Bladder Cancer

The Use of Urine Mycobacterium tuberculosis Complex Polymerase Chain Reaction as a Predictive Factor for Recurrence and Progression After Intravesical Bacillus Calmette-Guérin Therapy in Patients with Non–muscle-invasive Bladder Cancer

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Abstract

Background: Intravesical bacillus Calmette-Guérin (BCG) instillation is a standard treatment for non–muscle-invasive bladder cancer (NMIBC); however, not all patients benefit from BCG therapy. Currently, no surrogate marker exists to predict BCG efficacy, and thereby, identify patients who will benefit from this treatment. Objective: To evaluate the utility of urine Mycobacterium tuberculosis complex polymerase chain reaction (MTC-PCR) assay as a predictive marker for recurrence and progression following BCG therapy. Design, setting, and participants: A prospective analysis was carried out for of intermediate- or high-risk NMIBC patients who received BCG instillation for the first time. Urine samples, for MTC-PCR assay, were collected at baseline and annually for up to 10yr after the last BCG instillation, including induction and maintenance therapy. The first postoperative sample for MTC-PCR was taken at 1yr from the last instillation. Outcome measurements and statistical analysis: A survival analysis was performed using the Kaplan-Meier method, and risk factors for recurrence and progression after BCG treatment were assessed using Cox regression analysis. Results and limitations: During follow-up (median: 57 mo), 468/521 samples (89.8\%) were MTC-PCR positive, and 108/123 patients (87.8\%) exhibited MTC-PCR positivity at least once. Five-year recurrence- and progression-free survival in patients who were not MTC-PCR positive was significantly lower than in patients

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who were MTC-PCR positive at least once ($p < 0.001$). Using multivariable Cox regression analysis, MTC-PCR positivity at least once was a significant prognostic factor for recurrence (hazard ratio [HR]: $36.782$, $p < 0.001$) and progression (HR: $47.209$, $p < 0.001$).

**Conclusions:** Patients who were not MTC-PCR positive, even once after BCG therapy, were extremely likely to exhibit recurrence and progression. Urine MTC-PCR may be an extremely useful, noninvasive surrogate marker to predict recurrence and progression following BCG therapy.

**Patient summary:** Urine *Mycobacterium tuberculosis* complex polymerase chain reaction may be a novel biomarker capable of identifying patients at risk of recurrence and progression after bacillus Calmette-Guérin (BCG) immunotherapy.

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1. **Introduction**

Adjuvant intravesical instillation of bacillus Calmette-Guérin (BCG) has been used effectively against non–muscle-invasive bladder cancer (NMIBC) [1]. While numerous guidelines globally [2–6] recommend BCG for intermediate- or high-risk NMIBC patients, not every patient will benefit from BCG therapy [7]. The risk of failure of BCG treatment may be >40–50% in long-term follow-up [8,9], and patients with NMIBC receiving BCG instillation exhibit 1- and 5-yr recurrence rates of approximately 25% and 40%, respectively [10].

Currently, markers used to predict the effects of BCG include clinicopathological [9] and molecular factors [11], but these markers lack utility in predicting BCG response [10]. As a consequence, the lack of adequate and highly specific noninvasive prognostic surrogate biomarkers that can accurately predict response to BCG remains an unmet medical need [12,13].

Our hypothesis that long-term presence of BCG DNA in urine may predict intravesical BCG treatment responsiveness is based on reports that, while a delay in infectious BCG presentation is rare, it has been reported up to 3 yr after last BCG instillation [14,15].

The aim of this single-center study is to examine whether urine mycobacterial DNA detected by *Mycobacterium tuberculosis* complex polymerase chain reaction (MTC-PCR) in NMIBC patients after BCG instillation is useful for predicting the long-term effects of BCG therapy.

