Prognostic impact of variant histologies in urothelial bladder cancer treated with radical cystectomy

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Objectives

To evaluate variant histologies (VHs) for disease-specific survival (DSS) in patients with invasive urothelial bladder cancer (BCa) undergoing radical cystectomy (RC).

Materials and Methods

We analysed a multi-institutional cohort of 1082 patients treated with upfront RC for cT1-4aN0M0 urothelial BCa at eight centres. Univariable and multivariable Cox' regression analyses were used to assess the effect of different VHs on DSS in overall cohort and three stage-based analyses. The stages were defined as 'organ-confined' (≤pT2N0), 'locally advanced' (pT3-4N0) and 'node-positive' (pTanyN1-3).

Results

Overall, 784 patients (72.5%) had pure urothelial carcinoma (UC), while the remaining 298 (27.5%) harboured a VH. Squamous differentiation was the most common VH, observed in 166 patients (15.3%), followed by micropapillary (40 patients [3.7%]), sarcomatoid (29 patients [2.7%]), glandular (18 patients [1.7%]), lymphoepithelioma-like (14 patients [1.3%]), small-cell (13 patients [1.2%]), clear-cell (eight patients [0.7%]), nested (seven patients [0.6%]) and plasmacytoid VH (three patients [0.3%]). The median follow-up was 2.3 years. Overall, 534 (49.4%) disease-related deaths occurred. In uni- and multivariable analyses, plasmacytoid and small-cell VHs were associated with worse DSS in the overall cohort (both $P = 0.04$). In univariable analyses, sarcomatoid VH was significantly associated with worse DSS, while lymphoepithelioma-like VH had favourable DSS compared to pure UC. Clear-cell ($P = 0.015$) and small-cell ($P = 0.011$) VH were associated with worse DSS in the organ-confined and node-positive cohorts, respectively.

Conclusions

More than 25% of patients harboured a VH at time of RC. Compared to pure UC, clear-cell, plasmacytoid, small-cell and sarcomatoid VHs were associated with worse DSS, while lymphoepithelioma-like VH was characterized by a DSS benefit. Accurate pathological diagnosis of VHs may ensure tailored counselling to identify patients who require more intensive management.

Keywords

bladder urothelial carcinoma, radical cystectomy, variant histology, survival, prognosis, #BladderCancer, #blcsm, #utuc, #uroonc

Introduction

Urothelial carcinoma (UC), which encompasses bladder cancer (BCa) and upper tract urothelial carcinoma (UTUC), represents the sixth most commonly diagnosed cancer in Western countries [1]. Radical cystectomy (RC) with pelvic lymph node dissection (PLND) is a mainstay in the treatment of muscle-invasive bladder cancer (MIBC) and high-risk non-muscle-invasive bladder cancer (NMIBC) [2].

Genetic susceptibility in BCa carcinogenesis has been well recognized over recent decades and genetic predisposition combined with lifestyle factors has been shown to be associated with an increased risk of cancer development [3]. UC has a remarkable propensity for divergent differentiation. Several variant histologies (VHs) have been recognized by the 2016 WHO classification as potential drivers of clinical and therapeutic management [4]. The incidence of VHs in RC specimens has recently been described in almost 25% of cases [5]. Despite the association of VH with biological aggressiveness, there is a lack of agreement about the influence of VH on cancer-specific outcomes [6,7]. Such understanding could enhance both tailored counselling and the formulation of risk-adapted strategies to identify patients who would benefit from more intensive treatment protocols including neoadjuvant (NAC) and adjuvant chemotherapy (AC) [6].

In this context, the objective of our study was to evaluate the impact of VHs on disease-specific survival (DSS) using pathology review and individual patient data (IPD) from a large multi-institutional dataset.

Materials and Methods

Patient Selection and Data Collection

Clinico-pathological and outcome data were retrospectively collected for 1082 non-consecutive patients who underwent RC and bilateral PLND for cT1-4aN0M0 BCa between October 1986 and March 2020. Eight tertiary referral centres provided the IPD (Figure S1). The patients included in this analysis did not receive neoadjuvant treatment(s).

Appropriate ethical approval was obtained at each site. The variables collected included age at RC, gender, pathological tumour (pT) stage and grade, pathological nodal (pN) status, lymphovascular invasion (LVI), positive surgical margin (PSM) status, concomitant carcinoma *in situ* (CIS) and AC administration. Before RC, the patients underwent transurethral resection of bladder tumour (TURBT) and CT of the abdomen/pelvis and at least a chest X-ray. Patients were followed in accordance with individual site surveillance protocols.

Pathological Evaluation

All RC specimens were locally reviewed by a dedicated uro-pathologist. Pathology review was performed after the introduction of the 2016 WHO classification of bladder tumours [4]. Only pure UC and UC + VH were included in the current analysis. VH was defined as the combination of UC along with other morphologies. The included VHs were UC with divergent differentiation (squamous and glandular/adeno differentiation), micropapillary, nested (including large-nested), sarcomatoid, lymphoepithelioma-like, plasmacytoid (including signet-ring cells) and clear-cell VH. In cases of mixed tumours, in which more than one VH was present, the predominant VH was used. Small-cell histology admixed with UC was also included. Although it is classified as a distinct histological type in WHO 2016, herein it is referred to as small-cell VH for comparison purposes. Pure non-urothelial VHs were excluded. Percentage and threshold for VHs were not included in the analysis due to lack of universal reporting. Thus, we assumed that any VH would equally influence clinical outcome. Pathological stage was based on the 2017 TNM classification system (8th edition), while tumour grade was based on the 2004/2016 WHO system.

