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ORIGINAL ARTICLE

Challenging the prognostic impact of the new WHO and TNM classifications with special emphasis on HPV status in penile carcinoma

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Abstract

The evidence concerning prognostic parameters for clinical decision-making in penile cancer is either weak or missing. We therefore analysed the prognostic value of the revised TNM and WHO classification systems on relapse and survival with special emphasis on HPV status. We collected clinical data and tissue samples of 121 patients from centres in Germany and Russia. HPV genotyping and $p16^{INK4a}$ immunostaining were performed. The histological subtype and TNM were reclassified by two experienced uropathologists. Survival analyses were performed by Kaplan-Meier estimator and log-rank test. Uni- and multivariable analyses were performed by Cox proportional hazard model and Fisher's exact test for contingency analysis. HPV status was not found to be an independent prognostic factor. Histological subtypes differ in prognosis with the best outcome found in warty and the worst in basaloid carcinomas. Patients with pT1b defined by poor differentiation or lymphovascular invasion (LVI) had the shortest metastasis-free survival compared with pT1a (log-rank, $p = 0.02$). Lymph node metastasis and LVI were significantly associated with poor metastasis-free, cancer-specific and overall survival and could be identified as the only independent prognostic parameters. Prognostic value of TNM could not be improved using the 8th versus the 7th edition. In contrast to HPV status, histological subtypes are of prognostic value and should be an essential part of pathologic reports. The impact of the HPV status needs to be analysed in a subtype-specific manner. Parameters describing lymphatic dissemination have the highest impact on prognosis. Inclusion of tumour grade and LVI into a single T-category (pT1b) seems questionable.

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Introduction

Penile tumours are mainly squamous cell carcinomas (pSCC) and originate from the inner mucosal lining of the foreskin, glans, or coronal sulcus (approx. 90%) but rarely at the penile shaft (approx. 10%) [1, 2]. The incidence is increasing with age (mean age ~ 60 years) and varies regionally depending in part on socioeconomic conditions such as the lack of hygiene and preventive health care as well as religious habits like circumcision in childhood, which exhibits a protective effect [3, 4]. The age-standardised rate per 100,000 men ranges from 0 to 3.3 cases. The two most important risk factors are phimosis and infection with high-risk human papillomaviruses (hrHPV) [3, 5, 6]. The HPV prevalence in pSCC varies between 20 and 70% [7–10] and depends on histological subtypes [1, 2, 8, 10]. The usual type is the most common subtype (50–70%) followed by warty-basaloid (9–17%), papillary (7–15%), warty (3–10%) and basaloid (4–10%) carcinomas [2, 10–12]. Based on the hypothesis of independent pathways of carcinogenesis [10], the 4th edition of the WHO classification categorises pSCC regarding HPV followed by morphological features [1, 13]. Establishing prognostic parameters for pSCC is of high clinical impact in order to identify patients who are likely to benefit from invasive therapies such as early inguinal lymph node dissection or adjuvant systemic chemotherapy. Whether there is a prognostic value of HPV similar to that in oropharyngeal carcinomas [14] is controversially discussed in pSCC because of contradictory data [2, 8]. In order to improve risk stratification for patients with urologic cancers, the 8th edition of the TNM classification of malignant tumours updated staging parameters [15, 16]. However, the prognostic impact of the new system was not validated yet, which is certainly difficult because of the low incidence of the disease. Single centres hardly can collect a sufficient case number for validation studies. The aim of our international multicentre study was therefore to evaluate the prognostic value of the revised TNM and WHO classifications with special focus on the HPV status.

Material and methods

Cohort and study design

The cohort includes patients treated in Russia and Germany between 1992 and 2015.

Representative formalin-fixed and paraffin-embedded tissue (FFPE) samples from the primary tumour and metastasis and clinical data of 235 pSCC patients were collected and anonymised. Complete data sets including outcome data became available for 121 patients. Haematoxylin and eosin (H&E)–stained sections of all cases were reviewed by two experienced uropathologists and histological subtypes, tumour grade and lymphovascular invasion (LVI) were defined according to the 2016 WHO classification [1]. Tumours have been (re)classified according to the 7th and 8th editions of TNM classification of malignant tumours [15, 17]. pT3 and pT4 cases were grouped together because of the low number of pT4 tumours ($n = 2$).

