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Clinical-Bladder cancer

Predictive clinico-pathological factors to identify BCG, unresponsive patients, after re-resection for T1 high grade non-muscle invasive bladder cancer

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Abstract

Introduction: Seventy-five percent of bladder cancers are non-muscle invasive. The treatment strategy includes the transurethral resection of bladder tumor (TURB) followed by intravesical immunotherapy with the bacillus of Calmette-Guerin (BCG) or chemotherapy, depending on the grade of bladder tumor. Despite a proper BCG intravesical instillations schedule, up to 40% of patients present a failure within 2 years. The aim of this retrospective study was to investigate the predictive factors in the response to BCG in patients with a high-grade non-muscle invasive bladder cancer diagnosis.

Materials and methods: Patients with non-muscle invasive bladder cancer from 13 hospitals and academic institutions were identified and treated, from January 1, 2002, until December 31, 2012, with TURB and a subsequent re-TURB for restaging before receiving BCG. Follow-up was performed with urine cytology and cystoscopy every 3 months for 1 year and, successively every 6 months. Univariate and multivariate Cox regression models addressed the response to BCG therapy. Kaplan-Meier overall survival (OS) and cancer-specific survival (CSS) estimates were determined for BCG responsive vs. BCG unresponsive patients.

Results: A total of 1,228 patients with non-muscle invasive bladder cancer were enrolled. Of 257 (20.9%) patients were BCG unresponsive. Independent predictive factors for response to BCG were: multifocality (HR: 1.4; 95% CI 1.05−1.86; $P = 0.019$), lymphovascular invasion (HR: 1.75; 95% CI 1.22−2.49; $P = 0.002$) and high-grade on re-TURB (HR: 1.39; 95% CI 1.02−1.91; $P = 0.037$). Overall survival was significantly reduced in BCG-unresponsive patients compared to BCG-responsive patients at 5 years (82.9% vs. 92.4%, $P < 0.0001$) and at 10 years (44.2% vs. 74.4%, $P < 0.0001$). Similarly, cancer-specific survival was reduced in BCG-unresponsive patients at 5 years (90.6% vs. 97.3%, $P < 0.0001$) and at 10 years (72.3% vs. 87.2%, $P < 0.0001$).

Conclusion: Multifocality, lymphovascular invasion, and high-grade on re-TURB were independent predictors for response to BCG treatment. BCG-unresponsive patients reported worse oncological outcomes.

Keywords: BCG; re-TURB; Non-muscle invasive bladder cancer

1. Introduction

Bladder cancer (BC) accounts for 3% of global cancer diagnoses and 2.1% of all cancer deaths, representing the sixth most incident neoplasm in the developed world [1,2]. Males report a significative higher incidence of BC compared to females with, respectively, 425,000 and 125,000 cases diagnosed in 2018 [2]. Similarly, Age Standardized Incidence Rate per year (ASR) is 9.6 per 100,000 for males and 2.4 per 100,000 for females, although a wider variability is reported between geographical regions [3]. Among risk factors, tobacco smoking is recognized as the most important risk factor for BC and is estimated to account for 50% of all cases. In addition, other recognized environmental risk factors are carcinogens belonging to the categories of aromatic amines, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons, accounting for roughly 20% of all cases [4]. BC is divided, depending on the grade of invasion, into non-muscle invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) [5]. Furtherly, BC is divided into different histological subtypes which comprehend: urothelial carcinoma (>90%), squamous epithelial carcinoma (2%−5%), and adenocarcinoma (0.5%−2%) [6,7]. Considering that 75% of BC are NMIBC, the treatment strategy includes the transurethral resection of bladder tumor (TURB) followed by intravesical immunotherapy with the bacillus of Calmette-Guerin (BCG) or, alternatively, intravesical chemotherapy, depending on the grade of bladder tumor [8]. In particular, for high-grade (HG) NMIBC, the standard care, both for the American Urological Association (AUA) and the European Association of Urology (EAU), includes, prior to a repeated TURB within 2 to 6 weeks, the use of BCG as adjuvant therapy via an empirical induction cycle of 6 weeks followed by a variable maintenance cycle up to 3 years [9,10]. It is well established that this schedule, compared to TURB or TURB plus induction alone, permits to obtain a higher reduction in tumor recurrence and progression, prolonging recurrence-free survival (RF) [11]. Unfortunately, despite a proper BCG intravesical instillations schedule, up to 40% of patients present a failure within 2 years, requiring radical cystectomy or bladder-preserving therapies, thus belonging to the category of BCG unresponsive patients [12]. Among this group are included those with BCG refractory tumors and those who develop a high-grade recurrence within 6 months or, alternatively, a carcinoma in situ (CIS) within 12 months from the completion of an adequate BCG schedule [13]. As result, the ability to promptly identify patients who are least likely to respond to BCG immunotherapy is crucial, allowing better management of refractory disease and, consequently, better outcomes for patients' survival. The aim of this retrospective study was to investigate predictive factors in response to BCG in patients with a T1HG/G3 NMIBC diagnosis.

