinas *et al.*, who found no influence of UC variants on cancer-specific mortality on multivariable Cox analysis when adjusting for several factors like HP stage, age, gender, TNM staging [9]. On the other hand, Monn *et al.* found statistically significant influences of micropapillary and plasmacytoid UC variants (p=0.004) on OS. The corresponding hazard ratios were 2.2 [95% CI: 1.28–3.78] and 2.42 [95% CI: 1.33–4.42], respectively, thus reflecting the more aggressive status of these tumors [23]. On a larger cohort, Moschini *et al.* found that only the small cell variant of UC had a statistically significant influence on the survival (p=0.002), whereas other variants had no such effect [8].

Our study has several limitations. First, it is a retrospective, record-based study, and its results should be interpreted in this context. Second, the present study could refer only to OS for the included patients, as the necessary data for estimating cancer-specific survival and recurrence-free survival were not available for the study team. Third, some results are limited as a consequence of small samples of patients that harbor a certain variant of UC, making outcome assessment of patients with rare UC variants difficult (*e.g.*, syncytiotrophoblastic, nested).

Conclusions

To the best of our knowledge, this is the first study aimed to assess the impact of demographic factors and morphological tumor characteristics on the OS rates in patients treated by RC in a Romanian population. We found that age, tumor stage, lymph node involvement and positive surgical resection margins represent prognostic factors influencing the survival of patients with RC. The presence of a UC variant was associated to a higher TNM stage and a higher probability for positive surgical margins, although it had no statistically significant influence on the OS rate of these patients. Assessment of the outcome of patients with UC variants remains challenging also due to the small number of cases. Further multicentric prospective studies including larger numbers of cases with complete follow-up data are vital going forward.

Conflict of interests

None to declare.

Acknowledgments

This work was supported by the University of Medicine and Pharmacy of Tîrgu-Mureş Research Grant number 15609/5/29.12.2017.

References

[1] Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer, 2018, 144(8):1941–1953.

[2] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2018, 68(6):394–424.

[3] Moch H, Humphrey PA, Ulbright TM, Reuter VE. Tumours of the urinary tract. In: Moch H, Humphrey PA, Ulbright TM, Reuter VE (eds). World Health Organization (WHO) Classification of tumours of the urinary system and male genital organs. 4th edition, WHO Classification of Tumours No. 8, International Agency for Research on Cancer (IARC) Press, Lyon, France, 2016, 81–99.

[4] Stepan AE, Ciurea RN, Drăgoescu PO, Florescu MM, Stepan MD. Immunoexpression of transcription factors in urothelial bladder carcinomas. Rom J Morphol Embryol, 2017, 58(3):863–869.

[5] Witjes JA, Bruins M, Cathomas R, Compérat E, Cowan NC, Gakis G, Hernández V, Lorch A, Ribal MJ, Thalmann GN, Heijden AG, Van Der Veskimäe E. European Association of Urology (EAU) Guidelines on muscle-invasive and metastatic bladder cancer, 2019 edition. EAU Guidelines Office, Arnhem, The Netherlands, 2019, 10–11.

[6] Krasnow RE, Drumm M, Roberts HJ, Niemierko A, Wu CL, Wu S, Zhang J, Heney NM, Wszolek MF, Blute ML, Feldman AS, Lee RJ, Zietman AL, Shipley WU, Efstathiou JA. Clinical outcomes of patients with histologic variants of urothelial cancer treated with trimodality bladder-sparing therapy. Eur Urol, 2017, 72(1):54–60.

[7] Cai T, Tiscione D, Verze P, Pomara G, Racioppi M, Nesi G, Barbareschi M, Brausi M, Gacci M, Luciani LG, Liguori G, Gontero P, Campodonico F, Simonato A, Boddi V, Di Stasi SM, Colombo R, Serretta V, Carmignani G, Malossini G, Altieri V, Carini M, Terrone C, Bassi P, Montorsi F, Ficarra V, Selli C, Mirone V, Bartoletti R. Concordance and clinical significance of uncommon variants of bladder urothelial carcinoma in transurethral resection and radical cystectomy specimens. Urology, 2014, 84(5):1141–1146.

[8] Moschini M, Dell'Oglio P, Luciano' R, Gandaglia G, Soria F, Mattei A, Klatte T, Damiano R, Shariat SF, Salonia A, Montorsi F, Briganti A, Colombo R, Gallina A. Incidence and effect of variant histology on oncological outcomes in patients with bladder cancer treated with radical cystectomy. Urol Oncol, 2017, 35(6):335–341.

[9] Xylinas E, Rink M, Robinson BD, Lotan Y, Babjuk M, Brisuda A, Green DA, Kluth LA, Pycha A, Fradet Y, Faison T, Lee RK, Karakiewicz PI, Zerbib M, Scherr DS, Shariat SF. Impact of histological variants on oncological outcomes of patients with urothelial carcinoma of the bladder treated with radical cystectomy. Eur J Cancer, 2013, 49(8):1889–1897.