Isolation of genomic DNA and HPV genotyping

DNA was isolated from FFPE tissue sections by QIAamp DNA FFPE Tissue Kit (Qiagen) following the manufacturer's protocol. HPV PCR was performed as described in [18] with GP5+/6+ primers [19] using the LightCycler 1.5 instrument (Roche Diagnostics GmbH). After initial denaturation at 95 °C for 15 min, 45 PCR cycles followed with denaturation at 95 °C for 10 s, annealing at 45 °C for 5 s and elongation at 72 °C for 18 s. SYBR green and gel electrophoresis were used for detection. HPV16- and HPV18 DNA served as positive controls. For HPV genotyping, the amplified DNA was sequenced by seq-it GmbH and analysed using the Basic Local Alignment Search Tool (BLAST, NCBI). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) PCR was performed as described in [20].

Tissue microarray construction

H&E-stained sections were digitalised. For tissue microarray construction, representative areas from the tumour centre, invasion front and normal foreskin as well as lymph node metastases were selected (Software: Panoramic Viewer, Version 1.15.4; 3DHistech Ltd.). Three cylinders per area (tumour centre, normal skin and lymph node tissue) with a diameter of 0.6 mm and 2 cylinders (invasive front) with a diameter of 2 mm were punched and transferred to respective recipient block using the TMA Grandmaster (Sysmex) as described previously [21].

Immunohistochemistry

In order to determine the HPV status, detection of HPV DNA by PCR and $p16^{INK4a}$ protein as a surrogate for HPV oncoprotein activity is recommended [22]. Tissue microarrays were stained with a monoclonal antibody against $p16^{INK4a}$ (Abcam, clone 1D7D2A1). The FFPE sections (4 μm) were deparaffinised and rehydrated (30 min, 60 °C; 3 × 5 min in

xylene; 2 × 1 min in 99% ethanol; 2 × 1 min in 70% ethanol; 2 × 1 min in double distilled water). The primary antibody was diluted 1:4000 in antibody diluent (Dako) and incubated for 1 h. Signal detection was performed using the EnVison™ Detection System (Dako). Between every antibody incubation step, a wash step (threefold 5 min) with TBS-Tween (50 mM Tris, 150 mM NaCl, 0.1% Tween-20) was implemented. The sections were dehydrated (1 min in 70% ethanol; 1 min in 99% ethanol; 3 × 5 min in xylene) and embedded. All steps were done at room temperature.

Statistical analyses

Statistical analyses were performed using R (version 3.5.1) and R-Studio (version 1.1.453). Fisher's exact test was used for contingency analysis. Kaplan-Meier estimator and log-rank test were performed for survival analyses. Uni- and multivariable analyses were performed by Cox proportional hazard analysis. A p value of < 0.05 was considered statistically significant.

Results

Pathological classification and HPV genotyping

The mean age of patients was 61.6 years (range, 25–88), and the mean follow-up time was 46.8 (1–176) months. The histological subtypes and HPV distribution within these subtypes is depicted in Table 1. The analysis of HPV DNA was successful in 115 tumours; DNA quality was insufficient in 6 patients (5%). HPV DNA could be detected in 49 (40.5%) patients. Of these, 3 were infected with low-risk types (HPV6 ($n = 1$) and HPV11 ($n = 2$)) and 44 with high-risk types (HPV16 ($n = 42$), HPV18 ($n = 1$), HPV59 ($n = 1$)). Sequencing failed in 2 cases. In HPV-positive metastasised tumours, only hrHPV was detected. HPV type of the primary tumour was concordant with that detected in the corresponding lymph node and organ metastases in all cases. Overexpression of $p16^{INK4a}$ was found in 72 (59.5%) cases. Of these, 26 cases were negative for HPV DNA and 2 hrHPV DNA positive cases were negative for $p16^{INK4a}$. Because of absent p16INK4a staining, these 2 cases were classified as HPV negative. In 106 patients, reclassification according to the 8th TNM system was possible based on the available data. Table 2 depicts the distribution of cases between the 7th and 8th edition.

Association of HPV with histopathological and clinical parameters

Correlations have been examined based on the 8th edition of the TNM classification. Only the histologic grade correlated with HPV. With increasing grade, the frequency of hrHPV-infected tumours increased significantly (Fisher's exact test, $p = 0.0001$) (Suppl. Table 1). This observation could be explained by the histologic subtype. G3 tumours were mainly (64%) represented by the HPV-related basaloid and warty-basaloid subtypes (Suppl. Table 2).