2. Materials and methods

We identified 1,228 NMIBC (HG/G3T1) patients from January 1, 2002, until December 31, 2012, from 13 hospitals and academic institutions. Patients underwent TURB and restaging with re-TURB before receiving BCG. Classification of tumors has been performed at each academic institution according to the TNM system of the Union for

International Cancer Control (UICC) and to the 1973 World Health Organization (WHO) grading system. In 4 to 6 weeks after re-TURB, patients had 6 weekly instillations of BCG as induction therapy and successively maintenance therapy (every week for 3 weeks, and then up to 3 years after the start of the instillations). Patients who had a BCG induction cycle without the maintenance cycle were not enrolled in the study. The urothelial tract was screened for concomitant tumors as well. All patients signed written informed consent. Follow-up was performed with urine cytology and cystoscopy every 3 months for the first year from the initial TURB and BCG treatment and then every 6 months. BCG unresponsive patients included all BCG refractory patients (T1G3/HG tumor at 3 months; TaG3/HG tumor after 3 months and/or at 6 months, after either re-induction or first course of maintenance; CIS, without concomitant papillary tumor, at 3 months and persisting at 6 months after either re-induction or first course of maintenance; HG tumor during BCG maintenance therapy) and those who develop T1Ta/HG recurrence within 6 months or CIS within 12 months from the completion of adequate BCG exposure, according to the latest EAU Guideline [9]. Progression was defined as the presence of a muscle-invasive tumor or metastatic disease. Patients with progression to MIBC on re-TURB and patients with recurrence and progression during BCG therapy were subjected to radical cystectomy [13]. Smoking status was categorized in smokers and former smokers vs. non-smokers. Smoking status has been assessed at the first outpatient visit before TURB. Patients with incomplete BCG treatment and MIBC at re-TURB were not enrolled in the study. Re-TURB consisted of the resection of the primary tumor bed, bladder neck for CIS, and other visible patches.

2.1. Statistical methods

Association between categorical variables was tested using the chi-squared test. Differences in continuous variables across categorical variables were tested using the Mann−Whitney *U*-test or Kruskal−Wallis tests. Univariate and multivariate logistic regression analyses, as well as univariate and multivariate Cox regression models, addressed the response to BCG therapy. The Kaplan-Meier overall survival (OS) and cancer-specific survival (CSS) estimates were determined for BCG responsive vs. BCG unresponsive patients and survival distributions were compared using Log-Rank test. $P < 0.05$ (two-sided) was considered statistically significant. All statistical tests were performed with STATA 11 statistical software (Stata Corp., College Station, TX, USA).

3. Results

The associations between clinical and pathological variables with BCG response in 1,228 patients with initial T1HG NMIBC are shown in Table 1. In total 257 (20.9%) patients were BCG unresponsive. BCG unresponsive patients had statistically significant more multiple tumors, lymphovascular invasion (LVI) on the first TURB, an NPAR>18, and T1HG and LVI on the re-TURB specimen (Table 1).

Univariable Logistic regression analysis showed that were predictive factors for response to BCG: multifocality (HR: 1.41; 95% CI 1.07−1.86; $P = 0.01$), lymphovascular invasion (LVI)(HR: 1.81; 95% CI 1.28−2.55; $P = 0.001$), neutrophil percentage to albumin ration (NPAR, HR: 1.33; 95% CI 1−1.77; $P = 0.049$), T1HG on re-TURB (HR: 1.5; 95% CI 1.11−2.04; $P = 0.008$) and LVI on re-TURB (HR: 1.73; 95% CI 1.04−2.89; $P = 0.034$). In multivariable analysis, multifocality (HR: 1.4; 95% CI 1.05−1.86; $P = 0.019$), LVI (HR: 1.75; 95% CI 1.22−2.49; $P = 0.002$) and T1HG on re-TURB (HR: 1.39; 95% CI 1.02−1.91; $P = 0.037$) remained independent predictive factors for BCG response (Table 2).