Endpoint Definition

The primary endpoint of the current analysis was DSS. Disease-related death was determined by the treating physicians following chart review or was corroborated by death certificates. Survival was defined as the time interval between RC and the date of last imaging and/or clinical visit

Table 1 Patient and tumour characteristics of the 1082 patients with invasive, non-metastatic bladder cancer (cT1-4aN0M0) who underwent radical cystectomy and urinary diversion according to histology pattern.

Variable	Overall	Pure UC	Squamous	Micropapillary	Sarcomatoid
Patients, *n* (%)	1082 (100.0)	784 (72.5)	166 (15.3)	40 (3.7)	29 (2.7)
Age, years, median (IQR)	68 (59–75)	67 (59–75)	67 (59–75)	68 (60–75)	72 (60–78)
Gender, *n* (%)					
Male	822 (76.0)	608 (77.6)	113 (68.1)	32 (80.0)	19 (65.5)
Female	260 (24.0)	176 (22.4)	53 (31.9)	8 (20.0)	10 (34.5)
pT stage, *n* (%)					
NMIBC (pTa/is/1)	108 (10.0)	97 (12.4)	3 (1.8)	6 (15.0)	0 (0.0)
pT2	252 (23.3)	183 (23.3)	30 (18.1)	12 (30.0)	9 (31.0)
pT3	509 (47.0)	364 (46.4)	90 (54.2)	16 (40.0)	11 (38.0)
pT4	213 (19.7)	140 (17.9)	43 (25.9)	6 (15.0)	9 (31.0)
Grade (WHO 2004), *n* (%)					
Low	34 (3.1)	31 (4.0)	1 (0.6)	2 (5.0)	0 (0.0)
High	1048 (96.9)	753 (96.0)	165 (99.4)	38 (95.0)	29 (100.0)
pN stage, *n* (%)					
pN0	664 (61.4)	483 (61.6)	108 (65.1)	14 (35.0)	16 (5.22)
pN1	138 (12.8)	100 (12.8)	18 (10.8)	9 (22.5)	4 (13.8)
pN2	268 (24.8)	195 (24.9)	37 (22.3)	17 (42.5)	8 (27.6)
pN3	12 (1.1)	6 (0.8)	3 (1.8)	0 (0.0)	1 (3.4)
PSMs, *n* (%)	101 (9.3)	71 (9.1)	12 (7.2)	6 (15.0)	3 (10.3)
Concomitant CIS, *n* (%)					
Absence	676 (62.5)	475 (60.6)	114 (68.7)	22 (55.0)	18 (62.1)
Presence	406 (37.5)	309 (39.4)	52 (31.3)	18 (45.0)	11 (37.9)
LVI, *n* (%)					
Absence	543 (50.2)	407 (51.9)	78 (47.0)	16 (40.0)	16 (55.2)
Presence	539 (49.8)	377 (48.1)	88 (53.0)	24 (60.0)	13 (44.8)
Adjuvant chemotherapy, *n* (%)	331 (30.6)	238 (30.4)	47 (28.3)	17 (42.5)	6 (20.7)
Year of RC, *n* (%)					
1986–2003	410 (37.9)	315 (40.2)	54 (32.5)	15 (37.5)	9 (31.0)
2004–2020	672 (62.1)	469 (59.8)	112 (67.5)	25 (62.5)	20 (69.0)
Disease-specific events, *n* (%)	535 (49.4)	392 (50.0)	74 (44.6)	24 (60.0)	18 (62.0)
Follow-up, years, median (IQR)	2.3 (0.9–5.0)	2.4 (1.0–5.0)	2.2 (0.8–5.0)	2.0 (1.0–3.3)	0.5 (1.2–3.7)
Survivor follow-up, years, median (IQR)	4.2 (2.1–7.4)	4.5 (2.2–7.5)	3.8 (1.7–7.5)	4.1 (2.1–7.3)	6.4 (1.8–7.3)

CIS, carcinoma in situ*; IQR, interquartile range; LE, lymphoepithelioma; LN, lymph node; LVI, lymphovascular invasion; NMIBC, non-muscle invasive bladder cancer; PSM, positive surgical margin; RC, radical cystectomy; UC, urothelial carcinoma.*

(censored), or of documented (disease-related) death. To allow comparison of pure UC to the VHs, three disease stages were defined as follows: 'organ-confined' (≤pT2N0), 'locally advanced' (pT3-4N0) and 'node-positive' (pTanyN1-3).

Statistical Analysis

Descriptive analysis included frequencies and proportions for categorical variables. Medians and interquartile range (IQR) were reported for continuous variables. The Mann–Whitney *U*-test or Kruskal–Wallis test were used for comparison of the continuous data and chi-squared or Fisher's exact test for categorical data. All tests were two-sided with a level of significance set at $P < 0.05$. The Kaplan–Meier method was used to estimate DSS, stratified by histological type using log rank. Univariable and multivariable Cox regression models were used to assess the hazard ratio (HR) with 95% CI, testing the association between the VHs and DSS. Beyond that, we relied on 1:1 nearest-neighbour propensity-score matching [8] without replacement to account and adjust for potential baseline differences in patient characteristics. Thus, the propensity-score-matched cohort was balanced according to age, pT stage (NMIBC vs pT2 vs pT3 vs pT4) and pN stage (negative vs positive). After matching, all standardized mean differences for the covariates were below 0.1. Subsequently, the propensity-score-matched population was used to repeat all univariable and multivariable analyses among both overall and stage-based cohorts. Statistical analyses were performed using R v.3.6.3 (R-Foundation for Statistical-Computing).

