subsequent tumour recurrence and stage progression.

CONFLICT OF INTEREST

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Abbreviations: **HR**, hazard ratio; **NMIBC**, non-muscle-invasive bladder cancer; **CIS**, carcinoma *in situ*; **TURBT**, transurethral resection of bladder tumour; **MMC**, mitomycin C.

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Prognostic significance of Bacillus Calmette-Guérin failure classification in non-muscle-invasive bladder cancer

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Study Type – Prognosis (case series)
Level of Evidence 4

What's known on the subject? and What does the study add?
Adjuvant intravesical BCG therapy is the most effective regimen for non-muscle-invasive bladder cancer. Previously, patients who experienced recurrences after BCG therapy tended to be lumped together as patients with 'BCG failure', but BCG failure was defined inconsistently in each study and several studies indicated that patients with a particular pattern of BCG failure had a worse prognosis.

We divided patients with BCG failure into four groups, which were based mainly on the responsiveness to BCG therapy and duration until tumour recurrence. Patients in the BCG-refractory group, in particular, had a higher risk for subsequent stage progression and disease-specific death over a long duration compared with patients in the other BCG-failure groups. As the definitions of BCG failure used to date have been decidedly heterogeneous, we recommend that standardized treatment decisions, protocols and recommendations be established according to individual BCG failure patterns.

OBJECTIVE

- To investigate the differences in the clinical features and subsequent stage progression and disease-specific survival among patients with Bacillus Calmette-Guérin (BCG) failure, after dividing these patients into BCG-refractory, -resistant, -relapsing, and -intolerant groups.

PATIENTS AND METHODS

- We identified 173 patients with initial BCG failure from 521 patients who had undergone induction BCG therapy for non-muscle-invasive bladder cancer, excluding CIS, between 1987 and 2009.
- Patients were stratified into four BCG-failure groups, and each prognostic outcome was evaluated.

RESULTS

- Median follow-up period from initial BCG failure was 4.7 years.
- A total of 42 patients (24.3%) were stratified into the BCG-refractory, three (1.7%) into the BCG-resistant, 106 (61.3%) into the BCG-relapsing, and 22 (12.7%) into the BCG-intolerant group.
- Twenty-four patients (13.9%) experienced stage progression during follow-up.
- Multivariate analysis showed that pathological G3 at BCG failure ($P = 0.014$; risk ratio 2.84) and BCG-refractory ($P < 0.001$; risk ratio 4.68) were independent predictors for stage progression. The 10-year progression-free survival rates were 53.2%, 91.1% and 93.8% in the BCG-refractory, BCG-relapsing and BCG-intolerant groups, respectively. The stage progression rate was higher in the BCG-refractory than in the BCG-relapsing ($P < 0.001$) and BCG-intolerant ($P = 0.007$) groups.
- Similarly, the 10-year disease-specific survival rate in the BCG-refractory group was significantly worse than those in the other BCG failure groups ($P < 0.001$).

CONCLUSIONS

- Stratification of BCG failure into the above-mentioned four groups can identify patients with BCG-failure in terms of their prognosis.
- The potential risk for critical adverse events was higher in the BCG-refractory group than in the other BCG-failure groups, despite the fact that patients in each group all underwent induction BCG therapy, therefore, treatment decisions, protocols and recommendations should be established based on each individual BCG-failure pattern.

KEYWORDS

non-muscle-invasive bladder cancer, BCG failure, BCG refractory, stage progression

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INTRODUCTION

Approximately 75–85% of all patients with bladder cancer are initially diagnosed with non-muscle-invasive disease (i.e. stage Ta, Tis or T1) [1]. Adjuvant intravesical BCG therapy is the most effective regimen for non-muscle-invasive bladder cancer (NMIBC) among common intravesical agents; however, some patients with NMIBC experience tumour recurrence after BCG therapy, and this has been termed 'BCG failure'.