Multivariable Cox regression analysis revealed that the same factors (multifocality, LVI, and T1HG on re-TURB) were independent predictors for response to BCG treatment. The C-index of the predictive model was 59.85 (Table 3). Kaplan−Meier overall survival (OS) analysis showed that BCG unresponsive patients had a significantly reduced OS, compared to BCG responsive patients, at 5 years, with 82.9% (CI 95% 75.25−88.3) vs. 92.4% (CI 95% 89.9 −94.2), and at 10 years, with 44.2% (CI 95% 29.35−58.07) vs. 74.4% (CI 95% 68.8−79.19), respectively ($P < 0.0001$) (Fig. 1). Similarly, Kaplan−Meier cancer-specific survival (CSS) analysis showed that BCG unresponsive patients had, compared to BCG responsive patients, a significantly reduced CSS at 5 years, with 90.6% (CI 95% 84.25−94.4) vs. 97.3% (CI 95% 95.5−98.3), and at 10 years, with 72.3% (CI 95% 57.9−82.4) vs. 87.2% (CI 95% 82.1−90.9), respectively ($P < 0.0001$) (Fig. 2).

4. Discussion

BCG unresponsiveness in NMIBC represents an important issue in the management of BC patients. Although intravesical BCG is the most effective regimen, up to one-third of patients will not respond to BCG. According to Shirakawa et al., patients with BCG failure could be divided into four groups based on responsiveness to BCG therapy and duration until recurrence: BCG refractory, which presents recurrence at 6 months or progression at 3 months after an induction cycle; BCG-resistant, which reports the disappearance of disease at 6 months despite the presence of a lesser degree or stage disease at 3 months after induction cycle; BCG-relapsing, which presents recurrence after disease-free status at 6 months; BCG-intolerant, which aggregates patients who present recurrence after an inadequate BCG therapy due to toxicity [14]. Different factors may explain BCG failure, ranging from the presence of occult invasive or metastatic disease to inadequate or waned immune response [15,16]. In addition, several gene

Table 1
Association of BCG response with clinical and pathologic characteristics of 1,228 patients after primary T1 HG/G3 NMIBC

	All patients	BCG responsive	BCG unresponsive	*P*
Total, n (%)	1,228	971 (79.1)	257 (20.9)	
Age, n (%)				
<70 yr	552 (45)	432 (44.5)	120 (46.7)	0.52
>70 yr	676 (55)	539 (55.5)	137 (53.3)	
Gender, n (%)				
Male	990 (80.6)	779 (80.2)	192 (82.1)	0.49
Female	238 (19.4)	192 (19.8)	46 (17.9)	
Smoking status				
never	334 (27.2)	276 (28.4)	58 (22.6)	0.06
current/former	894 (72.8)	695 (71.6)	199 (77.4)	
Multifocality, n (%)				
single	705 (57.1)	575 (59.2)	130 (50.6)	**0.01**
multiple	523 (42.6)	396 (40.8)	127 (49.4)	
Size, n (%)				
<3 cm	440 (35.9)	347 (35.8)	93 (36.2)	0.9
>3 cm	787 (64.1)	623 (64.2)	164 (63.8)	
Concomitant carcinoma in situ, n (%)				
No	1039 (84.6)	818 (84.3)	221 (86)	0.49
Yes	189 (15.4)	153 (15.7)	36 (14)	
LVI, n (%)				
No	1032 (84)	834 (85.9)	198 (77)	**0.001**
Yes	196 (16)	137 (14.1)	59 (23)	
TIHG on re-TURB, n (%)				
No	920 (74.9)	744 (76.6)	176 (68.5)	**0.007**
Yes	308 (25.1)	227 (23.4)	81 (31.5)	
LVI on re-TURB, n (%)				
No	1153 (93.9)	919 (94.6)	234 (91)	**0.032**
Yes	75 (6.1)	52 (5.4)	23 (9)	
NPAR, n (%)				
<18	832 (67.8)	671 (69.1)	161 (62.7)	**0.049**
>18	396 (32.2)	300 (30.9)	96 (37.3)	
NLR, n (%)				
<3	571 (46.5)	460 (47.4)	111 (43.1)	0.23
>3	657 (53.5)	511 (52.6)	146 (56.8)	

LVI = lymphovascular invasion; NLR = neutrophil-to-lymphocytes ratio; NMIBC = non-muscle invasive bladder cancer; NPAR = neutrophil percentage to albumin ratio; TURB = transurethral resection of bladder tumor.
Bold values represents statistically significant values ($p < 0.05$).

