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The Impact of Histological Variant on Oncological Outcomes in Patients With Urothelial Carcinoma of the Bladder Treated With Radical Cystectomy

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Abstract. *Background/Aim: Bladder cancer with histological variant (HV) has different morphological features from usual urothelial carcinoma (UC). The aim of this study was to evaluate the oncological outcomes of HV in patients with bladder cancer. Patients and Methods: We retrospectively evaluated data from 102 patients with UC of the bladder treated with radical cystectomy between 1998 and 2017. Pathological findings including HV were assigned by one dedicated pathologist. Recurrence-free survival (RFS) and cancer-specific survival (CSS) and overall survival (OS) were estimated by Cox regression models. Results: In total, 26 patients (25.5%) had HV, and the most common variant was squamous differentiation, followed by glandular differentiation and a mixed variant consisted of squamous and glandular differentiation. The presence of HV was associated with RFS and CSS (p=0.018, p=0.036, respectively). Conclusion: HV has more aggressive tumor biological features compared to those with pure UC. The presence of HV was associated with poor survival.*

Urothelial carcinoma (UC) is the most common type of bladder cancer, and generally 80% of bladder cancers are represented by pure UC. On the other hand, about 20% of bladder cancers have histological variant (HV), and it has several different biological features deviated from pure UC. There are some reports showing that the presence of HV caused aggressive tumor invasion and poor oncological outcomes compared to pure UC (1, 2). World Health Organization (WHO) published the 2004 guidelines for classification of UC and chose to recognize distinct HV, and in this perspective, WHO 2016 has recently highlighted the importance of histological variant (HV) (3). There is a possibility that HV may be a predictor of clinical course and therapeutic managements.

The aim of this study was to investigate the prognostic impact of HV on oncological outcomes of patients with UC treated with radical cystectomy (RC).

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Key Words: Urothelial carcinoma, histological variant, radical cystectomy, clinical outcomes.

Patients and Methods

An electronic data search was performed from our Institutional files between January 1998 and March 2017, and we collected data of 115 consecutive patients treated with RC due to UC at our Institution. In most cases, complete transurethral resection of the bladder (TURBT) was performed before radical cystectomy.

Excluded from the data were patients with lymph node metastases or distant metastases, as well as patients with concomitant nephron-ureterectomy due to upper urinary tract carcinoma. Patients were evaluated with whole-body computed tomography (CT) scan or magnetic resonance imaging (MRI) or bone scan for detecting distant metastasis before RC, and no patient had distant metastatic disease at the time of RC.

Almost all specimens were examined by a single dedicated pathologist. Tumor stage and nodal status were assessed according to the Tumor, Node, Metastasis (TNM) staging system according to the 2010 American Joint Committee on Cancer TNM staging system. We classified HV according to the WHO classification of tumors (3), and tumor grade was assessed according to the 1998 WHO grading system (4). When neuroendocrine tumor was suspected, immunohistochemical markers panel consisting of neuroendocrine markers, chromogranin, synaptophysin, and CD56 were examined. We did not use a percentage of threshold for HV. Patients were diagnosed as HV when it was included in surgically resected bladder, regardless of the amount.

All patients were seen postoperatively at least every 3-4 months for 2 years after RC, and physical examination with laboratory testing, radiological imaging with CT scan, urine cytology were carried out. Additional radiographic evaluations (bone scans, MRI, PET-CT, *etc*.) were performed at the discretion of the treating physician when clinically indicated. After second year, same follow-up protocol was done per 4-6 months.

Disease recurrence was defined as local failure in the operative site, reginal lymph nodes, or distant metastasis. Patients who did not experience disease recurrence were censored at the time of last follow-up for recurrence-free survival (RFS) analysis. Cancer specific survival (CSS) was defined as the percentage of people who have not died from UC of bladder.