Owing to the lack of a standard classification system for BCG failure, previous studies have noted erratic rates of stage progression after BCG therapy [2–8]. Some studies classified patients as having BCG failure after a single induction course of BCG [9,10], while others used failure after two courses as their definition [5,6,11]. In addition, patients with persistent disease and patients with recurrent disease after an initial response have been combined in some studies [9,10]. A few reports combined all patients who could not complete the BCG therapy because of toxicity [11,12]. Meanwhile, in the most general sense, any recurrence of disease after BCG therapy can be referred to as 'BCG failure'. Most studies did not indicate the disease-free interval after the last BCG induction. These inconsistencies have led to comparisons of outcome in a very heterogeneous population, and this has resulted in confusion regarding treatment decisions in patients classified as having BCG failure.

To avoid unnecessary salvage therapy and to accurately evaluate the results of salvage therapy, Herr and Dalbagni [13] noted the need for a standard definition of BCG failure. Nieder *et al.* [14] stratified BCG-failure patterns into four major groups, BCG-refractory, BCG-resistant, BCG-relapsing, and BCG-intolerant, which were based mainly on the responsiveness to BCG therapy and duration until tumour recurrence. They recommended that the classification of BCG failure into specific types of BCG failure should be used whenever possible to provide more uniformity in reporting.

In the present study, using Nieder *et al.*'s classification, we divided patients with NMIBC who had undergone induction BCG therapy that had resulted in failure into the four major BCG-failure groups. We then investigated whether the defined classification of BCG failure could successfully identify patients with a higher malignant potential.

PATIENTS AND METHODS

Data were retrospectively reviewed from the medical records of patients who had received induction BCG therapy that had resulted in BCG failure for NMIBC, excluding carcinoma *in situ* (CIS), between 1987 and 2009 at Keio University Hospital and Saiseikai Central Hospital. During this period, >1800 patients (>1400 patients at Keio University Hospital and 400 patients at Saiseikai Central Hospital) were diagnosed with NMIBC and treated using transurethral resection of bladder tumour (TURBT). After excluding patients with CIS, 521 patients who underwent induction BCG therapy were included in the study population. Some patients had undergone TURBT and intravesical chemotherapy one or more times before the initial BCG therapy. Of these 521 patients, 173 patients with BCG failure (142 men and 31 women), who matched the BCG-failure criteria, were selected for the study. Nieder *et al.* [14] defined the groups as follows: BCG-refractory as the presence of the disease at 6-month follow-up after BCG therapy, or any progression in stage, grade, or disease extent at 3-month follow-up, BCG-resistant as the disappearance of disease at 6-month follow-up, despite presence of disease that was of a lesser degree, stage, or grade 3 months after induction BCG therapy, BCG-relapsing as recurrence after disease-free status at 6-month follow-up, accompanied by a complete response to induction BCG therapy, and BCG-intolerant as recurrence after administering inadequate BCG therapy because of BCG toxicity. Using this classification system, we divided the 173 patients into their corresponding groups.

The patients were treated with Tokyo 172 strain or Connaught strain induction BCG therapy scheduled for weekly administration for 6–8 weeks at a full dose of BCG in 40 mL of saline with retention for 1–2 h. Patients were assessed at follow-up using urine cytology and cystoscopy at 3-month intervals during the initial 2 years, every 6 months for the next 3 years and yearly thereafter. I.v. urography, ultrasonography, or CT was used to evaluate distant metastasis and the upper urinary tract every year for at least 5 years. Stage progression was defined as confirmed histological muscle invasion or distant metastases.

In the analysis of stage progression-free survival, the starting point was defined as the date of BCG failure, and the endpoint as the date of stage progression. In the analysis of disease-specific survival, the starting point was defined as the date of BCG failure, and the endpoint as the date of disease-specific death. Stage progression-free survival and disease-specific survival rate curves were constructed using the Kaplan–Meier method and were compared using the log-rank test. A P value of <0.05 was considered to indicate statistical signficance. Univariate and multivariate analyses of data were performed using the Cox proportional hazards model with stepwise forward selection. The variables used were age, sex, pathological grade, stage, tumour multiplicity at the time of both initial induction BCG therapy and BCG failure, and BCG-failure classification. These analyses were performed using a JMP version 8.02 statistical software package (SAS Institute, Cary, NC, USA).