2. **Patients and methods**

2.1. **Clinical outline**

Patients were eligible for inclusion in this prospective study if they presented with histologically confirmed NMIBC (intermediate or high recurrence risk according to the European Organization for Research and Treatment of Cancer [EORTC] risk table) [10], had undergone a transurethral resection of bladder tumor (TURBT), with or without random biopsies, followed by BCG. For patients with a bladder perforation during TURBT, the single immediate postoperative instillation of intravesical chemotherapy was not administered. If the detrusor muscle was not included in the primary TURBT specimen, patients with high-grade tumors underwent a second TURBT.

Bladder cancers (BCs) were staged according to the 2010 American Joint Committee on Cancer TNM classification system [16] and graded using the World Health Organization/International Society of Urologic Pathology classification system [17]; Patient characteristics, including recurrence risk classification according to EORTC recurrence risk classification [10] and Club Urologico Espanol de Tratamiento Oncologico (CUETO) recurrence risk classification [18], were documented.

All patients received intravesical instillation of BCG (Connaught strain, $3.77 \times 10^8$ colony-forming units [CFU]; Sanofi S.A., Paris, France, or Tokyo 172 strain, $48.77 \times 10^6$ CFU [19]; Japan BCG Supply, Tokyo, Japan) 6 wk after TURBT as induction therapy, given six to eight times weekly. Some patients underwent further instillations every 3–6 mo for 2 yr as maintenance therapy, which was generally three times at weekly intervals. The continuation of maintenance therapy was at the discretion of the attending physician and patient preference.

This study was performed in accordance with the Declaration of Helsinki. The internal review board and the local ethics committee authorized and approved the study (13-198). Written informed consent was obtained from each patient.

2.2. **DNA extraction and urine MTC-PCR**

Urine samples were collected for analysis at baseline (after TURBT and before initiation of BCG instillation), and every year after the last BCG instillation, including induction and maintenance for up to 10 yr. MTC-PCR results acquired during BCG treatment (induction and maintenance) were excluded from the data sets. MTC-PCR results acquired after the end of BCG treatment (induction or maintenance) were included in the data sets. A minimum of 50 ml freshly voided, midstream, clean catch urine was collected and stored immediately in aliquots at −80 °C until analysis. DNA was purified from the pellet using a QIAamp DNA kit (Qiagen, Hilden, Germany), and at least 2 ml of collected urine remained after centrifugation (5000 g/20 min).

MTC-PCR was used to detect the presence of mycobacterial DNA (from *M. tuberculosis* complex [MTC] organisms) in urine samples, as previously described [20]. Primer pair A (5′-agatggcaggcctctgacag/247 (5′-tttcacggcataacgcgacaa) [21,22] amplifies a 590 bp fragment, which contains the sequences for *M. tuberculosis* complex (MTC) [23]. MTC-PCR controls were prepared from urine collected 1 h after intravesical BCG instillation (positive control), and from the urine of patients without BC and urine tuberculosis on general wards (negative control; Fig. 1A).
controls were on positive follow-up. PCR Quantitative Calmette-Guérin; detected instillation as Group regression intravesical treatment of the general statistical models were performed with R software v.3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

![Image](image_url)

**Fig. 1** – Analysis of MTC-PCR products from DNA in urine. The products were separated by gel electrophoresis on a 1% agarose gel. (A) MTC-PCR controls were prepared from urine collected 1 h after intravesical BCG instillation (positive control), and from the urine of patients without BC on general wards (negative control). MTC-PCR for DNA coding for 16S rRNA resulted in the expected fragment of about 590 bp for the positive urine test. (B) We evaluated MTC-PCR using urine from enrolled patients. We have demonstrated that mycobacterial DNA can be detected in this way. (C) Percentage of positive and negative urine MTC-PCR results after intravesical BCG instillation therapy during the 10-yr follow-up. There was no significant difference in urine MTC-PCR positive rate for each year (p = 0.337). BC = bladder cancer; BCG = bacillus Calmette-Guérin; bps = base pairs; MTC-PCR = M. tuberculosis complex polymerase chain reaction; N = negative; P = positive; PCR = polymerase chain reaction.