Survival analysis

Comparing the most common histological subtypes within our cohort, highest metastasis-free (MFS), cancer-specific (CSS) and overall (OS) survival was observed in patients with warty carcinomas. In contrast, shortest MFS occurred in patients with basaloid carcinomas (Fig. 1a, Table 3). MFS in patients with warty carcinomas favourably and significantly

Table 1 Histological subtype regarding the 2016 WHO classification and HPV genotyping

Histologic type (WHO 2016)		n	%	HPV negative		High-risk HPV		Not evaluable
				n	%	n	%	
Carcinoma in situ		2	1.7	1	50.0	1	50.0	–
Non-HPV-related squamous cell carcinomas	Usual type	61	50.4	44	80.0	11	20.0	6
	Pseudohyperplastic	6	5.0	6	100.0	0	0.0	–
	Pseudoglandular	1	0.8	–	–	–	–	1
	Pure verrucous	6	5.0	5	100.0	0	0.0	1
	Carcinoma cuniculatum	1	0.8	1	100.0	0	0.0	–
	Papillary	1	0.8	1	100.0	0	0.0	–
HPV-related squamous cell carcinomas	Basaloid	18	14.9	4	22.2	14	77.8	–
	Papillary-basaloid	1	0.8	0	0.0	1	100.0	–
	Warty	8	6.6	4	66.7	2	33.3	2
	Warty-basaloid	15	12.4	2	14.3	12	85.7	1
	Clear cell	1	0.8	0	0.0	1	100.0	–

Table 2 Comparison of the 7th vs. 8th edition of the TNM classification and clinico-pathologic parameters. *n/a* not available

TNM classification of malignant tumours ($n = 106$)			7th edition		8th edition	
			n	%	*n*	%
Primary tumour		pTis	2	1.9	2	1.9
		pT1a	36	34.0	35	33.0
		pT1b	13	12.3	14	13.2
		pT2	35	33.0	30	28.3
		pT3	18	17.0	23	21.7
		pT4	2	1.9	2	1.9
Regional lymph Nodes		N0	70	66.0	70	66.0
		cN0	34	32.0	34	32.0
		pN0	36	34.0	36	34.0
		pN1	11	10.4	11	10.4
		pN2	5	4.7	5	4.7
		pN3	14	13.2	14	13.2
		n/a	6	5.7	6	5.7
Distant metastasis		pM0	100	94.4	100	94.4
		pM1	4	3.7	4	3.7
		n/a	2	1.9	2	1.9
Histologic grade			*n*		%	
		G1	15		14.2	
		G2	58		54.7	
		G3	32		30.2	
		n/a	1		0.9	
Tumour characteristics			*n*		%	
Lymphovascular invasion		L0	92		86.8	
		L1	13		12.3	
		n/a	1		0.9	
Vascular invasion		V0	86		81.1	
		V1	19		18.0	
		n/a	1		0.9	
Perineural invasion		Pn0	88		83.0	
		Pn1	15		14.2	
		n/a	3		2.8	
Tumour extension	Corpus spongiosum	No	65		61.3	
		Yes	41		38.7	
	Corpus cavernosum	No	81		76.4	
		Yes	25		23.6	
	Urethra	No	87		82.1	
		Yes	18		17.0	
		n/a	1		0.9	
	Adjacent structures	No	104		98.1	
		Yes	2		1.9	

differed (log-rank, $p < 0.05$) from those with usual type. Furthermore, we observed a statistical trend towards a longer MFS compared with basaloid tumours (log-rank, $p = 0.08$). No significant prognostic difference could be demonstrated for warty-basaloid carcinomas versus other subtypes (log-rank, $p = 0.14$). For CSS and OS, no differences were observed.

HPV status was not associated with survival (Fig. 1b, Table 3).

For T-category or N-status, no survival difference between the 8th and 7th TNM classification could be demonstrated (Fig. 1c, Suppl. Fig. 2D, Suppl. Fig. 1A, Suppl. Fig. 2C). Patients with pT1b tumours are characterised by worst MFS. This phenomenon was more pronounced using the 8th edition