RESULTS

Table 1 shows the clinicopathological profiles of the 173 patients divided by the four BCG-failure groups. The mean (range) age of the patients was 65.5 (35–89) years. Of these 173 patients, 42 (24.3%) were assigned to the BCG-refractory group, three (1.7%) to the BCG-resistant group, 106 (61.3%) into the BCG-relapsing group and 22 (12.7%) into the BCG-intolerant group. The median follow-up period from TURBT just before initial induction BCG therapy was 6.7 years. The median (range) follow-up duration from initial BCG failure was 4.7 (1.0–13.3) years. Of 173 patients, five (2.9%) underwent total cystectomy before stage progression during the follow-up period owing to recurrence. Of these five patients, three were in the BCG-refractory group, one was in the BCG-relapsing group and one was in the BCG-intolerant group. Twenty-four patients (13.9%) experienced stage progression during follow-up. The mean (range) length of time from initial BCG failure to stage progression was 2.4

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TABLE 1 Clinicopathological profiles of 173 patients divided into the four BCG-failure groups

	Study population	BCG-failure groups			
		Refractory	Resistant	Relapsing	Intolerant
No. of patients	173	42	3	106	22
Mean (range) age, years	65.5 (34–89)	65.6 (34–88)	72.9 (68–79)	65.1 (34–89)	65.8 (44–87)
Sex, *n* (%)					
Male	142 (82)	30 (71)	1	92 (87)	19 (86)
Female	31 (8)	12 (29)	2	14 (13)	3 (14)
Findings before initial BCG therapy					
Grade, *n* (%)					
G1-2	111 (64)	20 (48)	1	75 (71)	15 (68)
G3	62 (36)	22 (52)	2	31 (29)	7 (32)
Stage, *n* (%)					
Ta	100 (58)	19 (45)	0	67 (63)	14 (64)
T1	73 (42)	23 (55)	3	39 (37)	8 (36)
Concurrent CIS, *n* (%)					
Yes	21 (12)	10 (24)	0	9 (8)	2 (9)
No	152 (88)	32 (76)	3	97 (92)	20 (91)
Tumour multiplicity, *n* (%)					
Solitary	39 (23)	7 (17)	0	25 (24)	7 (32)
Multiple	134 (77)	35 (83)	3	81 (76)	15 (68)
Pathological category, *n* (%)					
TaG1-2	68 (39)	12 (29)	0	44 (42)	12 (55)
T1G1-2	33 (19)	7 (17)	0	23 (22)	3 (14)
TaG3	42 (24)	8 (19)	1	31 (29)	2 (9)
T1G3	30 (17)	15 (36)	2	8 (8)	5 (23)
Findings at BCG failure					
Grade, *n* (%)					
G1-2	126 (73)	27 (64)	2	82 (77)	15 (68)
G3	47 (27)	15 (36)	1	24 (23)	7 (32)
Stage, *n* (%)					
Ta	96 (55)	16 (38)	2	62 (58)	16 (73)
T1	72 (42)	25 (60)	1	41 (39)	5 (23)
Tis	5 (3)	1 (2)	0	3 (3)	1 (5)
Concurrent CIS, *n* (%)					
Yes	10 (6)	5 (12)	1	3 (3)	1 (4)
No	163 (94)	37 (88)	2	103 (97)	21 (95)
Tumour multiplicity, *n* (%)					
Solitary	83 (48)	17 (40)	2	53 (50)	11 (50)
Multiple	90 (52)	25 (60)	1	53 (50)	11 (50)
Pathological category, *n* (%)					
TaG1-2	76 (44)	11 (26)	2	50 (47)	13 (59)
T1G1-2	50 (29)	16 (38)	0	32 (30)	2 (9)
TaG3	20 (12)	5 (12)	0	12 (11)	3 (14)
T1G3	22 (13)	9 (21)	1	9 (8)	3 (14)

