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Original Article

A novel grading approach predicts worse outcomes in stage pT1 non-muscle-invasive bladder cancer

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Objective

To develop a prognostically relevant scoring system for stage pT1 non-muscle-invasive bladder cancer (NMIBC) incorporating tumour budding, growth pattern and invasion pattern because the World Health Organisation grading system shows limited prognostic value in such patients.

Patients and Methods

The tissue specimens and clinical data of 113 patients with stage pT1 NMIBC who underwent transurethral resection of bladder tumour were retrospectively investigated. Tumour budding, and growth and invasion patterns were evaluated and categorised into two grade groups (GGs). GGs and other clinical and histopathological variables were investigated regarding recurrence-free survival (RFS), progression-free survival (PFS), cancer-specific survival (CSS) and overall survival (OS) using univariable and multivariable Cox regression analyses.

Results

The integration of two tumour budding groups, two growth patterns, and two invasion patterns yielded an unfavourable GG ($n = 28$; 24.7%) that had a high impact on oncological outcomes. The unfavourable GG was identified as an independent RFS and OS predictor ($P = 0.004$ and $P = 0.046$, respectively) and linked to worse PFS ($P = 0.001$) and CSS ($P = 0.001$), irrespective of the European Association of Urology risk group. The unfavourable GG was associated with higher rates of BCG-unresponsive tumours ($P = 0.006$). Study limitations include the retrospective, single-centre design, diverse therapies and small cohort.

Conclusions

We present a morphology-based grading system for stage pT1 NMIBC that correlates with disease aggressiveness and oncological patient outcomes. It therefore identifies a highest risk group of stage pT1 NMIBC patients, who should be followed up more intensively or receive immediate radical cystectomy. The grading incorporates objective variables assessable on haematoxylin and eosin slides and immunohistochemistry, enabling an easy-to-use low-cost approach that is applicable in daily routine. Further studies are needed to validate and confirm these results.

Keywords

bladder cancer, EMT, grade group, NMIBC, tumour budding

Introduction

Urothelial carcinoma of the bladder is the 10th most common malignancy worldwide, accounting for 3% of global cancer diagnoses [1]. Approximately 75% of patients present with non-muscle-invasive bladder cancer (NMIBC) at initial diagnosis [2]. The term NMIBC covers a heterogenous spectrum of tumours, prompting the European Association of Urology (EAU) to stratify patients into four prognostic risk groups for tumour progression: low, intermediate, high and very-high risk [2]. The classification was updated in 2021 and proposed tumour stage, grading, concomitant carcinoma

in situ (CIS), number of tumours, tumour diameter, and age as independent risk factors for tumour progression [2,3]. Stage pT1 NMIBC is characterised by lamina propria invasion and makes up the majority of high-risk and very-high-risk patients. Due to potential residual disease (approximately 50%) and potential understaging (approximately 10%), a second resection within 2–6 weeks is recommended [2,4]. Disease management remains complicated considering that up to 44% of patients show progression to muscle-invasive disease within 5 years, while another 30% never experience disease recurrence [3]. Immediate radical cystectomy (RC) is considered the safest approach from an oncological point of view; however, this is associated with potentially severe complications and restrictions to quality of life and may represent overtreatment in certain patients [2,5].

Conventional pathological grading according to the WHO has limited prognostic value in the context of muscle-invasive bladder cancer (MIBC) and stage pT1 NMIBC, since the majority are considered high grade [6–9]. Recently, a novel pathological MIBC grading approach was proposed [10]. This incorporates histomorphological phenotypes, stromal tumour infiltrating lymphocytes (sTILs), tumour budding, and growth and spreading patterns, which were merged to form four grade groups (GGs) with high impact on survival outcomes [10]. Tumour budding, invasion patterns and growth patterns are well recognised as survival predictors in several cancers, including bladder cancer [7,11–15].

The aim of the current study was to investigate a prognostically relevant histological grading approach for stage pT1 NMIBC based on the above-mentioned histopathological variables.

Patients and Methods

Study Population

The retrospective patient cohort consisted of patients with histopathologically proven initial stage pT1 NMIBC who underwent transurethral resection of bladder tumour (TURBT) between 2007 and 2015 at the Department of Urology of the University of Regensburg at the Caritas St. Josef Medical Center in Regensburg. All patients underwent re-resection or RC, and patients with upper urinary tract urothelial cancer were excluded. Patients were treated according to current EAU guidelines and at the discretion of physicians. The tissue samples were stored as formalin-fixed paraffin-embedded blocks.