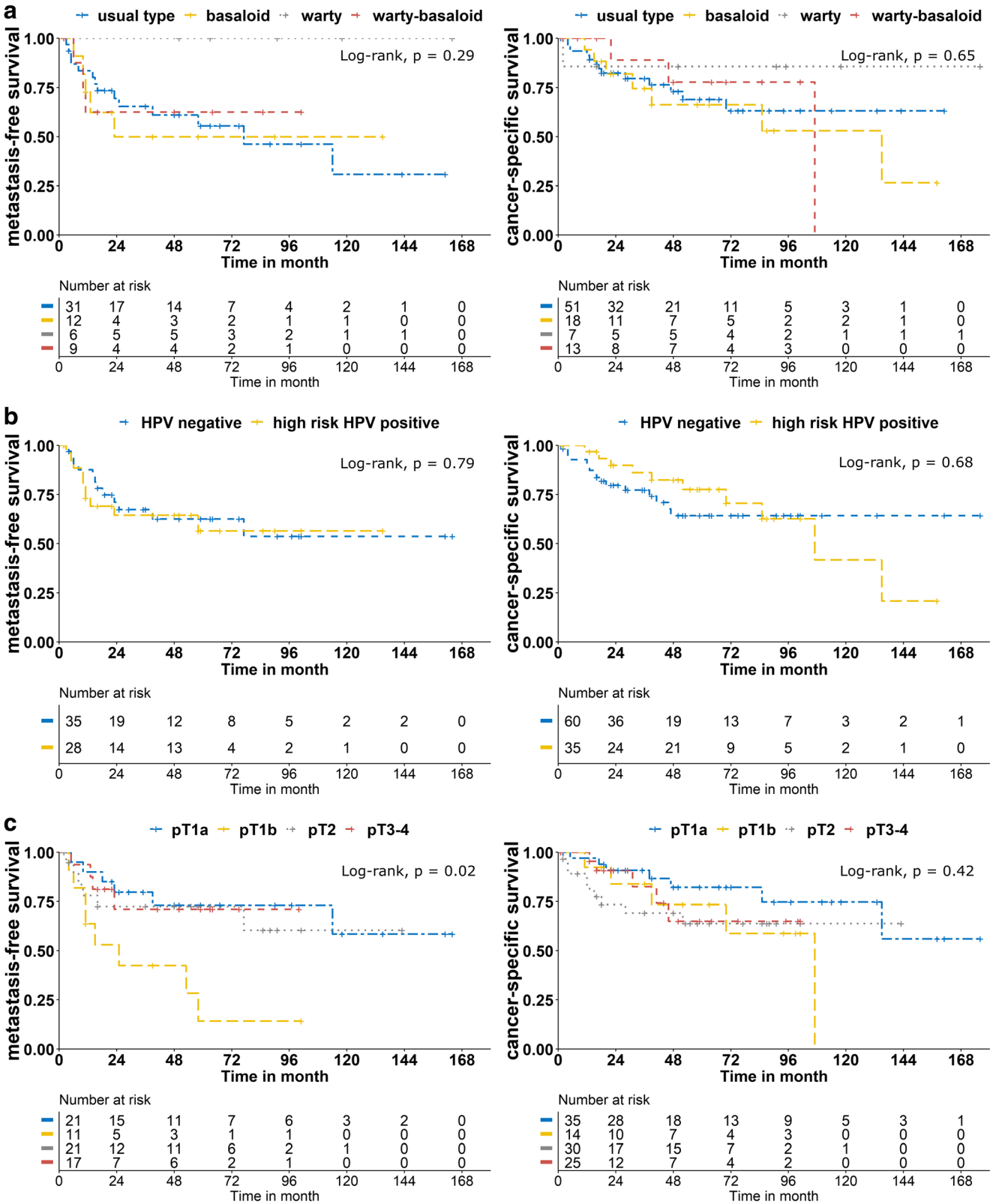

Fig. 1 Survival estimation by Kaplan-Meier for metastasis-free and cancer-specific survival by the histological subtype (**a**), the infection with high-risk HPV (**b**) and the T-category regarding the 8th TNM classification (**c**)

Table 3 Median survival times (log-rank) and 5-year survival rate regarding the histological subtype, HPV and clinico-pathologic parameters. *ND* not determinable