polymorphisms have been hypothesized to influence patient response to BCG treatment, such as those regarding *NOS2, NOS3, NRAMP1,* and *hGPX1* genes [17,18]. In the present study, we investigated clinical and pathological factors associated with unresponsiveness to BCG therapy. In particular, multifocality, LVI, and high-grade tumor on re-TURB were independent predictors for inadequate response to BCG treatment. The role of high-grade tumors on recurrence and progression is well known. The association between T1HG tumors and subsequent disease progression is likely a reflection of the understaging occurring in up to 50% of patients with presumed NMIBC [19,20]. Although better outcomes regarding PFS and RFS are reported in the literature in patients who underwent re-TURB before BCG immunotherapy, no data is currently reported regarding the influence of T1HG tumor at re-TURB on BGC response [21]. The role of re-TURB is indeed more evaluated due to its capacity to maximize staging accuracy, clear residual cancer, and promptly identify patients who are candidates for immediate radical cystectomy [22]. Recently, however, also the real efficacy of re-TURB in completely resected T1HG is under question [23]. To our knowledge, only Herr, in a study of 2005, reported, in small sample size, a better response to BCG in patients who underwent re-TURB compared who did not [24]. As previously stated, this finding could be however limited to the issues regarding incomplete resection and wrong staging of patients. Conversely, we clearly reported worse responses to BCG immunotherapy in patients who reported high-grade tumors at re-TURB, both at univariable (HR: 1.5; 95% CI 1.11−2.04; $P = 0.008$) and multivariable analysis (HR: 1.39; 95% CI 1.02−1.91; $P = 0.037$). Regarding multifocality, although a recent meta-analysis limited the impact of this factor on BCG response, two large studies (EORTC and CUETO) identified the presence of multiple tumors at TURB as a risk factor for BCG unresponsiveness, reporting HR ranging from 1.1 to 1.7 [25−27]. Consistent with the related literature, we reported multifocality as a risk factor for BCR

Table 2
Univariable and multivariable logistic regression for predicting BCG response in 1,228 patients with primary T1HG/G3 NMIBC

Preoperative prognostic factors	BCG response					
	Univariable			Multivariable		
	HR	CI	*P*	HR	CI	*P*
Age cat.	0.91	0.69–1.2	0.52	0.89	0.67–1.19	0.46
Gender	0.88	0.61–1.26	0.49	0.9	0.62–1.3	0.6
Smoking	1.36	0.98–1.88	0.06	1.35	0.96–1.89	0.07
Multifocality	1.41	1.07–1.86	**0.01**	1.4	1.05–1.86	**0.019**
Size	0.98	0.73–1.3	0.9	0.92	0.68–1.23	0.59
Concomitant CIS	0.87	0.58–1.28	0.49	0.75	0.49–1.13	0.17
LVI	1.81	1.28–2.55	**0.001**	1.75	1.22–2.49	**0.002**
T1HG re-TURB	1.5	1.11–2.04	**0.008**	1.39	1.02–1.91	**0.037**
LVI re-TURB	1.73	1.04–2.89	**0.034**	1.44	0.85–2.46	0.17
NPAR cat.	1.33	1–1.77	**0.049**	1.3	0.93–1.84	0.12
NLR cat.	1.18	0.89–1.56	0.23	0.97	0.7–1.36	0.9

CI = Confidence interval; CIS = carcinoma in situ; HR = Hazard ratio; LVI = lymphovascular invasion; NLR = neutrophil-to-lymphocytes ratio; NPAR = neutrophil percentage to albumin ratio; P = P value; TURB = transurethral resection of bladder tumor.
Bold values represents statistically significant values ($p < 0.05$).

unresponsiveness, both at univariable (HR: 1.41; 95% CI 1.07–1.86; $P = 0.01$) and multivariable analysis(HR: 1.4; 95% CI 1.05–1.86; $P = 0.019$). The role of LVI is well established in MIBC as well as in non-seminomatous testicular tumors, being an important decision-making factor in providing adjuvant chemotherapy to affected patients [28,29]. Fukumoto et al. evaluated the impact of LVI on clinical outcomes in patients with T1 NMIBC, reporting an independent association of this factor with stage progression and tumor recurrence in patients who received BCG immunotherapy (HR 2.19 and 3.76) [30]. However, no conclusions were made regarding the influence of LVI on BCG response in NMIBC. As reported in our study instead, LVI, both at primary TURB and re-TURB is independently associated with a worse response to BCG. Interestingly, NPAR, which was already investigated by a previous study regarding its association with OS and CSS in MIBC, reported, although only at univariable analysis and for values >18, an association with worse response to BCG [31]. To our knowledge, this is the first time this biomarker is associated with BCG response. In addition, considering a previous study by Racioppi et al., we investigated also the value of NLR as a prognostic factor in BCG response, without obtaining, however, significant results [32]. Finally, we did