### 3. Results

#### 3.1. Clinical and pathological characteristics

Of the 139 patients initially included in the study, 16 had missing data or their data were unavailable (Supplementary Fig. 1). Of the 123 evaluable patients (88.5%), 67 (54.5%) patients were excluded from the statistical analysis.

| Characteristics | Evaluable patients (n = 123) |
|-----------------|-------------------------------|
| Age (yr), median (IQR) | 68 (63–75) |
| Gender, n (%) | Male 102 (82.9) |
| | Female 21 (17.1) |
| Smoking history, n (%) | Smoker 64 (52.0) |
| | Non-smoker 27 (22.0) |
| | Unknown 32 (26.0) |
| PS, n (%) | 0 117 (95.1) |
| | 1 6 (4.9) |
| Recurrence history, n (%) | Primary 60 (48.8) |
| | Recurrent 63 (51.2) |
| cT, n (%) | cT1 67 (54.5) |
| | cT2 39 (31.7) |
| | cT3 17 (13.8) |
| Tumor grade, n (%) | High 86 (69.9) |
| | Low 37 (30.1) |
| EORTC recurrence risk, n (%) | Intermediate-low 39 (31.7) |
| | Intermediate-high 56 (45.5) |
| | High 28 (22.8) |
| EORTC progression risk, n (%) | Intermediate 51 (41.5) |
| | High-low 39 (31.7) |
| | High-high 33 (26.8) |
| CUETO recurrence risk, n (%) | 0–4 27 (22.0) |
| | 5–6 26 (21.1) |
| | 7–9 40 (32.5) |
| | 10–16 30 (24.4) |
| CUETO progression risk, n (%) | 0–4 36 (29.3) |
| | 5–6 20 (16.3) |
| | 7–9 25 (20.3) |
| | 10–16 42 (34.1) |
| Immediate postoperative instillation of intravesical chemotherapy, n (%) | Yes 30 (24.4) |
| | No 93 (75.6) |
| BCG strain | Connaught 67 (54.5) |
| | Tokyo 172 56 (45.5) |
| Maintenance BCG, n (%) | Yes 25 (20.3) |
| | No 98 (79.7) |

BCG = bacillus Calmette-Guérin; cT = clinical T disease; CUETO = Club Urologico Español de Tratamiento Oncologico; EORTC = European Organization for Research and Treatment of Cancer; IQR = interquartile ranges; PS = performance status; TURBT = transurethral resection of bladder tumor.

#### 2.3. Statistical analysis

Quantitative variables are shown as median and range with interquartile ranges (IQRs), and categorical variables as frequencies and proportions (percentages). Disease first recurrence was defined as the first pathologically confirmed BC relapse after adequate intravesical BCG treatment (a minimum of five or six induction therapies and two or three first maintenance therapies [6]), regardless of tumor stage and grade.

The primary endpoint of recurrence-free survival (RFS) was defined as the period between the last TURBT before BCG treatment and the date of the first pathologically confirmed recurrence.

The second endpoint was progression-free survival (PFS). Disease progression was defined according to the International Bladder Cancer Group consensus definition, in the presence of a ≥ T2 stage or lymph node positive (N+) disease or distant metastases [24].

Time to RFS and PFS was analyzed using Kaplan-Meier estimates and log rank tests. To assess the significant prognostic factors related to recurrence and progression during follow-up periods after the last intravesical BCG instillation, receiver-operating characteristic (ROC) curves, and univariate and multivariate Cox proportional hazard regression models were used to estimate hazard ratios (HR) with 95% confidence intervals (CIs).

All tests were two-sided, and p < 0.05 was considered significant. All statistical tests were performed with R software v.3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).
underwent instillation with the Connaught strain and 56 (45.5%) with the Tokyo 172 strain (Table 1). The median total number of BCG instillations was 8 (IQR: 8–9), and the overall median duration of follow-up was 57 mo (IQR: 33–100 mo).