Clinico-pathological parameters		Metastasis-free survival			Cancer-specific survival			overall survival		
		Median survival		5-year survival rate	Median survival		5-year survival rate	Median survival		5-year survival rate
		Month	*p*	Survival (95%CI)	Month	*p*	Survival (95%CI)	Month	*p*	Survival (95%CI)
Histologic subtype	Usual type	77	0.29	0.55 (0.39–0.79)	ND	0.65	0.69 (0.55–0.86)	ND	0.48	0.64 (0.50–0.82)
	Basaloid	23		0.50 (0.16–0.95)	135		0.66 (0.46–0.96)	85		0.58 (0.37–0.91)
	Warty	ND		*1.00 (1.00–1.00)*	ND		0.86 (0.63–1.00)	ND		0.86 (0.63–1.00)
	Warty-basaloid	ND		0.63 (0.37–1.00)	107		0.78 (0.55–1.00)	107		0.58 (0.36–0.94)
HPV	Negative	ND	0.79	0.63 (0.47–0.83)	ND	0.68	0.64 (0.51–0.81)	ND	0.78	0.64 (0.51–0.81)
	High-risk positive	ND		0.56 (0.38–0.83)	107		0.78 (0.63–0.96)	107		0.66 (0.51–0.86)
Primary tumour	pT1a	ND	*0.02*	0.73 (0.55–0.97)	ND	0.42	0.82 (0.69–0.98)	ND	0.29	0.76 (0.62–0.94)
	pT1b	25		0.14 (0.02–0.82)	107		0.73 (0.51–1.00)	99		0.73 (0.51–1.00)
	pT2	ND		0.72 (0.55–0.96)	ND		0.64 (0.47–0.86)	ND		0.54 (0.38–0.78)
	pT3–4	ND		0.71 (0.50–1.00)	ND		0.65 (0.43–0.97)	100		0.61 (0.40–0.93)
Regional lymph nodes	pN0, cN0	ND	*0.004*	0.70 (0.57–0.87)	ND	*0.0001*	0.83 (0.71–0.96)	ND	*0.001*	0.79 (0.68–0.92)
	pN1	114		0.60 (0.33–1.00)	ND		0.60 (0.36–1.00)	ND		0.55 (0.32–0.94)
	pN2–3	14		0.33 (0.15–0.74)	39		0.36 (0.16–0.83)	46		0.31 (0.13–0.76)
Histologic grade	G1	ND	0.21	0.68 (0.37–1.00)	ND	0.29	0.73 (0.47–1.00)	99	0.53	0.68 (0.53–1.00)
	G2	114		0.68 (0.54–0.87)	ND		0.73 (0.61–0.88)	135		0.65 (0.52–0.81)
	G3	58		0.46 (0.26–0.79)	107		0.69 (0.53–0.90)	100		0.66 (0.50–0.87)
Vascular invasion	V0	ND	0.06	0.70 (0.58–0.85)	135	0.38	0.75 (0.64–0.87)	135	0.28	0.69 (0.58–0.81)
	V1	23		0.32 (0.12–0.88)	ND		0.63 (0.43–0.92)	99		0.54 (0.33–0.88)
Lymphovascular invasion	L0	ND	*0.0003*	0.72 (0.60–0.86)	ND	*0.001*	0.75 (0.64–0.87)	135	*0.002*	0.71 (0.61–0.83)
	L1	16		0.00	31		0.18 (0.03–0.94)	31		0.29 (0.10–0.84)
Perineural invasion	Pn0	114	*0.01*	0.69 (0.56–0.85)	ND	*0.03*	0.76 (0.66–0.88)	107	0.17	0.69 (0.58–0.81)
	Pn1	20		0.25 (0.08–0.80)	135		0.54 (0.32–0.90)	135		0.54 (0.32–0.90)

Italics entries indicate $p < 0.05$

(log-rank, $p = 0.02$) compared with the 7th edition (log-rank, $p = 0.04$) of TNM classification (Fig. 1c, Table 3, Suppl. Fig. 1A). Depth of tumour invasion serves as separation criterion between pT2 (corpus spongiosum) and pT3 (corpus cavernosum) (Suppl Table 3). In 5 patients, tumours have been upstaged from pT2 into pT3 due to corpus cavernosum infiltration (Table 2). However, no significant differences in survival could be observed between these categories (Fig. 1c).

Because of the low number of pN2 ($n = 5$) patients, pN2 and pN3 were grouped together. With increasing number of lymph node metastases, MFS, CSS and OS decrease significantly (log-rank, MFS, $p = 0.004$; CSS, $p = 0.0002$; OS, $p = 0.001$) (Fig. 2a, Table 3, Suppl. Fig. 2E).

With increasing histological grade, MFS, CSS and OS become worse, but without reaching the level of significance (log-rank, MFS, $p = 0.21$; CSS, $p = 0.29$; OS, $p = 0.53$) (Table 3, Suppl. Fig. 2B, Suppl. Fig. 2F). Vascular invasion was not significantly associated with survival (log-rank, MFS, $p = 0.06$; CSS, $p = 0.38$; OS, $p = 0.28$) (Table 3, Suppl. Fig. 1C, Suppl. Fig. 2G).

Lymphovascular and perineural invasions distinguish stages pT1a and pT1b. Survival significantly decreases for patients exhibiting lymphovascular (log-rank, MFS; CSS and OS $p \leq 0.001$, respectively) or perineural (log-rank, MFS, $p = 0.01$; CSS, $p = 0.03$; OS, $p = 0.17$) invasions (Fig. 2b, c, Table 3, Suppl. Fig. 2H-I).

In univariable testing, pT1b and perineural invasions were significantly associated with MFS and CSS but failed to reach significance in multivariable analysis. Only lymph node metastases and LVI were significantly associated with MFS, CSS and OS in both univariable and multivariable testing (Table 4).