[10] Mitra AP, Bartsch CC, Bartsch G Jr, Miranda G, Skinner EC, Daneshmand S. Does presence of squamous and glandular differentiation in urothelial carcinoma of the bladder at cyst-

ectomy portend poor prognosis? An intensive case-control analysis. Urol Oncol Semin Orig Investig, 2014, 32(2):117–127.
[11] Marks P, Gild P, Soave A, Janisch F, Minner S, Engel O, Vetterlein MW, Shariat SF, Sauter G, Dahlem R, Fisch M, Rink M. The impact of variant histological differentiation on extranodal extension and survival in node positive bladder cancer treated with radical cystectomy. Surg Oncol, 2019, 28:208–213.
[12] Warrick JI. Clinical significance of histologic variants of bladder cancer. J Natl Compr Canc Netw, 2017, 15(10):1268–1274.
[13] Moschini M, Shariat SF, Lucianò R, D'Andrea D, Foerster B, Abufaraj M, Bandini M, Dell'Oglio P, Damiano R, Salonia A, Montorsi F, Briganti A, Colombo R, Gallina A. Pure but not mixed histologic variants are associated with poor survival at radical cystectomy in bladder cancer patients. Clin Genitourin Cancer, 2017, 15(4):e603–e607.
[14] Arrabal-Polo MA, Del Carmen Cano-García M, Fabiano P, Martínez-Pagán V, Barayobre M, García JG. Perirectal recurrence and intraperitoneal dissemination of urothelial carcinoma as plasmacytoid variant: clinical-imaging-histological findings. Int Urol Nephrol, 2015, 47(8):1361–1362.
[15] Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO Classification of tumours of the urinary system and male genital organs – Part B: Prostate and bladder tumours. Eur Urol, 2016, 70(1):106–119.
[16] Eble JN, Sauter G, Epstein JI, Sesterhenn IA (eds). Pathology and genetics of tumours of the urinary system and male genital organs. 3rd edition, WHO Classification of Tumours, IARC Press, Lyon, France, 2004, 90–110.
[17] Cseke A. Ordinul Ministerului Sănătăţii numărul 1323/2010 privind aprobarea Ghidurilor de practică medicală pentru specialitatea urologie. 794th edition, Ed. Monitorul Oficial, Bucureşti, România, 2010, 1–16 (in Romania).
[18] Loghin A, Chibelean C, Orsolya M, Nechifor-Boilă A, Nechifor-Boilă A, Borda A. Micropapillary urothelial carcinoma: an aggressive variant of urothelial carcinoma. Rom J Morphol Embryol, 2014, 55(3):939–945.
[19] Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, Compton CC, Hess KR, Sullivan DC, Jessup JM, Brierley JD, Gaspar LE, Schilsky RL, Balch CM, Winchester DP, Asare EA, Madera M, Gress DM, Meyer LR. American Joint Committee on Cancer (AJCC) Cancer Staging Manual. 8th edition, American College of Surgeons (ACS), Chicago, IL, USA, Springer International Publishing, 2017, 765–775.
[20] Cheng L, Weaver AL, Leibovich BC, Ramnani DM, Neumann RM, Scherer BG, Nehra A, Zincke H, Bostwick DG. Predicting the survival of bladder carcinoma patients treated with radical cystectomy. Cancer, 2000, 88(10):2326–2332.
[21] Sargos P, Baumann BC, Eapen L, Christodouleas J, Bahl A, Murthy V, Efstathiou J, Fonteyne V, Ballas L, Zaghloul M, Roubaud G, Orré M, Larré S. Risk factors for loco-regional recurrence after radical cystectomy of muscle-invasive bladder cancer: a systematic-review and framework for adjuvant radiotherapy. Cancer Treat Rev, 2018, 70:88–97.
[22] Madersbacher S, Hochreiter W, Burkhard F, Thalmann GN, Danuser H, Markwalder R, Studer UE. Radical cystectomy for bladder cancer today – a homogeneous series without neoadjuvant therapy. J Clin Oncol, 2003, 21(4):690–696.
[23] Monn MF, Kaimakliotis HZ, Pedrosa JA, Cary KC, Bihrle R, Cheng L, Koch MO. Contemporary bladder cancer: variant histology may be a significant driver of disease. Urol Oncol, 2015, 33(1):18.e15–18.e20.

Corresponding author
Bogdan Călin Chibelean, Lecturer, MD, PhD, Department of Urology, "George Emil Palade" University of Medicine, Pharmacy, Science, and Technology of Târgu Mureş, 38 Gheorghe Marinescu Street, 540139 Târgu Mureş, Romania; Phone +40745–326 359, e-mail: calinchibelean@yahoo.com

Received: October 7, 2019

Accepted: January 22, 2020