Results

Patient and Pathological Characteristics

In total, RC specimens of 1082 patients were included, of whom 784 (72.5%) had pure UC, while the remaining 298 (27.5%) had VH. Squamous differentiation was the most common VH, and was present in 166 patients (15.3%), followed by 40 patients (3.7%) with micropapillary, 29 (2.7%) with sarcomatoid, 18 (1.7%) with glandular, 14 (1.3%) with lymphoepithelioma-like, 13 (1.2%) with small-

Glandular	LE-like	Small-Cell	Clear-Cell	Nested	Plasmacytoid	*P*
18 (1.7)	14 (1.3)	13 (1.2)	8 (0.7)	7 (0.6)	3 (0.3)	
67 (65–74)	61 (50–69)	65 (55–77)	77 (73–78)	68 (71–74)	73 (70–78)	0.06
16 (88.9)	9 (64.3)	10 (76.9)	7 (87.5)	6 (85.7)	2 (66.7)	0.18
2 (11.1)	5 (35.7)	3 (23.1)	1 (12.5)	1 (14.3)	1 (33.3)	
1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	<0.001
4 (22.2)	9 (64.3)	2 (15.4)	2 (25.0)	1 (14.3)	0 (0.0)	
10 (55.6)	4 (28.6)	6 (46.2)	2 (25.0)	5 (71.4)	1 (33.3)	
3 (16.7)	1 (7.1)	5 (38.5)	4 (50.0)	0 (0.0)	2 (66.7)	
0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.5
18 (100.0)	14 (100.0)	13 (100.0)	8 (100.0)	7 (100.0)	3 (100.0)	
13 (72.2)	11 (78.6)	9 (69.2)	4 (50.0)	4 (57.1)	2 (66.7)	0.03
2 (11.1)	1 (7.1)	2 (15.4)	1 (12.5)	1 (14.3)	0 (0.0)	
3 (16.7)	2 (14.3)	2 (15.4)	2 (25.0)	1 (14.3)	1 (33.3)	
0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	1 (14.3)	0 (0.0)	
2 (11.2)	1 (7.1)	2 (15.4)	2 (25.0)	0 (0.0)	2 (66.7)	0.04
12 (66.7)	10 (71.4)	7 (53.8)	8 (100.0)	7 (100.0)	3 (100.0)	0.05
6 (33.3)	4 (28.6)	6 (46.2)	0 (0.0)	0 (0.0)	0 (0.0)	
10 (55.6)	10 (71.4)	2 (15.4)	1 (12.5)	3 (42.9)	0 (0.0)	0.02
8 (44.4)	4 (28.6)	11 (84.6)	7 (87.5)	4 (57.1)	3 (100.0)	
7 (38.9)	4 (28.6)	4 (30.8)	3 (37.5)	4 (57.1)	1 (33.3)	0.6
4 (22.2)	7 (50.0)	5 (38.5)	0 (0.0)	1 (14.3)	0 (0.0)	0.08
14 (77.8)	7 (50.0)	8 (61.5)	8 (100.0)	6 (85.7)	3 (100.0)	
7 (38.9)	2 (14.3)	9 (69.2)	5 (62.5)	1 (14.3)	3 (100.0)	0.02
2.2 (1.2–3.5)	5.9 (3.3–8.0)	0.9 (0.5–3.0)	1.1 (0.8–1.9)	1.1 (2.3–2.7)	0.5 (0.5–0.8)	0.01
2.6 (1.6–4.0)	5.9 (3.4–8.2)	7.7 (5.1–9.8)	1.1 (0.8–3.0)	2.0 (0.9–2.9)	0.5 (0.5–0.5)	0.05

cell, 8 (0.7%) with clear-cell, seven (0.6%) with nested and three (0.3%) with plasmacytoid VH. Descriptive clinico-pathological features of the cohort are shown in Table 1.

The median (IQR) age at RC was 68 (59–75) years and the majority of patients were male (76%). No significant differences in patient proportions were noted between the histology groups with regard to age, gender, tumour grade and AC administration. In general, VH was associated with more aggressive disease at RC as indicated by higher pT stage ($P < 0.001$), PSM rates ($P = 0.04$), presence of LVI ($P = 0.02$) and worse pN status ($P = 0.03$). No significant differences were found in terms of incidence of VH when considering the year of RC (Table 1).