(0.1–10.5) years. Of the 24 patients who experienced stage progression, 14 (58.3%) were in the BCG-refractory group, eight (33.3%) were in the BCG-relapsing group, one (4.2%) was in the BCG-resistant group, and one (4.2%) was in the BCG-intolerant group at the time of the first BCG failure. Metastases appeared in 12 patients (50.0%) and muscle invasion was observed in 12 patients (50.0%). The clinicopathological characteristics of the patients were compared between the BCG-refractory group and the BCG-relapsing group. The pathological results at the time of both TURBT just before initial induction BCG therapy and BCG failure showed that the BCG-refractory group contained significantly more T1 or concurrent CIS components than the BCG-relapsing group. In addition, the pathological results at the time of TURBT just before initial induction BCG therapy showed that the BCG-refractory group contained more G3 components than the BCG-relapsing group.

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TABLE 2 Univariate and multivariate analysis for stage progression

	Univariate	Multivariate		
	P	*P*	Hazard ratio	95% CI
Age (<65 vs ≥65)	0.058			
Sex (Male vs Female)	0.634			
Finding at initial BCG therapy				
Grade (G1–2 vs G3)	0.029			
Stage (pTa vs pT1)	0.307			
Concurrent CIS (Yes vs No)	0.122			
Tumour multiplicity (Solitary vs Multiple)	0.975			
Findings at BCG-failure				
Grade (G1–2 vs G3)	0.003	0.014	2.84	1.24–6.52
Stage (pTa vs pT1 and pTis)	0.252			
Concurrent CIS (Yes vs No)	0.053			
Tumour multiplicity (Solitary vs Multiple)	0.113			
BCG failure group (BCG-refractory vs Others)	<0.001	<0.001	4.68	2.07–10.97

FIG. 1. Stage progression survival curves for each BCG-failure group.

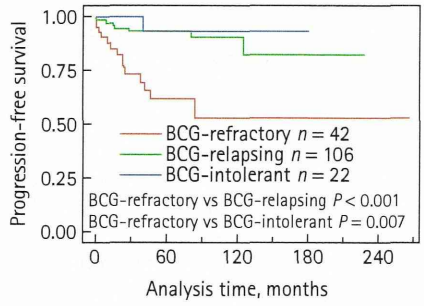

FIG. 2. Disease-specific survival rate curves for each BCG-failure group.

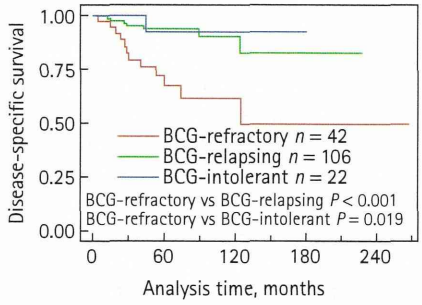

Table 2 shows the results of univariate and multivariate analysis of stage progression. Multivariate analysis showed that pathological grade G3 at BCG failure ($P = 0.014$; risk ratio 2.84; 95% CI 1.24–6.52) and BCG-refractory group ($P < 0.001$; risk ratio 4.68; 95% CI 2.07–10.97) were independent predictors for stage progression. The 10-year progression-free survival rates in the BCG-refractory group, BCG-relapsing group and BCG-intolerant group were 53.2%, 91.1%, and 93.8%, respectively (Fig. 1). Kaplan–Meier curve analysis showed that the BCG-refractory group progressed more than the BCG-relapsing group ($P < 0.001$) and BCG-intolerant group ($P = 0.007$).

A total of 20 patients (11.6%) died from their disease during follow-up. At the time of initial BCG failure, 12 (60.0%) were in the BCG-refractory group, seven (35.0%) in the BCG-relapsing group, and one (5.0%) in the BCG-intolerant group. The 10-year disease-specific survival rates in the BCG-refractory group, BCG-relapsing group and BCG-intolerant group were 61.8%, 90.6% and 92.9%, respectively (Fig. 2). Kaplan–Meier curve analysis showed that patients in the BCG-refractory group were significantly more likely to die from their disease than patients in the BCG-relapsing group ($P < 0.001$) and the BCG-intolerant group ($P = 0.019$).