Immunohistochemical Staining for Pancytokeratin and Assessment of Tumour Budding, sTILs, Growth and Invasion Patterns, and GGs

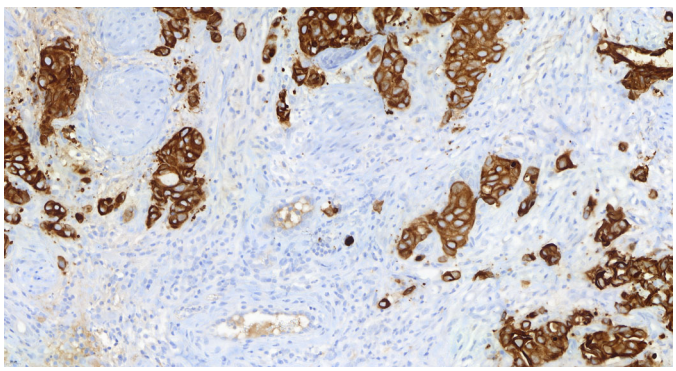
Immunohistochemical staining was performed on 4-µm tissue sections using an automated Ventana Benchmark Ultra autostainer (Ventana, Tucson, AZ, USA). Tissue sections were deparaffinised, antigens were retrieved by heat treatment in a Tris/Borate/EDTA solution pH 8.4 (Ventana), and endogenous peroxidase was blocked with 1% H_2O_2. Pancytokeratin was stained using an AE1AE3-antibody cocktail (Zytomed, Friedrichshafen, Germany). The stained tissue sections were scanned using a Panoramic P250 slide scanner (3DHistech, Budapest, Hungary), and tumour budding was evaluated using the open source software QuPath with a digital slide viewer [16].

Evaluation of tumour budding, sTILs, and growth and invasion patterns was performed by an experienced uropathologist (M.E.) without knowledge of the clinical characteristics and the clinical course, as described previously, and by the first author (M.H.), a resident in urology [10]. The morphological categories were defined by strict criteria as described previously [10]. In that previous article it was demonstrated that all categories were highly reproducible with excellent intra-class correlations and substantial/perfect interobserver agreement [10].

M.H. received 1 day of training and carried out scoring independently after a short training phase. Subsequently, all cases were independently re-revaluated by the pathologist.

As defined previously, tumour budding was quantified in five representative high-power fields (HPFs) under 400× magnification. Tumour buds were defined as single cells or clusters of 2–5 cells in the stroma at the invasion front of the tumour without connection to the main tumour mass (Fig. 1). The median bud count was calculated as the median number of tumour buds in all evaluated HPFs [10,14,17]. A median bud count of ≥0.9 was identified as a reasonable cut-off, indicating a worse prognosis in the context of stage pT1 NMIBC in a prior study [14]. The specific threshold was adjusted to 1 for enhanced interpretation and applicability without causing any changes in the distribution of patients. The growth pattern of invasive carcinoma areas was evaluated in two categories: (1) 'cohesive', comprising predominantly

Fig. 1 Example of tumour budding at the invasion front.



large cohesive tumour sheets and only small amounts of desmoplastic stroma and (2) 'discohesive', comprising invasive tumour cells composed of small cell nests without larger cohesive areas. Detailed approaches to assessment of these patterns were recently described elsewhere [10]. Unlike in MIBC, we did not observe cases with pure single-cell growth patterns [10].

Invasion patterns were defined as 'compact' with a well circumscribed and easily distinguishable tumour bulk (tumour deposits within one 200 × HPF allowed) or as 'discontinuous' in case of diffusely spreading tumour deposits with a distance of more than one 200 × HPF from the main tumour mass, or in cases where there was no main tumour bulk at all, but just loosely arranged tumour nests, sheets, or single cells separated by desmoplastic stroma spaces, as described in the aforementioned publication [10].

Stromal tumour-infiltrating lymphocytes were assessed semiquantitatively in the desmoplastic tumour stroma on one selected haematoxylin and eosin (H&E) slide, as already described [18]. Necrotic or large fibrotic areas, which are rarely found in pT1 stage carcinomas, were excluded from sTILs scoring.

Statistical Analysis

Statistical analysis was conducted with SPSS version 29.0 (IBM, Eblingen, Germany) and GraphPad Prism version 10 (GraphPad, San Diego, CA, USA). Recurrence-free survival (RFS) was defined as time from diagnosis (TURBT) to the first histologically proven NMIBC (pTa, pT1 or CIS). Progression-free survival (PFS) was defined as time from diagnosis (TURBT) to the first histologically proven MIBC in TURBT or RC (≥pT2), or in case of metastatic disease, during further tumour monitoring. In patients who had already undergone RC, detection of local tumour recurrence or metastasis was considered as progression. Each tumour progression was also considered as a recurrence, unless a recurrence had occurred earlier. Overall survival (OS) was defined as time from diagnosis to death, irrespective of the cause of death. Cancer-specific survival (CSS) was defined as time from diagnosis to death, in case the death was due to the underlying bladder cancer. RFS, PFS, CSS and OS rates were calculated by Kaplan–Meier analysis and tested for significance with the log rank test for the overall population and for those patients who received BCG instillation therapy. Univariable and multivariable Cox regression analyses were used to assess the value of tumour budding, invasion pattern, growth pattern and clinical and histopathological parameters for RFS, PFS, CSS and OS. Differences between the favourable and unfavourable GGs were evaluated using chi-squared and Fisher's exact tests for categorical variables. P values <0.05 were taken to indicate statistical significance. All analyses were considered two-tailed.