Survival Estimates According to Histology

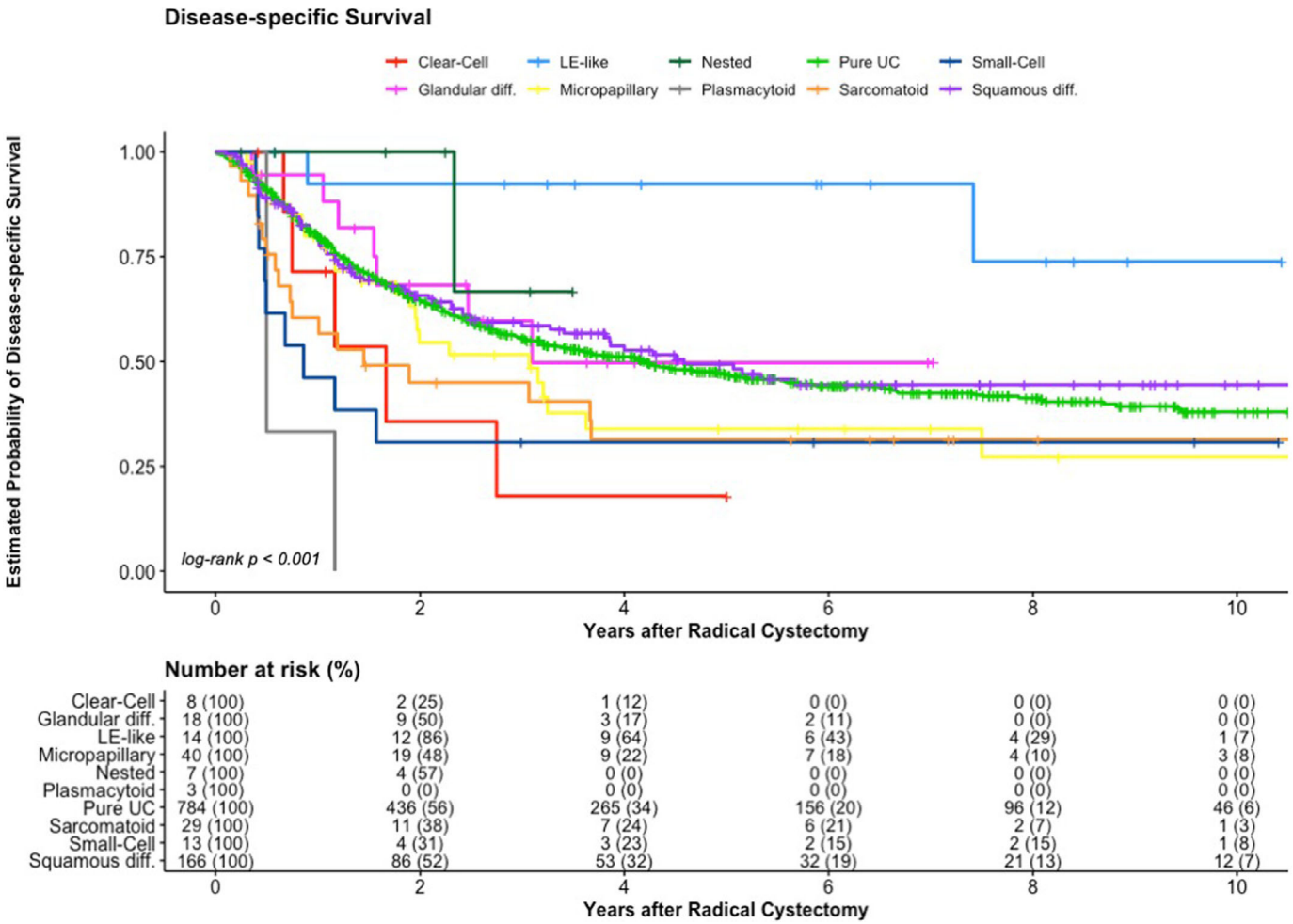
Within a median (IQR) follow-up of 2.3 (0.9–5.0) years, 535 (49.4%) disease-related deaths occurred. Of these, 392 (50.0%), 74 (44.6%), 24 (60.0%), 18 (62.0%), 7 (38.9%), 2 (14.3%), 9 (69.2%), 5 (62.5%), 1 (14.3%) and 3 (100.0%) occurred in the pure UC, squamous, micropapillary, sarcomatoid, glandular, lymphoepithelioma-like, small-cell, clear-cell, nested and plasmacytoid cohorts, respectively (Table 1). A Kaplan–Meier plot assessing DSS rates, stratified according to these histological groups, is shown in Fig. 1.

Cox Regression Analyses

Univariable Cox regression analyses assessing DSS across the overall cohort and the three disease stages are shown in Table S1. In the overall cohort, sarcomatoid, plasmacytoid and small-cell VHs exhibited worse DSS (HR 1.61, 95% CI 1.01–2.59 [$P = 0.047$], HR 6.21, 95% CI 1.99–19.4 [$P = 0.002$] and HR 1.93, 95% CI 1.09–3.73 [$P = 0.03$], respectively). Lymphoepithelioma-like VH showed better survival compared to pure UC (HR 0.20, 95% CI 0.05–0.82 [$P = 0.025$]).

Organ-confined disease was reported in 286 patients (26.4%), with 76 (26.6%) disease-related deaths. In this subgroup, clear-cell VH exhibited worse survival (HR 10.8, 95% CI 1.42–81.4 [$P = 0.021$]). Locally advanced disease was reported

Fig. 1 Kaplan–Meier survival curves for disease-specific survival (P log-rank [for trend], $P < 0.001$) stratified by histology group among the 1082 patients with clinically localized, non-metastatic (cT1-4aN0M0) bladder cancer undergoing radical cystectomy. Follow-up was curtailed at 10 years in the Kaplan–Meier plot. LE-like, lymphoepithelioma-like; UC, urothelial carcinoma.



in 378 patients (34.9%), with 174 (46.0%) disease-related deaths. Here, plasmacytoid VH was associated with worse DSS (HR 4.38, 95% CI 1.08–17.8 [$P = 0.04$]). Node-positive disease was reported in 418 patients (38.6%), with 285 (75.4%) disease-related deaths. In this subgroup, small-cell VH was associated with worse survival (HR 5.78, 95% CI 2.12–15.8 [$P < 0.001$]).