DISCUSSION

In the present study, we have shown that patients in the BCG-refractory group were at higher risk for subsequent stage progression and disease-specific death over a long duration compared with patients in the other BCG-failure groups. Our results indicated that a recurrent pattern after induction BCG therapy according to Nieder's classification had an association with subsequent clinical course and we could identify patients who had a markedly worse outcome after initial induction BCG therapy.

A close association between responsiveness to the initial induction BCG therapy and subsequent stage progression has been shown in previous studies. Lerner *et al.* [15] reported that failure to achieve a complete response during induction BCG before maintenance BCG therapy for CIS, Ta or T1 bladder cancer was associated with a significant risk for disease-specific death. The 5-year probability of survival for patients who achieved a complete response to induction BCG was 77% compared with 62% for the patients who did not. Herr *et al.* [16] showed that evaluation of patients with superficial bladder carcinoma at 6 months after BCG therapy best defined the probability of stage progression. Andius and Holmäng [17] concluded that the disappearance of disease at the time of the first cystoscopy after BCG therapy was an independent risk factor for stage progression. Although the patient profiles and protocols of BCG therapy were different among the studies, all of the studies noted that failure to achieve a complete response by about 6 months, which was defined as BCG-refractory in our population, could be a risk factor for subsequent stage progression.

Our results indicate that the term 'BCG failure' involves heterogeneous characteristics and behaviour with respect to stage progression and disease-specific survival. Current guidelines have their unique definitions of BCG failure and recommendations for its treatment. European Association of Urology guidelines state that the following is regarded as BCG failure: the detection of muscle-invasive disease, the presence of high grade, non-muscle-invasive tumour at both 3 and 6 months, or any worsening of the disease under BCG treatment in spite of an initial response [1]. National Comprehensive Cancer Network guidelines do not clearly define BCG failure and state that if high-risk NMIBC is managed conservatively and does not respond to BCG, a cystectomy should be performed. For patients with recurrent persistent NMIBC that responds to induction intravesical therapy, a second course of BCG or mitomycin C induction therapy is recommended. Meanwhile, treatment

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decision criteria for BCG-relapsing cases are not mentioned in any current guidelines. A clear and consistent definition of BCG failure should be used, such as BCG-refractory and BCG-relapsing, in future studies.

In the present study, we showed that definitive classification of BCG failure could estimate the different malignant potentials for subsequent stage progression and disease-specific survival. We found that more patients experienced stage progression (14/42 patients: 33.3%) and more patients died from their disease (12/42 patients: 28.6%) in the BCG-refractory group than in the other BCG-failure groups. We propose that patients in the BCG-refractory group should be advised of these potential worse outcomes during the follow-up. These patients might then be recommended to have more aggressive treatment, e.g. total cystectomy, at an early time because of this higher risk of stage progression and disease-specific death compared with the other BCG-failure groups. By contrast, total cystectomy does not appear to be directly indicated for patients in the BCG-relapsing group. Future studies concerning an appropriate treatment strategy for patients in the BCG-relapsing group are warranted.

The present study has several limitations. It was performed in a retrospective manner and included a small number of patients. BCG instillation with the maintenance schedule and second-look transurethral resection were not commonly practised at our institution during this period, and if they had been, they may have improved the results. Nevertheless, complete TURBTs until the muscle layer could be observed were performed in principle, except for tiny papillary tumours or CIS lesions. Moreover, all patients with a reddish or mossy area apart from the main tumour and oedema of the mucosa underwent biopsy of suspicious-appearing urothelium including biopsies from prostatic fossa. Disparities in treatments after BCG failure may have introduced bias into the results. Most of the patients had undergone an additional course of common intravesical agents, such as mitomycin C, epirubicin, pirarubicin or a second course of BCG.

In conclusion, patients in the BCG-refractory group had a potentially higher risk for subsequent stage progression and disease-specific death over a long duration compared with patients in the other BCG-failure groups. As the reporting definitions of 'BCG-failure' have been decidedly heterogeneous to date, standardized treatment decisions, protocols and recommendations should be established according to each individual BCG-failure pattern.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **NMIBC**, non-muscle-invasive bladder cancer; **TURBT**, transurethral resection of bladder tumour.