Ethics Approval

All findings, data acquisition and processing complied with the ethical standards described in the latest declaration of Helsinki. The study was approved by the local ethics committee of the University of Regensburg (Nr. 16-321-101).

Results

Patient Cohort

From 2007 to 2015, 141 patients were diagnosed with pT1 NMIBC through TURBT. Twenty-four patients with inadequate tissue for immunohistochemical staining were excluded and four patients were lost to follow-up. The remaining 113 patients (80.1%) were included in the study. The median age was 71 (63–79) years, with 83.2% males. Recurrence affected 43 patients (38.1%), 19 patients (16.8%) progressed to MIBC, and 32 patients (28.3%) died during the study (12.4% cancer-specific). The median (interquartile range) follow-up of surviving patients was 66 (39–104) months. Twenty-six patients (23%) underwent RC, 73 patients (64.6%) received BCG, 15 patients (13.3%) received mitomycin C, and 11 patients (9.7%) neither underwent RC nor received instillation therapy. Of the patients who underwent RC, 12 had received an instillation therapy pre-surgery (Table 1).

Table 1 Clinical characteristics and follow-up information.

Variable	
Patient data, *n* (%)	
Total patients	113 (100)
Male patients	94 (83.2)
Age, median (IQR) years	71 (63–79)
Treatment, *n* (%)	
Instillation therapy	88 (77.9)
BCG	73 (64.6)
MMC	15 (13.3)
RC, *n* (%)	26 (23)
Pathological T stage at RC, *n* (%)	
pT0	3 (11.5)
pTa/is/1	12 (46.1)
pT2	5 (19.2)
pT3	4 (15.4)
pT4	2 (7.7)
Follow-up information	
Follow-up of surviving patients, median (IQR) months	66 (39–104)
Maximum follow-up, months	160
Recurrence (≤pT1), *n* (%)	43 (38.1)
Progression (≥pT2), *n* (%)	19 (16.8)
Death, *n* (%)	32 (28.3)
Death by disease, *n* (%)	14 (12.4)

IQR, interquartile range; MIBC, muscle-invasive bladder cancer; MMC, mitomycin C; NMIBC, non-muscle-invasive bladder cancer; RC, radical cystectomy.

Histopathological Parameters and EAU Risk Groups

Concomitant CIS was evident in 44.2% of patients ($n = 50$), multifocal tumours in 62.8% ($n = 71$), tumours ≥30 mm in diameter in 52.2% ($n = 59$), WHO 2004/2016 high-grade tumours in 96.5% ($n = 109$) and WHO 1973 Grade 3 tumours in 80.5% ($n = 91$). According to the EAU NMIBC 2021 risk model, 50.4% of patients ($n = 57$) were high risk and 49.6% ($n = 56$) were very high risk (Table 2, Fig. 2). At RC, 15 patients (57.7%) were assessed as having the favourable GG and 11 patients (42.3%) the unfavourable GG. Upstaging was evident in 7/11 patients (63.6%) within the unfavourable GG and in 4/15 patients (26.7%) within the favourable GG. The differences were not statistically significant ($P = 0.109$).

Tumour Budding, sTILs, Growth and Invasion Pattern

Tumour features included a median bud count ≥1 in 68 patients (60.2%), a discontinuous invasion pattern in 11 patients (9.7%), and a discohesive growth pattern in 27 patients (23.9%). sTILs were categorised into tertiles: TIL1 (<15%), TIL2 (15 to <25%), and TIL3 (≥25%), comprising 26.5% ($n = 30$), 31.9% ($n = 36$), and 41.6% of patients ($n = 47$), respectively.

Factors Associated with Worse RFS, PFS and CSS

Multifocal tumour growth was associated with worse RFS ($P = 0.004$) and PFS ($P = 0.023$). A median bud count of ≥1 (p_1), discontinuous invasion pattern (p_2), and discohesive growth pattern (p_3) related to worse RFS ($p_1 = 0.002$, $p_2 < 0.001$, $p_3 < 0.001$) and CSS ($p_1 < 0.019$, $p_2 < 0.010$, $p_3 = 0.003$). A discontinuous invasion pattern (p_1) and discohesive growth pattern (p_2) were also associated with worse PFS ($p_1 = 0.005$, $p_2 = 0.002$). Details in Table 3.