3.2. **MTC-PCR**

At baseline, urine MTC-PCR was negative for all patients (Fig. 1B). Of the 521 urine samples analyzed by MTC-PCR assay, 468 samples (89.8%) were positive. During the 10-yr follow-up, there was no significant difference in the urine MTC-PCR positive rate for each year ($p = 0.337$; Fig. 1C). During follow-up, all patients ($n = 103$) in the nonrecurrence group exhibited MTC-PCR positivity at least once. In the recurrence group ($n = 20$), 15 patients (75.0%) were negative for all MTC-PCR tests during follow-up. There was no significant difference in time period until recurrence.

between the negative for all MTC-PCR tests group (median: 18 mo, IQR: 13–33.5 mo) and the MTC-PCR positive at least once group (median: 27 mo, IQR: 20–33 mo, $p = 0.293$).

Although there was no significant difference in the total number of intravesical BCG instillations (median: 8 vs 8, $p = 0.647$) between the MTC-PCR positive at least once group and the MTC-PCR negative for all MTC-PCR tests group, the number of patients with an MTC-PCR positive result at least once in the Connaught strain group was significantly higher (63/67 patients, 94.0%) than that in the Tokyo 172 strain group (45/56 patients, 80.4%, $p = 0.021$).

3.3. **Prognostic factors for recurrence after BCG instillation**

During follow-up, recurrences were observed in 20/123 patients (16.3%) after BCG, and in these patients, median time to recurrence was 19.0 mo (IQR: 14.75–34 mo). In all

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patients, RFS periods were 96.7% at 1 yr and 81.7% at 5 yr after the last BCG instillation (Fig. 2A).

Kaplan-Meier plots showed a significant difference in 5-yr RFS rate between the pre-TURBT urine cytology positive group (classes IV and V) and urine cytology negative group (classes II and III; \( p < 0.001 \); Fig. 2B), between initial BC (91.2%) and recurrent BC (72.1%, \( p = 0.017 \); Fig. 2C), and between the Connaught strain group (89.1%) and the Tokyo 172 strain group (71.3%, \( p = 0.009 \); Fig. 2D). The RFS rate in the maintenance group was significantly higher than that in the only induction group (\( p = 0.043 \); Fig. 2E). Although there was no significant difference in the RFS rate between EORTC recurrence risk groups (\( p = 0.552 \); Supplementary Fig. 2G), for patients grouped according to the CUETO recurrence risk classification, there were significant differences in the 5-yr RFS rate between recurrence score categories (\( p = 0.009 \); Fig. 2F).

Examining the relationship between MTC-PCR results and recurrence, 5-yr RFS rate in the group with a positive MTC-PCR result at least once (94.5%) was significantly higher than in patients with no positive MTC-PCR results (0.0%, \( p < 0.001 \); Fig. 2G). Only 4.6% of patients (5/108) with a positive MTC-PCR result at least once developed tumor recurrence within 5 yr, compared with 100% of patients with no positive MTC-PCR results during follow-up. The RFS rate in patients with a positive MTC-PCR result from 1 to 4 yr after the last BCG instillation was significantly higher than that in patients with a negative MTC-PCR result (\( p < 0.001 \); Fig. 2H–K). There were no significant differences in RFS between other clinicopathological factors, including gender, smoking habits, performance status, clinical T disease (cT), presence or absence CIS, disease grade, EORTC recurrence risk group, second TURBT, and immediate postoperative instillation of intravesical chemotherapy (Supplementary Fig. 2A–l), as determined by log-rank statistical analyses.

Univariate Cox analysis indicated that age (\( p = 0.003 \)), urine cytology before TURBT (\( p < 0.001 \)), tumor status (primary or recurrent; \( p = 0.024 \)), BCG strain (\( p = 0.012 \)), CUETO recurrence risk (\( p = 0.003 \)), and urine MTC-PCR outcome (\( p < 0.001 \)) were significant predictive factors for disease recurrence in NMIBC patients treated with intravesical BCG (Table 2). Multivariate Cox analysis indicated that MTC-PCR positivity at least once was an independent risk factor for recurrence (\( p < 0.001 \); Table 2). MTC-PCR positivity at least once (by ROC curve analysis) was most predictive of recurrence during follow-up periods: sensitivity, 0.750; specificity, 1.000 (Supplementary Table 1); and area under the curve (AUC), 0.868 (Fig. 2L).