Table 3
Uni- and Multivariable Cox regression analyses predicting BCG response in 1,228 patients treated with BCG after primary T1 HG/G3 NMIBC

Variables	Univariable			Multivariable		
	HR	95% CI	*P*	HR	95% CI	*P*
Age cat. (<70 vs. >70 yr)	0.96	0.64–1.21	0.43	0.97	0.76–1.25	0.85
Gender (male vs. female)	0.88	0.64–1.21	0.43	0.89	0.64–1.24	0.51
Smoking (no vs. yes)	1.27	0.95–1.7	0.1	1.22	0.9–1.64	0.19
Size (<3 vs. ≥3) cm	0.98	0.76–1.72	1.27	0.96	0.74–1.24	0.77
Multifocality (single vs. multiple)	1.33	1.04–1.7	**0.02**	1.31	1.02–1.69	**0.03**
Concomitant CIS (no vs. yes)	0.89	0.62–1.27	0.53	0.78	0.54–1.12	0.18
LVI (no vs. yes)	1.63	1.22–2.19	**0.001**	1.54	1.14–2.09	**0.004**
LVI on re-TURB (no vs. yes)	1.81	1.17–2.78	**0.007**	1.54	0.99–2.4	0.055
T1 HG/G3 on re-TURB (no vs. yes)	1.47	1.13–1.92	**0.004**	1.37	1.04–1.88	**0.024**
NPAR cat. (<18 vs. ≥18)	1.3	1.01–1.67	**0.04**	1.28	0.95–1.72	0.09
NLR cat. (<3 vs. ≥3)	1.17	0.91–1.5	0.19	0.98	0.73–1.31	0.92
Harrell's C Index			59.85			

BCG = Bacillus Calmette-Guérin, CI = confidence interval; CIS = carcinoma in situ; HG = high grade; HR = hazard ratio; LVI = lymphovascular invasion; NPAR = neutrophil percentage to albumin ratio; NMIBC = non-muscle invasive bladder cancer; P = *P* value; TURB = transurethral resection of bladder tumor.
Bold values are those statistically significant, i.e. $p < 0.05$.

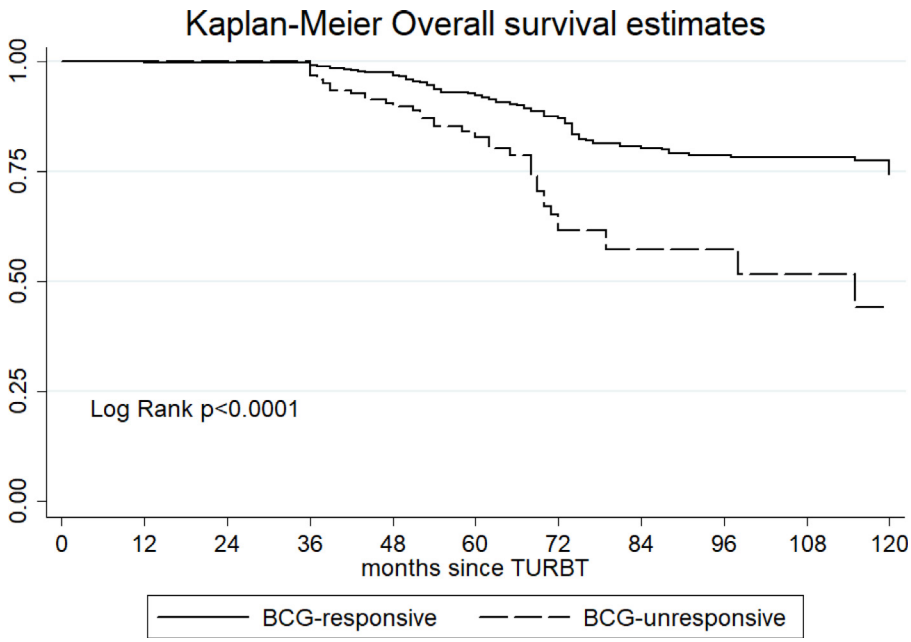

Fig. 1. Kaplan-Meier Overall survival estimates.