Multivariable Cox regression analyses are presented in Table 2. After adjusting for all clinico-pathological prognosticators, plasmacytoid and small-cell VHs remained independently associated with worse DSS in the overall cohort (HR 3.37, 95% CI 1.06–10.7 [$P = 0.04$] and HR 1.93, 95% CI 1.02–3.79 [$P = 0.04$], respectively). Among patients harbouring organ-confined disease, clear-cell VH remained independently associated with worse DSS (HR 14.8, 95% CI 1.70–128 [$P = 0.015$]). No significant associations were found with the different VHs for patients with locally advanced disease. In the node-positive cohort, the impact of small-cell VH on DSS was confirmed (HR 3.77, 95% CI 1.35–10.6 [$P = 0.011$]). Overall, other predictors of worse survival outcomes were increasing pT stage, positive pN status and presence of LVI.

Propensity-Score Matching

After 1:1 propensity-score matching, 596 patients remained. Organ-confined, locally advanced and node-positive disease were reported in 124 (20.8%), 238 (39.9%) and 234 patients (39.3%), respectively. Within a median (IQR) follow-up of 2.0 (0.8–4.5) years, 310 (52.1%) disease-related deaths occurred. Of these, 39 (12.6%), 110 (35.5%) and 161 (51.9%) occurred in the pure organ-confined, locally advanced and node-positive weighted cohorts, respectively. Similarly, after adjusting for all clinico-pathological prognosticators, small-cell VH remained associated with worse DSS in the node-positive-weighted cohort (HR 3.30,

Table 2 Multivariable Cox regression analysis stratified by histology group across the overall cohort (*n* = 1082), pT1-2N0 cohort (*n* = 286), pT3-4N0 cohort (*n* = 378) and pTany N1-3 cohort (*n* = 418).

Variable	Overall cohort		Organ-confined (T1-2N0)		Locally advanced (T3-4N0)		Node-positive (TanyN1-3)	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years), continuous	1.01 (1.00–1.02)	0.09	1.03 (1.00–1.05)	0.045	1.00 (0.99–1.02)	0.6	1.00 (0.99–1.02)	0.4
Gender								
Male	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-
Female	0.94 (0.76–1.15)	0.5	1.22 (0.73–2.04)	0.5	0.95 (0.65–1.38)	0.8	0.87 (0.65–1.17)	0.4
pT stage								
NMIBC	Ref. (1.00)	-	Ref. (1.00)	-	-	-	Ref. (1.00)	-
pT2	1.30 (0.83–2.02)	0.3	0.94 (0.56–1.60)	0.8	-	-	1.56 (0.37–6.60)	0.5
pT3	2.25 (1.48–3.44)	<0.001	-	-	Ref. (1.00)	-	2.25 (0.55–9.25)	0.3
pT4	3.05 (1.94–4.79)	<0.001	-	-	1.49 (1.02–2.17)	0.041	2.80 (0.67–11.6)	0.2
pN stage								
Negative	Ref. (1.00)	-	-	-	-	-	-	-
Positive	2.31 (1.87–2.85)	<0.001	-	-	-	-	-	-
LVI								
No	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-
Yes	1.40 (1.14–1.72)	0.001	0.75 (0.37–1.51)	0.4	1.39 (1.02–1.89)	0.04	1.45 (1.06–1.98)	0.02
Concomitant CIS								
No	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-
Yes	0.82 (0.69–0.98)	0.03	1.37 (0.83–2.25)	0.2	0.93 (0.68–1.29)	0.7	0.71 (0.56–0.91)	0.007
PSMs								
No	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-
Yes	1.30 (0.98–1.73)	0.07	1.14 (0.40–3.26)	0.8	1.31 (0.78–2.20)	0.3	1.29 (0.90–1.85)	0.2
AC								
No	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-
Yes	0.57 (0.47–0.71)	<0.001	2.57 (1.08–6.13)	0.034	0.70 (0.48–1.03)	0.07	0.46 (0.35–0.59)	<0.001
Histology of BCa								
Pure UC	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-	Ref. (1.00)	-
Squamous	0.80 (0.62–1.04)	0.09	1.28 (0.59–2.78)	0.5	0.71 (0.47–1.08)	0.11	0.84 (0.59–1.20)	0.3
Sarcomatoid	1.35 (0.84–2.17)	0.2	2.34 (0.71–7.69)	0.2	1.72 (0.75–3.96)	0.2	1.13 (0.57–2.25)	0.7
Clear-cell	1.02 (0.42–2.52)	>0.9	14.8 (1.70–128)	0.015	0.88 (0.12–6.43)	>0.9	0.99 (0.31–3.22)	>0.9
Glandular	0.72 (0.34–1.53)	0.4	1.58 (0.20–12.6)	0.7	0.90 (0.28–2.87)	0.9	0.50 (0.16–1.57)	0.2
Lymphoepithelioma-like	0.30 (0.07–1.20)	0.09	0.48 (0.06–3.55)	0.5	0.00 (0.00–Inf)	>0.9	0.39 (0.05–2.82)	0.4
Micropapillary	1.19 (0.79–1.81)	0.4	0.94 (0.29–3.08)	>0.9	0.79 (0.19–3.29)	0.7	1.32 (0.81–2.13)	0.2
Nested	0.50 (0.07–3.56)	0.5	4.37 (0.50–37.8)	0.2	0.00 (0.00–Inf)	>0.9	0.00 (0.00–Inf)	>0.9
Plasmacytoid	3.37 (1.06–10.07)	0.04	NA	NA	3.31 (0.79–13.09)	0.1	2.76 (0.37–20.8)	0.3
Small-cell	1.93 (1.02–3.79)	0.04	2.55 (0.32–20.6)	0.4	1.07 (0.39–2.97)	0.9	3.77 (1.35–10.6)	0.011