### 3.4. Prognostic factors for progression after BCG instillation

During follow-up, progression was observed in six of 123 (4.9%) patients after BCG, and three patients exhibited an increase in T stage or grade. The 5-yr PFS rate was 94.2% (Fig. 3A).

In the pre-TURBT urine cytology positive group (classes IV and V), PFS rate was significantly lower than that in the urine cytology negative group (classes II and III, \( p = 0.002 \); Fig. 3B). The PFS rate in the positive MTC-PCR results at least once group was significantly higher than that in patients with no positive MTC-PCR results (\( p < 0.001 \); Fig. 3C). Progression occurred in five of 15 patients (33.3%) in the no positive MTC-PCR results group, compared with just in one of 108 patients (0.9%) in the positive MTC-PCR result at least once group. The PFS rate in patients with a positive MTC-PCR result from 1 to 4 yr after the last BCG instillation was significantly higher than that in patients with a negative MTC-PCR result (\( p < 0.001 \); Fig. 3D–G). There were no significant differences in PFS for other clinicopathological

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**Table 2** – Univariate and multivariate Cox regression analyses for the prediction of disease recurrence in NMIBC patients treated with intravesical BCG

| NMIBC patients | Univariate analyses | | | | Multivariate analyses |
|---|---|---|---|---|---|
| | HR | 95% CI | \( p \) value | HR | 95% CI | \( p \) value |
| Age | 1.093 | 1.030–1.159 | **0.003** | 0.991 | 0.930–1.055 | 0.769 |
| Gender (male vs female) | 1.748 | 0.405–7.533 | 0.454 | | | |
| Smoking history (smoker vs nonsmoker) | 0.711 | 0.231–2.173 | 0.549 | | | |
| PS (I vs 0) | 0.862 | 0.115–6.442 | 0.885 | | | |
| Cytology (IV+V vs II+III) | 4.841 | 1.897–12.355 | **0.001** | 2.692 | 0.748–9.692 | 0.310 |
| Recurrence history (recurrent vs primary) | 3.222 | 1.170–8.872 | **0.024** | 2.499 | 0.592–10.549 | 0.213 |
| Second TURBT (yes vs no) | 0.641 | 0.231–0.763 | 0.389 | | | |
| cT (CT1 vs CTa) | 1.091 | 0.601–0.974 | 0.774 | | | |
| Tumor grade (high vs low) | 1.122 | 0.431–2.921 | 0.813 | | | |
| CIS (positive vs negative) | 1.866 | 0.743–4.685 | 0.184 | | | |
| Immediate postoperative instillation of intravesical chemotherapy (yes vs no) | 1.816 | 0.690–4.779 | 0.227 | | | |
| BCG strain (Connaught vs Tokyo72) | 0.304 | 0.120–0.770 | **0.012** | 0.424 | 0.142–1.267 | 0.125 |
| Maintenance BCG (yes vs no) | 0.162 | 0.022–1.210 | 0.076 | | | |
| Total number of BCG instillations | 0.986 | 0.883–1.101 | 0.805 | | | |
| EORTC recurrence risk | 1.388 | 0.781–2.466 | 0.265 | | | |
| CUETO recurrence risk | 2.154 | 1.305–3.557 | **0.003** | 1.459 | 0.529–4.024 | 0.466 |
| MTC-PCR | 63.050 | 20.131–197.473 | <**0.001** | 36.782 | 10.072–134.321 | <**0.001** |