Establishment of a Two-Tiered GG System

The presence or number of sTILs had no impact on the oncological outcomes in our cohort and therefore was not captured in our grading system (Table 3) [10]. The grading system was based on tumour budding, growth patterns, and invasion patterns, which were each weighted with 1 point. A score of ≥2 proved statistically significant and was therefore used as cut-off, creating favourable (0–1 point) and unfavourable (2–3 points) GGs (Table 2, Table S1).

Unfavourable GG is an Independent Predictor of RFS and OS, and is Associated with Worse PFS, and CSS, Irrespective of EAU Risk Group

The unfavourable GG comprised 28 patients (24.7%) and predicted worse RFS, PFS, CSS and OS independent of the EAU risk groups. Within the unfavourable GG, tumour

Table 2 Histopathological variables and resulting grade groups.

Variable	*n* (%)
Focality	
Unifocal	42 (37.2)
Multifocal	71 (62.8)
Tumour diameter (largest)	
<3 cm	54 (47.8)
≥3 cm	59 (52.2)
Concomitant CIS	
No	63 (55.8)
Yes	50 (44.2)
Grading WHO 1973	
G1	0 (0)
G2	22 (19.5)
G3	91 (80.5)
Grading WHO 2016	
Low-grade	4 (3.5)
High-grade	109 (96.5)
EAU risk classification	
High risk	57 (50.4)
Very high risk	56 (49.6)
Median bud count	
<1	45 (39.8)
≥1	68 (60.2)
Tumour invasion pattern	
Compact	102 (90.3)
Discontinuous	11 (9.7)
Tumour growth pattern	
Cohesive	86 (76.1)
Discohesive	27 (23.9)
Stromal tumour-infiltrating lymphocytes	
<15%	30 (26.5)
15 to <25%	36 (31.9)
25%	47 (41.6)
Grade group	
Favourable	
0 points	45 (39.8)
1 point	40 (35.4)
Total	85 (75.2)
Unfavourable	
2 points	18 (15.9)
3 points	10 (8.8)
Total	28 (24.7)

CIS, carcinoma in situ; EAU, European Association of Urology; IQR, interquartile range; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer.

recurrence (64.3% vs 29.4%; $P = 0.001$), progression to MIBC (35.7% vs 10.6%; $P = 0.002$), cancer-specific death (28.6% vs 7.1%; $P = 0.003$), and overall mortality (42.9% vs 23.5%; $P = 0.049$) were significantly higher (Fig. 2), which corresponds to a higher risk of recurrence ($P < 0.001$), progression to MIBC ($P = 0.001$), cancer-specific death ($P = 0.001$), and overall mortality ($P = 0.015$) in Kaplan–Meier analysis (Fig. 3). Multivariable Cox regression identified unfavourable GG (odds ratio [OR] 2.577, 95% CI 1.361–4.880; $P = 0.004$) and multifocal tumour growth (OR 2.308, 95% CI 1.029–5.177; $P = 0.042$) as RFS predictors. Unfavourable GG (OR 2.120, 95% CI 1.013–4.439; $P = 0.046$), age ≥70 years (OR 3.096, 95% CI 1.494–6.417; $P = 0.002$) and instillation therapy (OR 0.328, 95% CI 0.156–0.693; $P = 0.003$) were identified as OS predictors (Table 4). In order

Fig. 2 Distribution of age, gender, WHO 1973 grading, WHO 2004/2016 grading, European Association of Urology (EAU) risk group, treatment strategies (radical cystectomy and instillation therapy), and oncological outcomes (recurrence-free survival, progression-free survival, cancer-specific survival) with regards to the respective grade group. CS, cancer-specific; HG, high-grade; HR, high-risk; LG, low-grade; MMC, mitomycin C; VHR, very-high-risk.