We analyzed clinical differences between pure UC and HV, and investigated whether the extent of HV affects to oncological outcomes. The extent of HV was analyzed as continuous and categorical variables. Descriptive statistics of categorical variables were focused on frequencies and proportions. The Mann-Whitney test and Chi-square test were used to compare the statistical significance of differences. RFS, CSS and overall survival (OS) probabilities were estimated using the Kaplan-Meier method and differences between groups were assessed using the log-rank statistic. Cox regression analyses tested the effect of HV on recurrence, cancer-specific mortality, and overall mortality after accounting for all available confounders. All tests were 2-sided and $p<0.05$ was set to be statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

Results

Clinicopathological characteristics of pure UC and HV. In total, 13 patients were excluded from this study, due to metastasis of another carcinoma to the bladder (n=6), and missing clinical and pathological data (n=7). Finally, we identified a total of 102 patients who received RC for UC of the bladder. Of these, 76 patients (74.5%) had pure UC, whereas 26 patients (25.5%) had HV. In the HV group, squamous differentiation was the most common variant (n=14; 13.7%), followed by glandular differentiation (n=3; 2.9%) and a mixed variant consisted of squamous and glandular differentiation (n=3; 2.9%), and small cell carcinoma (n=3; 2.9%). Among other variants, there were sarcomatoid, micropapillary, and lymphoepithelioma-like variant (all n=1; 1.0% respectively) (Table I).

Table II shows the clinicopathological characteristics of patients. Patients with HV were significantly more likely to have advanced tumor stage ($p<0.01$) and vascular invasion ($p<0.01$) compared to patients with pure UC. There was no statistically significant difference in other evaluation items. Median follow-up for the cohort was 39.5 (18.0-70.5) months in all cases.

Recurrence-free survival and cancer-specific survival of pure UC and HV. In total, 34 patients (33.3%) experienced disease recurrence during follow-up. Recurrence occurred in 27.6% (n=21/76) patients of pure UC, and 50.0% (n=13/26) patients of HV. HV has significantly worse survival compared to pure UC in RFS ($p=0.018$) (Figure 1A). Furthermore, 27 patients (26.5%) died of UC. Among them, 21.1% (16/76) patients with pure UC and 42.3% (11/26) patients with HV were counted. CSS of HV had significantly worse survival than pure UC ($p=0.036$) (Figure 1B). On the other hand, there was no statistically significant difference in OS ($p=0.385$; Figure 1C).

Table I. *Pathological characteristics of 102 patients treated with radical cystectomy.*

Histology	Number of patients (%)
Pure UC	76 (74.5)
Histological variant	26 (25.5)
Squamous differentiation	14 (13.7)
Glandular differentiation	3 (2.9)
Mixed (Squamous + Glandular)	3 (2.9)
Small cell carcinoma	3 (2.9)
Sarcomatoid variant	1 (1.0)
Micropapillary variant	1 (1.0)
Lymphoepithelioma-like variant	1 (1.0)

Cox regression analyses and survival estimates. In multivariable Cox regression analyses predicting OS, the presence of HV was not associated with any survival endpoint. Otherwise, neoadjuvant chemotherapy was represented independent predictors for OS. Other predictors of survival were removed (Table III).

Neoadjuvant chemotherapy with the extent of HV. A total of 39 patients (38.2%) had received neoadjuvant chemotherapy. There were 38.2% (n=29/76) patients with pure UC, and 38.5% (n=10/26) patients with HV. Platinum-based chemotherapy (MVAC, gemcitabine/cisplatin, gemcitabine/carboplatin) was administered, and 4 cases underwent intra-arterial chemotherapy with radiation therapy. Since we diagnosed small cell carcinoma by the specimen of transurethral resection in only one case, we applied irinotecan/cisplatin as the neoadjuvant chemotherapy. Figure 2 shows RFS and CSS of HV patients received neoadjuvant chemotherapy. Although there was no statistically significant difference, compared to patients undergoing RC only, patients who received neoadjuvant chemotherapy tended to have worse RFS and CSS than patients with HV ($p=0.17$ and $p=0.08$, respectively).

Discussion

We found that patients with HV have worse survival compared to those with pure UC. To the best of our knowledge, this is the first study analyzing the effect of HV

Table II. *Association of histologies with clinicopathological characteristics of 102 patients treated with radical cystectomy.*