BCG = bacillus Calmette-Guérin; CI = confidence interval; CIS = carcinoma in situ; cT = clinical T disease; CUETO = Club Urológico Español de Tratamiento Oncológico; EORTC = European Organization for Research and Treatment of Cancer; HR = hazard ratio; MTC-PCR = urine M. tuberculosis complex polymerase chain reaction; NMIBC = non–muscle-invasive bladder cancer; PS = performance status; TURBT = transurethral resection of bladder tumor.
Fig. 3 – (A) PFS in all patients was 99.2% at 1 yr and 94.2% at 5 yr after BCG instillation. (B) PFS rate in the pre-TURBT urine cytology positive group (classes IV and V) was significantly lower than that in the urine cytology negative group (classes II and III; p = 0.002). (C) PFS rate in the group with positive MTC-PCR results at least once was significantly higher than in patients with no positive MTC-PCR results (p < 0.001). Compared with patients with a negative MTC-PCR result, RFS rate in the group with a positive MTC-PCR result at (D) 1, (E) 2, (F) 3, and (G) 4 yr after the last BCG instillation was significantly higher (p < 0.001). (H) ROC curve results showed the area under the curve (AUC) values of three factors that were significantly different in univariate Cox analysis. The AUC values of urine cytology, CUETO progression risk, and MTC-PCR were 0.765 (95% CI: 0.596–0.934), 0.752
factors (Supplementary Fig. 3A–M), as determined by log-rank analyses. Univariate Cox analyses demonstrated that urine cytology before TURBT ($p = 0.017$), CUETO progression risk ($p = 0.049$), and urine MTC-PCR outcome ($p < 0.0001$) were significant predictive factors of PFS (Table 3). Multivariate Cox analyses indicated that urine MTC-PCR outcome was a significant predictive factor of PFS ($p < 0.001$; Table 3). MTC-PCR positivity at least once (by ROC curve analysis) was most predictive of progression: sensitivity, 0.833; specificity, 0.915 (Supplementary Table 2), and AUC, 0.875 (Fig. 3H).

4. Discussion

Although BCG therapy is widely accepted as standard therapy after TURBT, nearly 5% of patients develop systemic BCG infection [15], and there is a high rate of tumor recurrence and progression [6]. Furthermore, some patients do not benefit from BCG therapy [25], and we are currently unable to predict which patients respond to this treatment [26]. We report here for the first time that for patients who were not MTC-PCR positive even once after BCG therapy, this was a significant predictive factor for post-BCG recurrence and progression. While previous studies have shown an association between a positive UroVysion fluorescent in situ hybridization (FISH) assay with an increased risk of recurrence among patients who have undergone BCG therapy [25], these studies had limitations [27–30]. FISH assays are generally more cumbersome to perform than polymerase chain reaction (PCR) tests and are also more expensive; PCR testing is a simpler assay to perform in a clinical setting.

The urine MTC-PCR assay used in this study to determine the response to BCG therapy is based on the amplification of the mycobacterial DNA fragment from the MTC [20]. The presence of long-term residual BCG organisms in urine has previously been reported. Bowyer et al. [14] found persistent acid-fast bacilli in the urine of some patients after intravesical BCG therapy, and the presence of long-lasting and persistent BCG DNA in the bladder, measured by PCR, has also been demonstrated [31,32]. The authors previously investigated urine culture for acid-fast bacilli for the detection of BCG DNA in urine, but only three of 58 (5.2%) MTC-PCR–positive patients had a positive urine culture after BCG instillation (data not shown), so this avenue was not pursued.

Our hypothesis was that the expression of BCG DNA in urine is crucial to sustain and determine the BCG treatment effect following therapy; MTC-PCR positivity persisted in many samples for a very long time after the last BCG instillation. There was no significant difference in the total number of BCG instillations in the MTC-PCR positive result at least once group, compared with the MTC-PCR negative group. Therefore, BCG instillation period does not appear to affect MTC-PCR positivity. However, the number of patients who were MTC-PCR positive at least once in the Connaught strain group was significantly higher than in the Tokyo 172 strain group. These findings may be the reason why a previous study by Niwa et al. [33] suggested that the Connaught strain may be more effective than the Tokyo 172 strain.