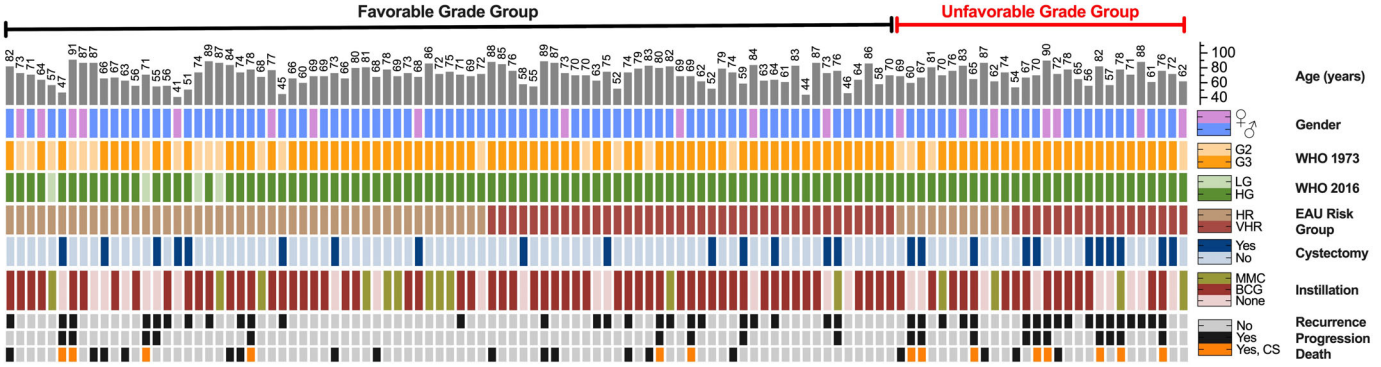


Table 3 Univariable Cox regression analyses of variables associated with recurrence-free, progression-free, cancer-specific and overall survival, respectively.

Variables	Endpoint: RFS (Events $n = 43$)		Endpoint: PFS (Events $n = 19$)		Endpoint: CSS (Events $n = 14$)		Endpoint: OS (Events $n = 32$)	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (continuous)	1.009 (0.982–1.037)	0.528	1.005 (0.965–1.046)	0.815	1.035 (0.985–1.088)	0.171	1.058 (1.022–1.095)	**0.001***
Age dichotomous (cut-off: 70 years)	1.322 (0.709–2.466)	0.380	1.667 (0.666–4.170)	0.275	2.571 (0.887–7.449)	0.082	2.532 (1.274–5.141)	**0.010***
Gender	0.901 (0.401–2.027)	0.801	0.562 (0.130–2.433)	0.441	0.834 (0.186–3.733)	0.812	0.710 (0.249–2.027)	0.522
Grading 1973	2.043 (0.803–5.196)	0.134	1.336 (0.389–4.591)	0.645	0.891 (0.248–3.203)	0.860	0.909 (0.392–2.106)	0.832
Grading 2016	1.926 (0.265–14.011)	0.517	0.742 (0.095–5.341)	0.742	0.538 (0.070–4.141)	0.551	1.366 (0.186–10.053)	0.760
Concomitant CIS	1.367 (0.752–2.487)	0.305	1.143 (0.464–2.816)	0.771	0.682 (0.228–2.036)	0.492	0.713 (0.348–1.459)	0.354
Multifocal tumour growth	3.110 (1.440–6.719)	**0.004***	5.503 (1.270–22.835)	**0.023***	3.899 (0.872–17.434)	0.075	1.463 (0.692–3.091)	0.319
Tumour size >30 mm	0.933 (0.511–1.701)	0.820	0.958 (0.389–2.359)	0.925	1.154 (0.400–3.330)	0.791	0.648 (0.321–1.308)	0.226
Instillation therapy	0.502 (0.268–0.941)	**0.031***	0.373 (0.149–0.929)	**0.034***	0.388 (0.134–1.121)	0.080	0.383 (0.187–0.784)	**0.009***
Stromal tumour-infiltrating lymphocytes	0.984 (0.678–1.429)	0.934	1.039 (0.592–1.824)	0.893	1.693 (0.824–3.478)	0.152	1.287 (0.828–2.002)	0.263
Median bud count ≥ 1	3.132 (1.506–6.513)	**0.002***	2.942 (0.969–8.929)	0.057	11.522 (1.493–88.946)	**0.019***	3.032 (1.333–6.896)	**0.008***
Discontinuous invasion pattern	4.854 (2.295–10.267)	<**0.001***	4.374 (1.569–12.189)	**0.005***	4.633 (1.447–14.840)	**0.010***	2.317 (0.884–6.069)	0.087
Discohesive growth pattern	3.314 (1.787–6.146)	<**0.001***	4.173 (1.691–10.299)	**0.002***	4.858 (1.682–14.031)	**0.003***	2.106 (1.006–4.405)	**0.048***
Unfavourable grade group	3.183 (1.715–5.908)	<**0.001***	4.035 (1.634–9.964)	**0.002***	4.774 (1.652–13.795)	**0.004***	2.383 (1.154–4.952)	**0.019***
EAU risk group	1.613 (0.875–2.976)	0.126	1.371 (0.551–3.411)	0.497	1.023 (0.359–2.918)	0.966	0.891 (0.444–1.786)	0.744