	All (n=102)	Pure UC (n=76)	HV (n=26)	*p*-Value
Age (years; median)	71 (65.0-76.8)	71.5 (65.0-76.3)	67 (62.0-76.7)	0.43
Gender (%)				
Male	74 (72.5)	55 (72.4)	19 (73.1)	1
Female	28 (27.5)	21 (27.6)	7 (26.9)	
ASA class (%)[a]				
1	3 (5.2)	1 (2.5)	2 (11.1)	0.06
2	47 (81.0)	31 (77.5)	16 (88.9)	
3	8 (13.8)	8 (20.0)	0	
Pathologic T stage (%)				
pT0	5 (4.9)	5 (6.6)	0	<0.01
pTa	1 (1.0)	1 (1.3)	0	
pTis	10 (9.8)	9 (11.8)	1 (3.8)	
pT1	21 (20.6)	20 (26.3)	1 (3.8)	
pT2	26 (25.5)	19 (25.0)	7 (27.0)	
pT3	29 (28.4)	16 (21.1)	13 (50.0)	
pT4	10 (9.8)	6 (7.9)	4 (15.4)	
Tumor grade (%)[b]				
G2	12 (12.4)	10 (14.1)	2 (7.7)	0.06
G3	85 (87.6)	61 (85.9)	24 (92.3)	
Lymphovasucular invasion (%)				
ly+	67 (65.7)	46 (60.5)	21 (80.8)	0.20
v+	32 (31.4)	17 (22.4)	15 (57.7)	<0.01
Lymph node status (%)[c]				
pN0	75 (73.5)	56 (73.7)	19 (73.1)	
pN+	15 (14.7)	10 (13.2)	5 (19.2)	0.75
NAC (%)	39 (38.2)	29 (38.2)	10 (38.5)	1
Follow-up (months;median)	39.5 (18.0-70.5)	40.5 (19.5-75.0)	38.5 (12.0-62.0)	0.23

NAC: Neoadjuvant chemotherapy. [a]ASA class can be evaluated in 58 patients. [b]Tumor grade was missing in 5 patients. [c]12 patients did not receive pelvic lymphadenectomy.

with regards to oncological outcomes in Japan. In this study, one out of four patients who underwent RC harbored HV, and squamous cell differentiation was the most common variant, followed by glandular differentiation. This result is consistent with a previous study (5-7), whereas the second most common variant is sometimes reported as sarcomatoid variant and micropapillary variant (8-10). Although the frequency of HV is uncertain, previous studies reported a broad range for the prevalence of HV from 7% to 81% (1, 11, 12). This broad range in the reported frequencies of HV is thought to be the lack of standardized reporting methodologies for UC. Moreover, awareness of the potential impact of HV may have led to more attention to small areas of all specimen. In addition, the prevalence of HV has increased consistently during the years. Indeed, Marco *et al*. (10) reported that the increasing number of patients diagnosed with HV in recent years when compared with historical patients (1990-2000: 21.3% *vs*. 1991-2013: 35.1%), and Monn *et al*. (9) reported 21.1% in 2008 to 28.9% in 2012. They say it was due to a direct consequence of the increasing awareness on this important element. This trend has been explained by transition of guidelines after the 2004 to 2016 WHO guidelines, which strongly recommends HV indication in the pathologic report (6). Shah *et al*. (13) reported that 44% of HV were not recognized by referring institutions. This fact affects badly to the oncological outcomes in patients with HV, so the recognition of HV has to be infiltrated more.

Whereas the importance of recognizing HV has increased, few large sample size studies on the clinical prognosis of HV have been reported (8-10). However, the background of patients in these studies (neoadjuvant and adjuvant chemotherapy, intravesical therapies before RC, the selection of variants, and so on) is unfortunately not consistent. In any case, there is no doubt that HV has more aggressive oncological features compared to those with pure UC. We found that patients with HV had poor RFS and CSS. This result is consistent with the findings of previous studies, which found that HV is associated with inferior oncological outcomes in patient with RC. We reported that there is no significant difference in OS, and as far as we investigated, there are no other reports that HV affects OS of patient with RC.