Discussion

In order to individualise the decision-making process with respect to early invasive therapies in patients with pSCC

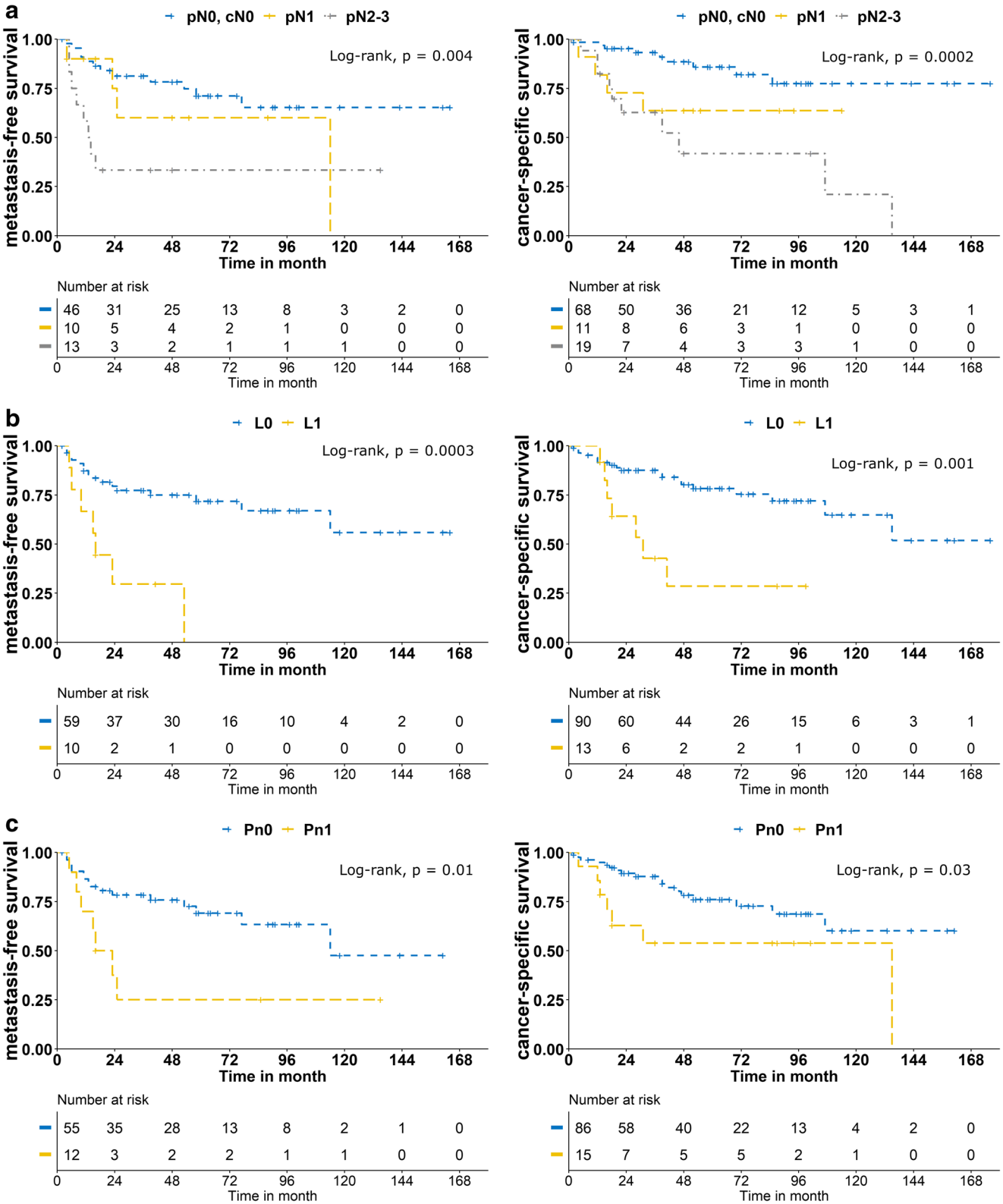

Fig. 2 Survival estimation by Kaplan-Meier for metastasis-free and cancer-specific survival by regional lymph node metastasis (**a**), lymphovascular (**b**) and perineural (**c**) invasion

without radiological evidence of metastases, robust prognostic parameters are warranted. Single-centre studies often are limited by small patient cohorts. We, therefore, collected a larger cohort by a comprehensive international multicentre attempt in order to overcome such limitations and to investigate putative prognostic parameters on the basis of a more