*CIS, carcinoma in situ; CSS cancer-specific survival; EAU, European Association of Urology; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival. Bold values indicate statistical significance. *Statistical significance.*

to avoid overfitting, the resulting GG was used rather than its individual parameters and multivariable Cox regression was not performed for PFS ($n = 19$) or CSS ($n = 14$), due to small sample size. EAU risk group had no impact on RFS ($P = 0.126$), PFS ($P = 0.497$), CSS ($P = 0.966$) or OS ($P = 0.744$). Worse RFS ($P < 0.001$), PFS ($P = 0.007$) and CSS ($P = 0.013$) persisted within the unfavourable GG across EAU risk groups (Fig. 3). For patients with BCG instillations ($n = 73$), the unfavourable GG showed worse RFS, indicating more BCG-unresponsive tumours ($P = 0.006$; Fig. S1, Table S2). PFS (26.7% vs 8.6%) and CSS (20% vs 6.9%) were also worse within the unfavourable GG, although those differences did not reach statistical significance ($P = 0.05$ and $P = 0.12$, respectively).

Discussion

Stage pT1 NMIBC is associated with a significant recurrence and progression risk [2,3]. Due to the high prevalence of high-grade tumours, the diagnostic relevance of current WHO grading remains questionable in those patients [8]. We aimed to establish an additional histological grading approach for stage pT1 NMIBC that should exhibit a strong discriminative effect, is easy to apply, and helps to identify

Fig. 3 Kaplan–Meier analyses of recurrence-free survival (RFS), progression-free survival (PFS), cancer-specific survival (CSS) and overall survival (OS) for (**A–D**) patients within a specific grade group (GG) and (**E–H**) patients within a specific GG and further stratified into two subgroups based on the European Association of Urology (EAU) risk category: favourable (Fav.) GG and high risk (HR; $n = 46$); Fav. GG and very high risk (VHR; $n = 39$); unfavourable (Unfav.) GG and HR ($n = 11$); unfavourable (Unfav.) GG and VHR ($n = 17$).