AC, adjuvant chemotherapy; BCa, bladder cancer; CIS, carcinoma in situ*; HR, hazard ratio; LVI, lymphovascular invasion; NMIBC, non-muscle invasive bladder cancer; PSM, positive surgical margin; Ref., reference; UC, urothelial carcinoma, VH, variant histology.*

95% CI 1.13–9.69 [*P* = 0.02]). Squamous VH was associated with survival benefit in both the overall and locally advanced-weighted cohorts (HR 0.74, 95% CI 0.56–0.98 [*P* = 0.037] and HR 0.60, 95% CI 0.38–0.95 [*P* = 0.03], respectively). Cox regression models after propensity-score matching are presented in Tables S2 and S3.

Discussion

In this retrospective multi-institutional analysis, we evaluated the impact of VHs in a large IPD cohort undergoing RC and PLND for non-metastatic BCa. We found that 27.5% of patients harboured a VH at time of RC. Consistent with previous reports, VHs were usually associated with worse disease features, such as advanced pT stage, LVI, lymph node metastases and PSMs [9–11]. However, after accounting for different pathological stages and controlling for clinico-pathological predictors, only few VHs had a different prognosis from pure UC.

We found several VHs associated with DSS in unadjusted analyses. Lymphoepithelioma-like VH accounts for fewer than 1% of all bladder tumours [5]. Lymphoepithelioma-like VH is characterized by a dense infiltrate of lymphoid and/or inflammatory cells including polyclonal T- and B-lymphocytes [12]. Our series included 14 (1.3%) cases of lymphoepithelioma VH. Our findings corroborate those of a systematic review, in which this VH showed low metastatic potential, favourable response to platinum-based chemotherapy and better survival if RC was performed [13]. Lymphoepithelioma-like VH exhibited the best survival trend in our analysis and, after controlling for standard predictors, this variant approached borderline significance. Hence, the

Table 3 Comparison of studies (number of patients per study and reference number are shown between brackets) regarding the incidence and the impact on disease-specific survival at the multivariable level of variant histologies compared to pure urothelial carcinoma in patients with clinically localized invasive bladder cancer treated with radical cystectomy.

Variable	Present series (*n* = 1082)		Moschini et al. (*n* = 1067) [9]		Stroman et al. (*n* = 430) [10]	
	n (%)	HR (95%CI)	*n* (%)	HR (95%CI)	*n* (%)	HR (95%CI)
Variant histology						
Squamous	166 (15.3)	0.80 (0.62–1.04)	109 (10.2)	1.29 (0.87–1.92)	41 (9.5)	**2.03 (1.21–3.42)**
Sarcomatoid	29 (2.7)	1.35 (0.84–2.17)	21 (2.0)	1.18 (0.48–2.91)	9 (2.1)	1.51 (0.55–4.18)
Clear-cell	8 (0.7)	1.02 (0.42–2.52)	-	-	1 (0.2)	3.92 (0.47–32.42)
Glandular	18 (1.7)	0.72 (0.34–1.53)	23 (2.2)	1.54 (0.55–4.34)	13 (3.0)	1.36 (0.57–3.24)
LE-like	14 (1.3)	0.30 (0.07–1.20)	10 (0.9)	1.32 (0.78–2.15)	-	-
Micropapillary	40 (3.7)	1.19 (0.79–1.81)	89 (8.3)	0.66 (0.35–1.26)	8 (1.9)	0.65 (0.09–4.77)
Nested	7 (0.6)	0.50 (0.07–3.56)	-	-	4 (0.9)	1.11 (0.25–4.88)
Plasmacytoid	3 (0.3)	**3.37 (1.06–10.07)**	-	-	3 (0.7)	4.33 (0.76–24.53)
Small-cell	13 (1.2)	**1.93 (1.02–3.79)**	19 (1.8)	**3.30 (1.56–7.02)**	-	-
Microcystic	-	-	-	-	4 (0.9)	0.68 (0.09–5.20)
Mixed variants	-	-	34 (3.2)	1.36 (0.71–2.61)	-	-
Other	-	-	33 (3.1)	1.41 (0.87–2.28)	-	-

*Statistically significant results in bold. HR, hazard ratio; LE, lymphoepithelioma. *Micropapillary, sarcomatoid and small-cell variants were analysed together. †Overall survival as endpoint of interest.*

present study suggested that lymphoepithelioma-like VH might be associated with better DSS compared to pure UC. Squamous VH was the most frequent type of VH, with 166 cases (15.3%). Presence of squamous differentiation appeared to have little impact on the prognosis of BCa patients treated with RC [9,11,14]. Furthermore, the SWOG8710 trial showed that this VH had a better response to cisplatin-based NAC [15]. Notably, both lymphoepithelioma-like and squamous variants were the only VHs with immunotherapy sensitivity in the PURE-01 trial. Possible explanations for this included both elevated tumour mutational burden and peculiar gene amplifications such as CD247 and PDCD1LG2 [16]. Accordingly, these VHs could be managed as would be appropriate for pure UC of the same stage considering both conventional chemotherapy regimens and data from immune checkpoint inhibitor trials.