Table 4 Uni- and multivariable Cox proportional hazard regression analysis

Univariable cox regression							
Clinico-pathological parameters		Metastases-free survival		Cancer-specific survival		Overall survival	
		HR (95%CI)	*p*	HR (95%CI)	*p*	HR (95%CI)	*p*
Primary tumour	pT1a	Reference		Reference		Reference	
	pT1b	4.1 (1.4–12.2)	*0.01*	2.2 (0.7–7.1)	0.18	2.0 (0.7–5.8)	0.19
	pT2	1.3 (0.4–4.2)	0.61	2.2 (0.8–6.0)	0.13	2.3 (1.0–5.5)	0.07
	pT3–4	1.2 (0.3–4.3)	0.82	1.7 (0.5–5.4)	0.41	1.8 (0.7–4.9)	0.26
Regional lymph nodes	pN0, cN0	Reference		Reference		Reference	
	pN1	1.9 (0.6–5.8)	0.28	2.9 (0.9–9.6)	0.07	2.4 (0.9–6.7)	0.17
	pN2–3	4.3 (1.7–10.7)	*0.002*	5.6 (2.3–13.9)	*< 0.001*	3.9 (1.8–8.7)	*< 0.001*
Histologic grade	G1	Reference		Reference		reference	
	G2	1.9 (0.4–8.9)	0.40	1.9 (0.4–8.5)	0.38	1.3 (0.4–3.7)	0.68
	G3	3.3 (0.7–15.1)	0.13	3.0 (0.7–13.8)	0.16	1.8 (0.6–5.5)	0.33
Vascular invasion	V0	Reference		Reference		Reference	
	V1	2.3 (0.9–5.6)	0.07	1.5 (0.6–3.8)	0.38	1.5 (0.7–3.4)	0.29
Lymphovascular invasion	L0	Reference		Reference		Reference	
	L1	4.8 (1.9–12.0)	*0.001*	4.0 (1.6–9.8)	*0.002*	3.4 (1.5–7.8)	*0.003*
Perineural invasion	Pn0	Reference		Reference		Reference	
	Pn1	3.4 (1.2–7.2)	*0.02*	2.6 (1.1–6.2)	*0.04*	1.8 (0.8–4.1)	0.18
Multivariable cox regression (age adjusted)							
Clinico-pathological parameters		Metastases-free survival		Cancer-specific survival		Overall survival	
		HR (95%CI)	*p*	HR (95%CI)	*p*	HR (95%CI)	*p*
Primary tumour	pT1a	Reference		Reference		Reference	
	pT1b	1.5 (0.3–8.2)	0.62	0.7 (0.1–6.0)	0.76	1.1 (0.2–6.6)	0.95
	pT2	0.5 (0.1–2.4)	0.38	1.9 (0.4–8.9)	0.44	1.8 (0.5–7.1)	0.39
	pT3–4	0.4 (0.1–2.2)	0.28	0.9 (0.2–4.2)	0.90	1.2 (0.3–4.5)	0.76
Regional lymph nodes	pN0, cN0	Reference		Reference		Reference	
	pN1	1.6 (0.4–6.2)	0.54	2.8 (0.7–12.0)	0.16	2.9 (0.9–9.9)	0.09
	pN2–3	3.7 (1.2–11.0)	*0.02*	12.4 (3.4–45.3)	*< 0.001*	8.0 (2.6–24.3)	*< 0.001*
Histologic grade	G1	Reference		Reference		Reference	
	G2	4.6 (0.6–38.6)	0.16	4.0 (0.4–43.4)	0.25	3.5 (0.6–20.4)	0.17
	G3	7.4 (0.9–63.0)	0.07	4.1 (0.4–48.8)	0.26	3.0 (0.5–19.2)	0.24
Vascular invasion	V0	Reference		Reference		Reference	
	V1	1.4 (0.4–5.3)	0.66	0.2 (0.05–0.9)	0.04	0.4 (0.1–1.3)	0.14
Lymphovascular invasion	L0	Reference		Reference		Reference	
	L1	9.6 (1.5–60.7)	*0.01*	28.0 (4.9–157.0)	*< 0.001*	15.8 (3.7–67.9)	*< 0.001*
Perineural invasion	Pn0	Reference		Reference		Reference	
	Pn1	0.5 (0.1–2.3)	0.39	0.4 (0.1–1.4)	0.13	0.3 (0.1–1.0)	*0.04*

Italics entries indicate $p < 0.05$

representative database. The situation is further complicated by the fact that about 50% of tumours are HPV associated. Therefore, at least two different molecular pathways have to be assumed. Consequently, the new histological classification differentiates between HPV- and non-HPV-related subtypes. One would therefore also assume a prognostic role of HPV infection as shown for head and neck tumours [14]; literature data, however, remained contradictory so far.

In our cohort, HPV could not be proven as a prognostic factor. We further investigated the association between histological subtypes and outcome. The data indicate that the histological subtype has prognostic potential. However, definition and report of subtypes is still not a standard in clinical routine. In accordance with other studies, the HPV-related basaloid and the non-HPV-related usual tumours represent more aggressive subtypes with high metastatic potential. On the other side of the spectrum, the HPV-related warty subtype exhibits no metastatic potential and an excellent survival suggesting that local treatment is sufficient. These findings indicate that the HPV status alone cannot define tumour aggressiveness. In addition, regardless of the categorisation into non-HPV- and HPV-related subtypes, HPV is not present or absent

with 100% reliability within each histological subtype [13]. The role of HPV therefore needs to be clarified within the particular subtypes in order to definitively answer the question of a prognostic and biological role of HPV in penile carcinomas. In our study, the case numbers in each subtype still were not sufficient to analyse the prognostic role of HPV within these subgroups.