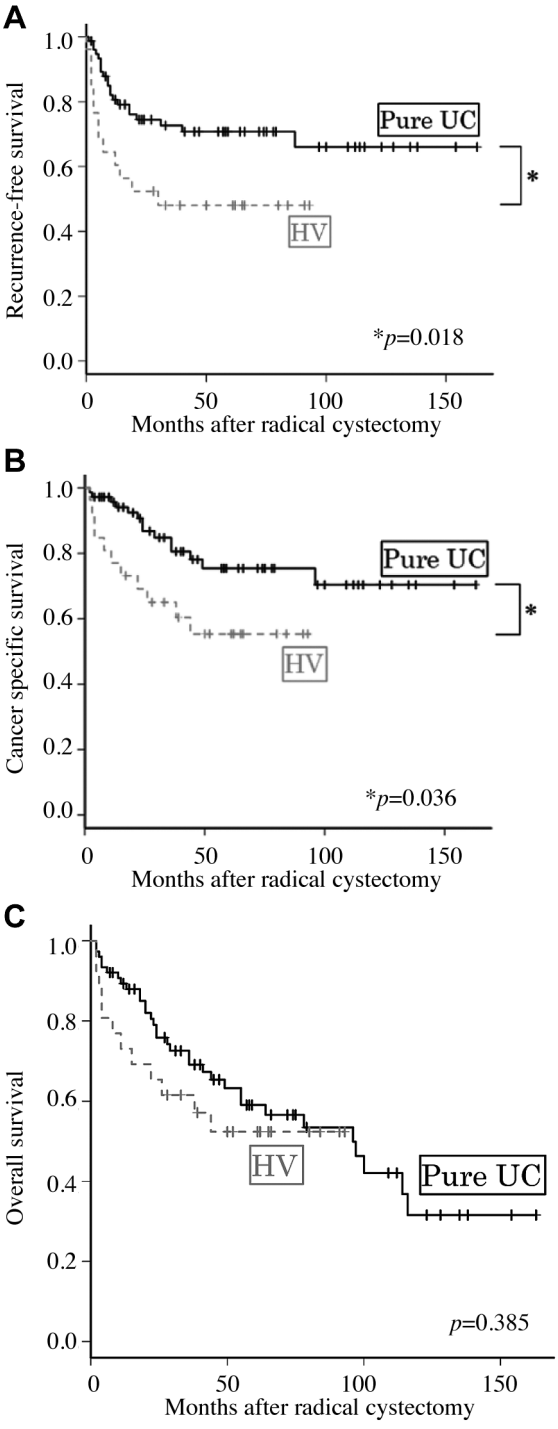

Figure 1. *The Kaplan-Meier analysis assessing recurrence-free survival (A), cancer-specific survival (B), and overall survival (C) of 102 patients treated with radical cystectomy.*

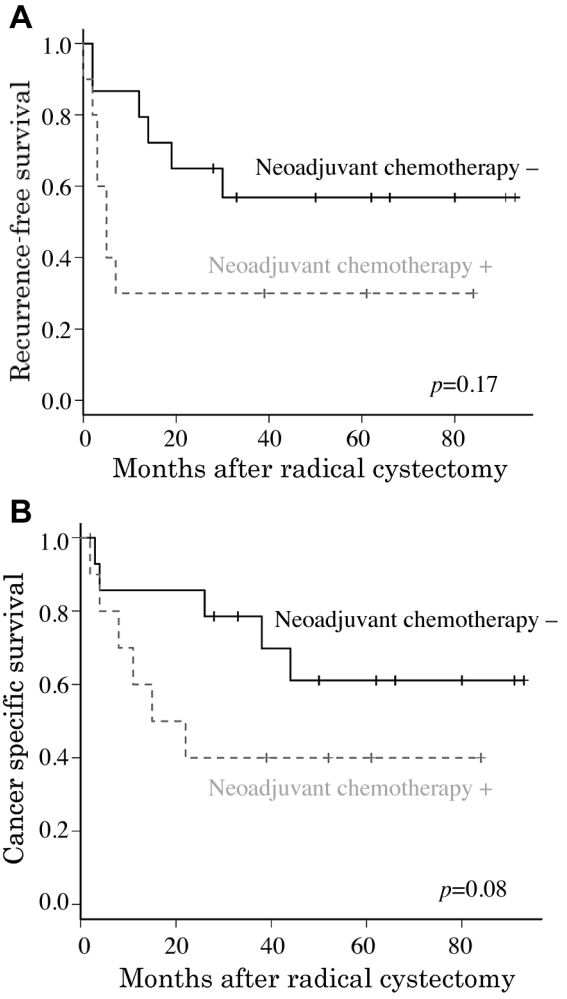

Figure 2. *The Kaplan-Meier analysis assessing recurrence-free survival (A), cancer specific survival (B) in patients with HV stratified by whether receive neoadjuvant chemotherapy.*

In our study, HV did not remain a statistically significant difference in multivariable analysis. Similarly, the greater part of investigators reported that HV was not a significant predictor for clinical outcomes after RC (8). On the other hand, several kinds of HV are recently reported as a significant predictor of poor prognosis. Monn *et al*. (9) reported that the micropapillary variant and the plasmacytoid variant was a significant predictor of survival, and Marco *et al*. (10) reported that small cell carcinoma was a significant predictor. If we diagnosed such kinds of variants including micropapillary variant, plasmacytoid variant, or small cell carcinoma, there is possibility that it might be good for oncological outcomes to give additional treatment such as adjuvant chemotherapy or radiation therapy, because these subtypes of HV have poorer survival than pure UC. Consequently, patients with HV received more frequent adjuvant chemotherapy (8), and this is consistent with those of previous studies, including upper urinary tract (12, 14, 15).