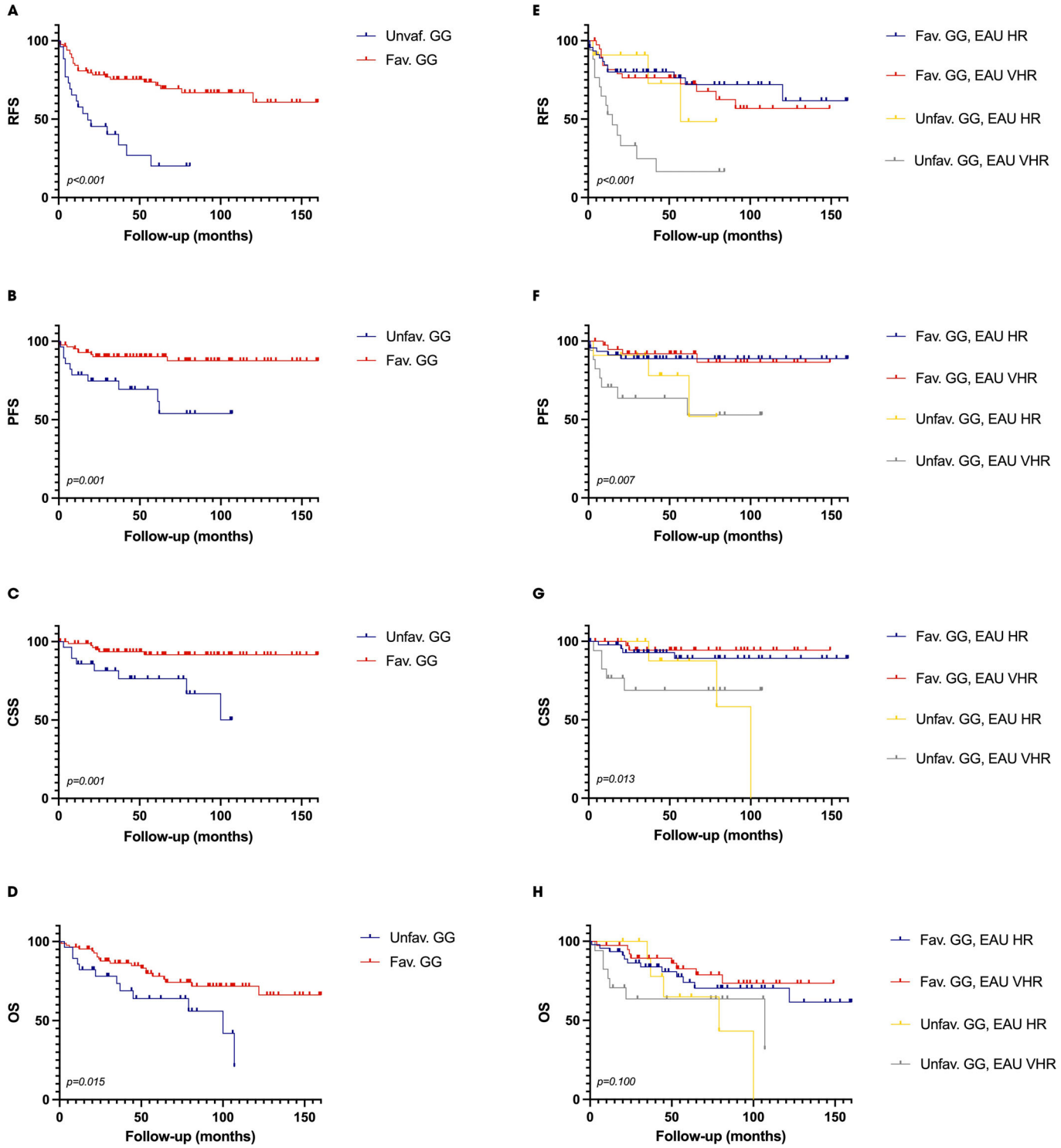


Table 4 Multivariable Cox regression analyses of variables associated with recurrence-free and overall survival including statistically significant variables from univariable Cox regression analysis.

Variables	Endpoint: RFS (Events $n = 43$)		Variables	Endpoint: OS (Events $n = 32$)	
	OR (95% CI)	*P* value		OR (95% CI)	*P* value
Multifocal tumour growth	2.308 (1.029–5.177)	**0.042***	Age dichotomous (cut-off: 70 years)	3.096 (1.494–6.417)	**0.002***
Unfavourable grade group	2.577 (1.361–4.880)	**0.004***	Unfavourable grade group	2.120 (1.013–4.439)	**0.046***
Instillation therapy	0.650 (0.341–1.238)	0.190	Instillation therapy	0.328 (0.156–0.693)	**0.003***

OR, odds ratio; OS, overall survival; RFS, recurrence-free survival. Bold values indicate statistical significance. **Statistical significance.*

prognostically unfavourable tumours, which necessitate intensive follow-up or immediate RC.

Disease management in stage pT1 NMIBC poses challenges, given that up to 44% of patients may progress to muscle-invasive disease within a 5-year period, while another 30% may never experience a tumour recurrence [2,3,19]. Immediate RC offers safety from an oncological point of view and is the recommended treatment option in very-high-risk patients according to current guidelines [2]. It is, however, associated with a high burden of disease raising concerns about quality of life and may represent overtreatment in a relevant proportion of patients [2,5]. It is therefore crucial to identify those patients who are most likely to benefit from RC.

The conventional WHO grading system, in relation to the entirety of patients with NMIBC, showed a balanced distribution, thus holding significance as a prognostic tool in this context [10]. In case of stage pT1 NMIBC, however, the vast majority of patients are considered high grade, which calls into question the prognostic relevance of conventional WHO grading in those patients [10]. Notably, 97% of the tumours in the current study were considered high grade (WHO 2004/2016), which further reinforces these doubts. In 2022, the International Society of Urological Pathology organised a consensus conference where current issues, including bladder cancer grading, were discussed. High-grade urothelial carcinomas were a primary focus and it was agreed to refine the current grading into a three-tier scheme with the division of WHO 2004/2016 high grade into clinically relevant categories [9].

Recently, a new potential grading approach for MIBC was proposed [10]. The grading system incorporates histomorphological phenotype, sTILs, tumour budding, and growth and spreading patterns. Individual parameters were merged to four GGs, independently predicting CSS, PFS and OS and therefore allowing for improved risk stratification compared with the method currently proposed by the WHO [10].

Elevated levels of sTILs indicated an effective anti-tumour immune response and were linked to enhanced survival in the aforementioned study [10]. Similarly, greater lymphocyte infiltration and high programmed death-ligand 1 (PD-L1) mRNA expression were linked to improved survival in stage pT1 NMIBC in prior research [20,21]. It is important to distinguish PD-L1 expression on tumour cells from PD-L1 expression on infiltrating lymphocytes since several studies associated a high PD-L1 expression on tumour cells with poor prognosis including worse OS and CSS, while PD-L1 expression on infiltrating lymphocytes had no negative impact on oncological outcomes [22]. One hypothesis for this is described in the literature as the ability of PD-L1 to promote immune escape. This process involves the formation of highly immunosuppressive microenvironments, which are promoted by the tumour and prevent the generation of an effective anti-tumour immune response through multiple mechanisms [23]. We were unable to reproduce an effect of sTILs either in one direction or the other, which suggests that semiquantitative sTILs scoring may not sufficiently reflect the subtle differences at the mRNA level or, alternatively, that the size of our cohort was too small.