We found sarcomatoid VH in 29 patients (2.7%), which is a slightly higher incidence compared to former studies [9,10,17]. Sarcomatoid VH showed features of epithelial-to-mesenchymal transition that has been historically associated with aggressive biological behaviour [5]. Although results from single reports were controversial [9,18], a pooled analysis showed no independent impact on survival [6]. Within a large National Cancer Database (NCDB) analysis, Sui et al. found no difference between patients treated with surgery alone and those who received either NAC or AC [18]. Pending further evidence, the treatment algorithm of sarcomatoid VH should be decided at referral centres within a multidisciplinary consensus. In general, centralization of such procedures and high service capability have been associated with better surgical outcomes compared with low-volume centres [19]. In this context, Sui et al. [20] queried the NCDB identifying 23 284 patients with BCa and VH who were treated at 1301 hospitals. The authors found that the management of VH BCa at high-volume centres was associated with improved overall survival.

After accounting for other variables, plasmacytoid and small-cell VH remained associated with worse DSS in multivariable analysis. A comparison with previous studies considering the incidence and survival outcomes at the multivariable level is summarized in Table 3 [9–11,17,21,22]. By relying on Weibull regression, Martini et al. [21] aimed to create a personalized follow-up scheme of patients who harboured VHs after RC. The authors considered urothelial variants, non-urothelial variants and mixed variants. However, only squamous (29%) and micropapillary (14%) VH entities were separately analysed as the most common. They showed that patients with VHs had a higher risk of recurrence and shorter median time to recurrence than those with pure UC, especially in the subgroup of patients with <pT2 disease at final pathological report [21]. Fu et al. [22] queried the NCDB as a non-IPD cohort to investigate how VHs impacted on pathological primary site and nodal downstaging after multi-agent NAC. They found that the VH group was more likely to have ypN-positive disease (26% vs 18%) and to be ypN2/3 ($P < 0.001$). For survival analysis purposes, the authors grouped all the VHs into one cohort, resulting in a significantly increased adjusted overall mortality risk at 5 years after RC [22]. In the present study, we relied on IPD from a large multi-institutional dataset using a dedicated pathology review capturing more VHs than in the previous literature (Table 3). Moreover, we separately analysed each VH and then used a propensity-score matching analysis to account and adjust for potential baseline differences in patient characteristics. Thus, a full comparison between our

Naspro et al. (*n* = 525) [17]		Xylinas et al. (*n* = 1983) [11]		Martini et al. (*n* = 2422) [21]		Fu et al. (*n* = 5335) [22]†	
n (%)	HR (95%CI)	*n* (%)	HR (95%CI)	*n* (%)	HR (95%CI)	*n* (%)	HR (95%CI)
78 (14.9)	-	227 (11.4)	-	155 (6.4)	-	63 (1.2)	-
9 (1.7)	2.6 (1.35–4.84)*	40 (2.0)	-	-	-	75 (1.4)	-
-	-	-	-	-	-	-	-
8 (1.5)	-	75 (3.8)	-	-	-	31 (0.6)	-
-	-	-	-	-	-	-	-
15 (2.9)	2.6 (1.35–4.84)*	34 (1.7)	-	74 (3.1)	-	73 (1.4)	-
8 (1.5)	-	-	-	-	-	-	-
6 (1.2)	-	7 (0.4)	-	-	-	-	-
7 (1.3)	2.6 (1.35–4.84)*	40 (2.0)	-	-	-	187 (3.5)	-
-	-	-	-	-	-	-	-
-	-	65 (3.3)	-	52 (2.1)	-	-	-
-	-	-	-	152 (6.3)	-	-	-

findings and the previous reports is challenging. Mori et al. [6] highlighted in their systematic review that several VHs have been commonly analysed, together or combined into specific groups. Hence, data on the impact of each individual VH on DSS was not always possible. Patients with plasmacytoid VH have a higher rate of locally advanced disease and PSMs after extirpative surgery than do patients with conventional UC [5]. Two meta-analyses described this variant as an independent predictor of overall mortality [7,23]. Although the absolute number of patients with plasmacytoid VH was low, we still found a link between plasmacytoid VH and worse DSS. As the role of conventional NAC or novel targeted regimens is still uncertain [24,25], it appears essential to strive for negative surgical margins at RC.

It is widely accepted that small-cell variants derive from a de-differentiation process of UC [26]. We included small-cell VH only combined with UC in this analysis. We found small-cell VH in 1.2% of our study cohort. This is a slightly higher proportion compared with the literature, in which the presence of small-cell VH ranges between 0.5% and 0.7% [5]. Small-cell VH is an aggressive tumour and more than 95% of patients with this VH are diagnosed at muscle-invasive stages or higher [27]. It should be noted that our study cohort included only patients with clinically localized BCa. In this setting, NAC combined with consolidative local cancer control represents the mainstay treatment. The high chemo-sensitivity may be explained by the expression of basal molecular subtype features, which have been shown to be associated with response to cisplatin-based therapy [28]. Comprising a similar cohort of patients, Moschini et al. showed results that mirrored ours: over a 23-year period, they found that small-cell VH was associated with worse disease-specific outcomes on multivariable analysis [9]. This was also consistent with a systematic review, in which this VH was associated with poor survival [6]. Thus, small-cell VH requires strict surveillance and ideally a multimodal treatment strategy.