Table III. *Univariable and multivariable Cox regression analysis predicting OS of 102 patients treated with radical cystectomy.*

Variables	Overall survival					
	Univariable analysis			Multivariable analysis		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age (<70 y *vs*. ≥70 y)	0.388	0.731-2.177	0.388			
Gender (Male *vs*. Female)	0.444	0.668-2.294	0.444			
pT (≤pT2 *vs*. >pT3)	0.885	0.441-1.651	0.710			
Tumor grade (Low *vs*. High)	0.731	0.360-1.689	0.437			
ly (Negative *vs*. Positive)	0.508	0.294-0.890	0.018	0.669	0.372-1.226	0.189
v (Negative *vs*. Positive)	1.706	0.861-3.181	0.121			
Lymph node status (Negative *vs*. Positive)	1.047	0.314-2.593	0.931			
NAC (Negative *vs*. Positive)	2.696	1.527-4.792	0.001	2.625	1.432-4.821	0.002
Variant (Negative *vs*. Positive)	1.477	0.763-2.715	0.237			

RC with neoadjuvant chemotherapy improved prognosis of muscle invasive bladder cancer patients compared to RC alone (16). However, this cannot be translated into an equal benefit for patients with HV. There is clear evidence that neoadjuvant chemotherapy provides a survival benefit in patients with neuroendocrine tumors (17). On the other hand, it is uncertain for the other variant whether neoadjuvant therapy provided benefit. In our study, there is a tendency that patients who received neoadjuvant chemotherapy were less likely to harbor RFS and CSS in HV patients compared to patients undergoing RC only. Vetterlein *et al*. (8) analyzed 2,018 patients with HV who underwent RC between 2003 and 2012. They reported that an overall survival benefit for neoadjuvant chemotherapy was only shown in patients with neuroendocrine tumors. This result may suggest that patients with HV should be treated with RC without neoadjuvant chemotherapy. However, even if the inferiority of neoadjuvant chemotherapy for patients with HV was proven, most of patients with HV will receive neoadjuvant chemotherapy, because the sensitivity of initial biopsy or transurethral resection for detecting HV is very low (18, 19). Abdle-Latif *et al*. (18) reported that the sensitivity of initial transurethral resection was 39%.

Recently, immunotherapy with checkpoint inhibitors (PD-1 and PD-L1 inhibitors) has revolutionized the treatment paradigm of bladder cancer. However, they vary in dose and frequency and cost burden, and only pembrolizumab has shown superiority over standard chemotherapy. There is a randomized, open-label, active control clinical trial (KEYNOTE-045) (20), and we can use this drug from December 2017 in Japan, while there are some reports of this drug in our country (21). According to this Phase III trial, there is a tendency that Pembrolizumab has greater effect in patients with HV than those with pure UC, however there is no statistically significant difference. Some reports show that PD-L1 or CTLA-4 expression is frequently seen in Luminal-infiltrated and Basal/Squamous subtypes (22). These findings suggest that checkpoint inhibitors might give better outcomes to patients with HV than conventional standard chemotherapy. Moreover, there is a possibility that patients with HV have more sensitivity to the checkpoint inhibitors than those with pure UC.

Our study has certain limitations. First, it was retrospective in its design, and was a single-center study. On the other hand, pathological findings including HV were assigned by one dedicated pathologist, so the pathological findings were unified. Second, we were unable to compare the subtypes of HV because of few data. With increasing recognition of HV, larger sample size studies will be conducted in the future, and we will be able to elucidate the characteristics of each subtype of HV. Furthermore, we can choose best treatment for each subtype.

Conclusion

There is no doubt that HV has more aggressive oncological features than pure UC. Therefore, appropriate treatment for each subtype of HV is needed. Further accumulation of cases and more analysis are required to determine the best strategy for patients with HV.

Conflicts of Interest

The Authors declare that there are no potential conflicts of interest relevant to this article.

Authors' Contributions

Study conception and design: KT and MK. Manuscript writing: KT, statistical analysis: KT, JT, KI, HN, AG, SI, TH and MT. Coordination of the team and final corrections: AM. Patient management: KT, YK, AG, and MK. All Authors read and approved the final manuscript.

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