We found an association of HPV with histological grade. HrHPV was more present in G3 tumours, which is in contrast to the association of HPV and better clinical outcome in oropharyngeal tumours [14]. A recent study also reports a positive correlation of hrHPV and high-grade pSCC [23], which may be explained by the high percentage of G3 tumours in the HPV-related basaloid and warty-basaloid carcinomas. In our cohort, the poorly differentiated tumours (G3) revealed a higher risk to metastasise and to cancer-related death as well as shortened median MFS and CSS, albeit without statistical significance. Other studies reported a clear difference between poorly and well/moderately differentiated tumours regarding recurrence and cancer-specific survival [24–26]. Nevertheless, we could not prove grade as an independent prognostic factor.

In addition to grade, TNM classification represents the most important prognostic factor in tumour diseases. In order to improve the prognostic value in pSCC, TNM was optimised in the 8th edition based on data suggesting a better correlation with lymph node involvement and outcome [16]. Nevertheless, we did not observe any outcome difference when comparing the 7th and the 8th edition. Surprisingly, the worst outcome was found in the pT1b group, especially when regarding MFS. pT1 is subdivided into pT1a and pT1b based on histologic grade (G), lymphovascular (L) and in the 8th edition also perineural (Pn) invasion, which was suggested to better correlate with inguinal lymph node involvement [16, 27]. Incorporation of parameters which are not related to tumour size or depth of invasion is uncommon in the TNM classification. The inclusion of tumour grade into the staging system may therefore be questionable. Moreover, the reproducibility of grading was shown to be poor even among pathologists with a focus in uropathology [28]. In our study, lymphovascular (uni- and multivariable) and perineural (univariable) invasions were the best pT-independent prognostic parameters. Therefore, G, L and Pn should be further evaluated as independent prognostic parameters and should not be incorporated into a TNM category. Lymphovascular invasion was the most important predictor for lymph node involvement in our study and could serve as an additional decision tool for lymphadenectomy. As shown in earlier studies, invasion into the corpus cavernosum is associated with a high risk of lymph node metastasis [29, 30]. These tumours became therefore categorised as pT3. In contradiction, pT2 is restricted to the invasion of the corpus spongiosum. However, when comparing the 7th with the 8th TNM edition, we did not observe a better differentiation between pT2 and pT3 tumours in terms of lymph node metastatic risk or long-term outcome, which was very recently demonstrated by Li et al. as well [31]. Whether nomograms as published by Sun et al. will be more clinically relevant compared with the current TNM classification has to be evaluated [30].

Our results indicate that parameters describing the dissemination potential of the primary tumour such as lymphovascular invasion and perineural invasion could have the highest impact for the prediction of metastasis and survival of patients with pSCC.

The limitations of this study are the retrospective character and the low case numbers in some of the subgroups limiting the statistical robustness and the use of more precise prediction models. Thus, the analysis for MFS and CSS considering competing risks [32] could not be performed due to small numbers of events. Furthermore, patients have been treated between 1992 and 2015 with heterogeneous therapeutic options. Therefore, we continue data acquisition in order to validate our results in independent cohorts and to develop more accurate prediction models for metastatic risk and survival.

Conclusions

HPV status has no prognostic impact on metastatic risk and survival after local treatment of pSCC. However, its prognostic role within subtypes of the disease has to be elucidated. Histological subtypes, on the other hand, have a prognostic impact and should therefore be an essential part of pathology reports.

Lymph node metastases and lymphovascular invasion have the highest prognostic impact and must therefore be regarded as the most important parameters for clinical decision-making. Mixing grading or independent prognostic invasion features into a particular T-category like pT1b seems questionable.

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Contribution statement Authors KJ, AH, SH and VM conceived and designed the study. SH, OK, AP, RB, PL, HL, HW, XK, SU, TP and CG acquired data. SH, AH, AA, SS, JH, MS, MJ, JS, SW, AP and KJ have been involved in data analysis and interpretation. SH, JH, KJ, MS, AH, AA and SS wrote and edited the manuscript. All authors reviewed the manuscript. All authors gave final approval for publication. Author KJ takes full responsibility for the work as a whole, including the study design, access to data and the decision to submit and publish the manuscript.

Compliance with ethical standards

Because of the retrospective character of this investigation, informed consent could be obtained only from a part of patients. Therefore, data have been analysed anonymously.

Conflict of interest The authors declare that they have no conflicts of interest.

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