Stratification according to disease stages allowed us to better understand stage-specific survival outcomes. To the best of our knowledge this approach has not been used before to compare pure UC with UC + VH. At the organ-confined stage, we found that clear-cell VH was independently associated with worse DSS. Because this VH is very rare, prognosis and management of clear-cell tumours are poorly defined [5]. A systematic review including only 27 patients described this VH as aggressive with a high propensity to progress [29]. Our findings confirmed this behaviour. Thus, clear-cell histology could be used to select patients who require an intensive surveillance protocol and might benefit from adjuvant treatments, even at organ-confined stages. We found no relevant differences at multivariable analysis for locally advanced disease. Small-cell VH remained significantly associated with worse outcomes in patients with lymph node involvement. In a stage-matched analysis within the Surveillance, Epidemiology and End Results registry, Deuker et al. showed that neuroendocrine BCa was independently associated with cancer-specific mortality among patients with lymph node metastases [30]. These findings mirror our own.

The impact of concomitant CIS on survival outcomes after RC is still controversial. Considering 23 studies, Kimura et al. found that concomitant CIS was a proxy for aggressive disease in terms of DSS among patients with organ-confined disease at time of RC [31]. Conversely, Zhang et al. evaluated 18 845 patients from 24 studies, showing no significant correlation between this entity and the prognosis of BCa

patients undergoing radical treatment [32]. We found 406 patients (37.5%) with concomitant CIS. In the present series, concomitant CIS was independently associated with survival benefit among both the overall and node-positive cohorts before and after propensity-score matching. Hence, there is still uncertainty about the role of concomitant CIS as a prognosticator after upfront RC. In this context, it should be noted that our cohort comprised a group with aggressive disease, potentially influencing the survival analyses.

Four of the nine VHs (squamous, glandular, nested and micropapillary VH) exhibited almost the same DSS as pure UC if all clinico-pathological predictors were considered and after stage-based stratification. This could be explained by the rare occurrence of some VHs in our analysis. Furthermore, we applied stage-based stratification and did not use NAC, which may have different efficacy in UC + VH compared to pure UC. It should also be noted that we only included UC + VH and not pure VH, which has been found to exhibit different biological behaviour [30]. More studies with even larger IPD cohorts are needed to confirm our findings.

Taken together, our findings emphasize the crucial role of early detection of VHs at time of TURBT. Although the awareness of VHs has increased consistently over the years, discordance between TURBT and RC specimens was found in up to 50% of cases [7]. Further understanding of the biology underlying VHs may allow a tailored and personalized treatment paradigm. Because driver genomic alterations and transcriptional profiles underline the difference among VHs of UC, molecular subtyping classification appeared promising to delineate a more refined classification [33].

This study has some limitations, including its retrospective nature and its time span of over 30 years, during which different temporal practice patterns may have existed. Competing risks were not captured for overall survival estimation. Data on tumour size as well as data on patients who progressed from NMIBC to MIBC vs patients who presented with *de novo* MIBC were not captured. Moreover, with a rate of disease-specific events of approximately 50% and a percentage of occult pathological node-positive patients of 38.6%, our series comprised a cohort of patients with aggressive disease, which might not be representative of more contemporary RC series. It may well be that the VH distribution would differ in RC series with less advanced disease. Although this study represents a large multi-institutional IPD series, we were not able to capture data for microcystic, giant-cell and lipid-rich VHs. Additionally, the relatively small sample sizes of most of the VH subgroups is a concern for reliable multivariable analyses for DSS. Because we limited the study to patients who underwent upfront RC, our cohort did not receive NAC, which is the current standard of care for MIBC.

Strengths of this study include its pathology review of the RC specimens of a relatively large set of homogeneously treated IPD extracted from eight tertiary referral centres to study pure UC vs UC + VH. Although challenging, such a setting is necessary to study such rare variants.

In conclusion, one out of four patients with UC harboured a VH at time of RC. VHs were correlated with worse pathological features. In univariable analyses, sarcomatoid VH was associated with worse DSS, while lymphoepithelioma-like VH could be linked to DSS benefit. Clear-cell, plasmacytoid and small-cell VHs were independently associated with worse DSS. Accurate pathological diagnosis is imperative to ensure tailored counselling and to identify patients who require an intensive surveillance protocol and who may benefit from more aggressive management.

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Disclosure of Interests

None declared.

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Abbreviations: AC, adjuvant chemotherapy; BCa, bladder cancer; CIS, carcinoma *in situ*; DSS, disease-specific survival; HR, hazard ratio; IPD, individual patient data; IQR, interquartile range; LVI, lymphovascular invasion; MIBC,

muscle-invasive bladder cancer; NAC, neoadjuvant chemotherapy; NMIBC, non-muscle-invasive bladder cancer; PLND, pelvic lymph node dissection; PSM, positive surgical margin; RC, radical cystectomy; TURBT, transurethral resection of bladder tumour; UC, urothelial carcinoma; UTUC, upper tract urothelial carcinoma; VH, variant histology.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Consort flow diagram.

Table S1 Univariable Cox regression analysis stratified by histology group across the overall cohort (n = 1082), pT1-2N0 cohort (n = 286), pT3-4N0 cohort (n = 378) and pTanyN1-3 cohort (n = 418).

Table S2 Univariable Cox regression analysis stratified by histology group across overall (n = 596), pT1-2N0 (n = 124), pT3-4N0 (n = 238) and pTanyN1-3 (n = 234) propensity-score-matched cohorts.

Table S3 Multivariable Cox regression analysis stratified by histology group across overall (n = 596), pT1-2N0 (n = 124), pT3-4N0 (n = 238) and pTanyN1-3 (n = 234) propensity-score-matched cohorts.