Using ROC curve analyses and multivariate Cox analyses, MTC-PCR outcome provided a good predictive factor for recurrence and progression after BCG treatment. Although BCG is difficult to differentiate from other MTC bacteria using conventional methods, including regular PCR [34], all patients in this study had no history or signs of urogenital tuberculosis, as well as a negative MTC-PCR result before BCG instillation. Taking these findings into consideration, we propose that this method can adequately detect long-term urinary BCG DNA in urine. Ongoing local antigen stimulation may have an important role to play in the mode of action of BCG; thus, the continuous presence of BCG in urine may stimulate the immune system to generate an antitumor response.

From these data, it is not possible to predict the usefulness of BCG from MTC-PCR before the start of treatment, and the optimal timing, frequency, and period of urine MTC-PCR positivity after the last BCG instillation are not fully known. While surveillance with cystoscopy and cytology for detecting the recurrence and progression of BC is essential standard of care, MTC-PCR tests may help predict post-treatment recurrence and progression in a timely manner. The recurrence and progression rates of the MTC-PCR positive from 1 to 4 yr group were significantly lower than the recurrence rates for patients in the MTC-PCR negative from 1 to 4 yr group ($p < 0.001$).

Study limitations included the relatively low number of patients (all from a single center), the low number of BCG bladder instillations (median number of doses, $n = 8$), and <20% of patients receiving maintenance therapy. Patients who discontinued BCG instillation were also included in the analyses. As the aim of the study was to examine the usefulness of the MTC-PCR assay in predicting the efficacy of BCG after the end of BCG administration, it was considered that stopping BCG administration early would not affect MTC-PCR assay results. Another limitation was the necessity to examine MTC-PCR results as a predictive factor according to the type of BCG failure category. Recommendations from specialists and professional guidelines devote significant attention to the definition of BCG failure [3,5,6,35–38], and recent evidence suggests that BCG-relapsing disease is associated with better outcomes than BCG-refractory disease [39].

(95% CI: 0.631–0.874), and 0.875 (95% CI: 0.710–1.041), respectively. There were no significant differences between these factors. BCG = bacillus Calmette-Guérin; CI = confidence interval; CUETO = Club Urogénico Español de Tratamiento Oncológico; FPF = false positive fraction; MTC-PCR = M. tuberculosis complex polymerase chain reaction; PFS = progression-free survival; ROC = receiver-operating characteristic; RFS = recurrence-free survival; TPF = true positive fraction; TURBT = transurethral resection of bladder tumor.
with recurrence in this study were in the BCG-relapse group.

5. Conclusions

This study reports the significantly low likelihood of tumor recurrence and progression among patients with at least one MTC-PCR positive result after BCG therapy, compared with that among patients with a negative MTC-PCR result for all follow-up tests. MTC-PCR test results may, therefore, be related to BCG therapeutic response and may influence prognosis. Post-BCG urine MTC-PCR analysis allows urologists the possibility to stratify patients according to the likelihood of relapse and progression after BCG therapy.

Author contributions: Satoru Muto had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Muto.
Acquisition of data: Lu, Saito, Kitamura, Noma, Koyasu, Hirano, Ashizawa.
Analysis and interpretation of data: Ide.
Drafting of the manuscript: Muto.
Critical revision of the manuscript for important intellectual content: Isotani, Nagata.
Statistical analysis: Muto.
Obtaining funding: Muto.
Administrative, technical, or material support: Yamaguchi.
Supervision: Horie.
Other: None.

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CRediT authorship contribution statement

Satoru Muto: Conceptualization, Methodology, Writing - original draft. Yan Lu: Methodology. Hisamitsu Ide: Validation, Writing - review & editing. Raizo Yamaguchi: Formal analysis. Keisuke Saito: Investigation. Kousuke Kitamura: Resources. Yasuhiro Noma: Data curation. Hiroki Koyasu: Data curation. Hisashi Hirano: Data curation. Takeshi Ashizawa: Data curation. Shuji Isotani: Project administration. Masayoshi Nagata: Visualization. Shigeo Horie: Supervision.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euros.2021.02.005.

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