Tumour budding adversely affects survival across cancers and was described as an easy and rapidly assessable variable, indicating a worse prognosis in the context of stage pT1 NMIBC [10,13,14,24]. Accordingly, the current study associated budding with worse oncological outcomes using a median bud count of ≥1. Pancytokeratin staining in 5 HPFs was previously validated in MIBC [10,25] and NMIBC [14], and proved reproducible and valuable. The aforementioned study found a positive correlation of tumour budding with lamina propria invasion (metric T1 substaging) [14]. Furthermore, tumour budding was the only variable that showed significant differences regarding RFS, PFS and CSS and was independent of the resection depth [14]. Tumour invasion beyond the muscularis mucosae or extensive tumour invasion in the lamina propria, which are covered by the term T1 substaging, have shown worse prognostic effects in prior studies [26,27]. Hence, T1 substaging is recommended in the current EAU guidelines [2]. A significant constraint in using the muscularis mucosae as a landmark is the absence of this layer in 22% of the specimens [27]. Consequently, the optimal method for determining T1 substaging remains to be identified.

Tumour budding, a discontinuous infiltration pattern, and a discohesive growth pattern were linked with worse oncological outcomes in the current study. These parameters display an aggressive and invasive T1 phenotype with loosely arranged and diffusely infiltrating tumour cells that may have undergone epithelial-mesenchymal transition (EMT), thus acquiring several metastatic properties of tumour cells with enhanced mobility and invasion [28,29]. EMT is recognised as a negative prognostic factor with tumour-promoting effects in bladder cancer and a transcriptional analysis of early-stage urothelial carcinoma associated the presence of EMT-related transcription factors in high-risk tumours with an unfavourable prognosis [28–30]. However, the exact relationship between the abovementioned histopathological parameters and EMT is still the subject of current research and further investigation is needed for a complete understanding.

For our new grading system, each variable was attributed 1 point, resulting in a score ranging from 0 to 3, with the threshold of ≥2 points used to define unfavourable GG. The unfavourable GG independently predicted RFS and OS and was associated with significantly worse PFS and CSS. The EAU risk group, by contrast, did not affect RFS, PFS, CSS or OS. With that said, according to the current data, unfavourable GG seemed to have greater impact on oncological outcomes than the respective EAU risk group. It is noticeable that EAU very-high-risk patients who were attributed to the unfavourable GG showed particularly high recurrence rates. Therefore, applying the proposed grading system does not preclude risk stratification according to the EAU NMIBC 2021 scoring model for tumour progression. On the contrary, the grading system seems to be a useful supplemental tool that further increases the prognostic value of the EAU scoring model. We would therefore argue that applying both systems identifies a highest risk group of stage pT1 NMIBC patients, necessitating a particularly thorough follow-up protocol. Additionally, the unfavourable GG was linked with BCG-unresponsive tumours and was associated with higher rates of upstaging at RC, emphasising its potential value as a decision-making tool for stratification into BCG instillation or immediate RC treatment groups.

Study limitations include the retrospective, single-centre design, creating the risk of recall and information bias, as well as the small and heterogenous cohort. Due to the small sample size, multivariable Cox regression analysis was limited to calculation of RFS and OS and we were not able to account for other clinically relevant variables in the context of PFS or CSS. We suggest further investigation in future prospective, multicentre studies in order to validate the diagnostic performance in a large cohort, which would allow clinically relevant but infrequently occurring parameters (such as lymphovascular invasion) as well as more homogenous subgroups (e.g., patients with a bladder-sparing approach only) to be investigated, and would address further questions such as interobserver variability or the impact of the TURBT resection technique (fractioned vs en bloc) on the outcome and assessment of histopathological variables.

In conclusion, we present a morphology-based grading system for stage pT1 NMIBC that correlates with disease aggressiveness and oncological patient outcomes. It therefore identifies a highest risk group of patients, who should be followed up more intensively or receive immediate RC. The grading system incorporates objective variables assessable on H&E slides and immunohistochemistry, enabling an easy-to-use low-cost approach that is applicable in daily routine. Further studies with a larger sample size are needed to validate and confirm these results.

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Disclosure of Interests

The authors declare no conflicts of interest.

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Abbreviations: CSS, cancer-specific survival; EAU, European Association of Urology; EMT, epithelial-mesenchymal transition; GG, grade group; H&E, haematoxylin and eosin; HPF, high-power field; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer; OR, odds ratio; OS, overall survival; PFS, progression-free survival; RC, radical cystectomy; RFS, recurrence-free survival; sTILs, stromal tumour infiltrating lymphocytes; TURBT, transurethral resection of bladder tumour.

Supporting Information

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

Fig. S1. Kaplan–Meier analysis of RFS, PFS, CSS and OS (A–D) for patients within a specific grade group, who received BCG instillation therapy throughout the follow-up period.

Table S1. Univariable regression analyses of the individual grading scores regarding RFS, PFS, CSS and OS, respectively.

Table S2. Patients, who received BCG instillation therapy, with regards to the respective grade group ($n = 73$).