not demonstrate the role of age in BCG responsiveness, although several studies reported a worse prognosis in older patients [33,34]. It has however to be noted that findings on this issue are still unclear and controversial [35,36]. Despite the significance of our findings, our study has different limitations. First of all, the retrospective nature of our work; secondly, albeit we included large centers with over 300 to 400 TURB per year, we did not have the precise data in the different institutions involved; thirdly, TURB is a procedure that is influenced by the interpersonal variability of surgeons involved. Nevertheless, the experience of urologists involved (which were far over the suggested learning curve of >100 TURB) and the re-TURB performed on every patient included in the study, could have limited the issue of this inter-variability in the surgery [37].

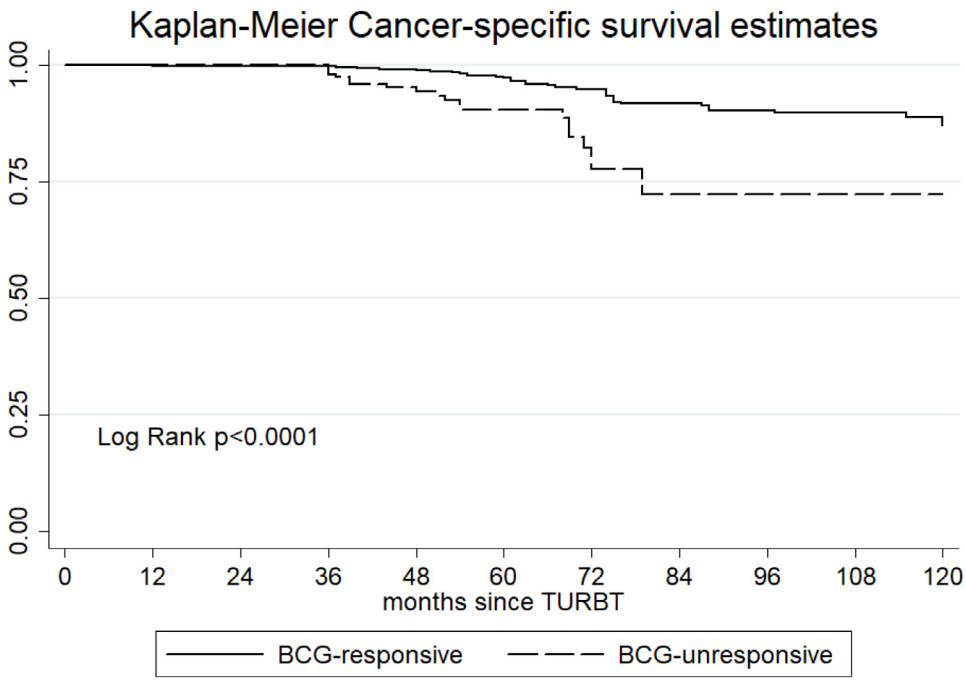

Fig. 2. Kaplan-Meier cancer-specific survival estimates.

5. Conclusion

We found that multifocality, lymphovascular invasion, and T1HG on re-TURB were independent predictors for response to BCG treatment. These findings were doubled by worse oncological outcomes of BCG unresponsive patients compared to those that showed treatment response. This finding could help to better select patients that may be candidates for an early radical cystectomy.

Author contributions

Conceptualization: MF, MDV, GL, OdC; Data curation: MF, BB, FC (Felice Crocetto), GL, GMB, FDG, MM (Martina Maggi), FC (Fabio Crocerossa), FC (Francesco Cantiello), RD, MB, PLB, RP, AM (Andrea Mari), SL, FS, MM (Michele Marchioni), ELC, DT, FAM, MP, AM (Andrea Marmiroli), GIR, LS, RH, RC, SP, PDP, VM, OST, GM, EM, OdC, MDV; Methodology: MF, MDV; Project administration: MF, MDV; Supervision: MF, MDV, BB, FC, Odc; Visualization: MF, MDV, BB, FC, GMB, OdC; Writing-original draft: MF, MDV, BB; Writing-review and editing: MF, MDV, BB.

Conflict of interests

The authors declare that they have no conflict of interest.

Data availability

The datasets are available from the corresponding author on request.

Ethics approval

This retrospective study was approved by the institutional research ethics committee of institutions involved and performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

Consent to publish was obtained from all individual participants included in the study.

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