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Original article

Expression profile of E-cadherin and N-cadherin in non-muscle-invasive bladder cancer as a novel predictor of intravesical recurrence following transurethral resection

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Abstract

The objective of this study was to investigate the impact of the expression profile of E-cadherin and N-cadherin in newly diagnosed non-muscle-invasive bladder cancer (NMIBC) on the probability of intravesical recurrence in patients undergoing transurethral resection (TUR). This study included 115 consecutive patients diagnosed as having NMIBC following TUR. Expression levels of E-cadherin and N-cadherin in TUR specimens from these patients were measured by immunohistochemical staining. In this series, intravesical recurrence occurred in 35 of 115 patients (30.4%). Immunohistochemical study showed that positive expression of E-cadherin and N-cadherin were noted in 62 (53.9%) and 48 (41.7%) specimens, respectively. Intravesical recurrence was detected in only 7 of 62 patients (11.3%) with positive E-cadherin expression, while 33 of 48 patients (68.8%) with positive N-cadherin expression developed intravesical recurrence. When patients were divided into 4 groups according to the positivities of E-cadherin and N-cadherin expression, intravesical recurrence was detected in 27 of 30 patients (90.0%) with negative E-cadherin as well as positive N-cadherin expression, and the intravesical recurrence-free survival of this group was significantly poorer than those of the remaining 3 groups. Furthermore, negative E-cadherin as well as positive N-cadherin expression was identified as the most powerful independent predictor for intravesical recurrence following TUR on multivariate analysis. These findings suggest that the loss of E-cadherin and gain of N-cadherin expression in on NMIBC appeared to be significantly associated with postoperative recurrence; therefore, the switch from E-cadherin to N-cadherin expression might be involved in the mechanism underlying intravesical recurrence of on NMIBC. © 2012 Elsevier Inc. All rights reserved.

Keywords: Non-muscle-invasive bladder cancer; Intravesical recurrence; E-cadherin; N-cadherin

1. Introduction

Approximately 80% of newly diagnosed bladder cancers are classified into non-muscle-invasive tumors that are limited to the urothelium or infiltrate no deeper than the lamina propria. The current standard treatment of patients with non-muscle-invasive bladder cancer (NMIBC) is complete removal of the visible tumor burden by transurethral resection (TUR), and the prognosis of such patients is generally favorable, achieving 5-year survival rates greater than 80% [1]. However, the high probability of intravesical recurrence in patients undergoing TUR has been reported to range between 30% and 80% in patients with NMIBC [1,2]. Intensive efforts, therefore, have been made to identify factors precisely predicting the clinical course of NMIBC following TUR, which are of great utility in planning both postoperative adjuvant therapy and follow-up schedule in an individual patient.

Cadherins are the transmembrane glycoproteins that mediate cell–cell adhesion through the extracellular domains and connect to the actin cytoskeleton by cooperating with catenins through the cytosolic domains [3]. In normal tissues, epithelial and mesenchymal cells mainly express E-cadherin and N-cadherin, respectively; however, down-regulation of E-cadherin and/or up-regulation of N-cadherin, which are observed in various kinds of malignant tumors, have been shown to be associated with the acquisition of

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aggressive phenotypes [4–11]. Furthermore, several recent studies have demonstrated the important role of a switch from E-cadherin to N-cadherin in cancer cells, which is regarded as one aspect of the epithelial-to-mesenchymal transition (EMT), in the malignant progression through their enhanced motility and invasive potential [12,13].

In bladder cancer, the significance of cadherins in invasive disease has been well characterized [6,7,10,11]; however, it remains largely unknown whether the expression profile of cadherins, particularly N-cadherin, is correlated to intravesical recurrence of NMIBC. In the present study, therefore, we evaluated the expression levels of E-cadherin and N-cadherin in newly diagnosed NMIBC specimens from 115 patients undergoing TUR, and then investigated the impact of these findings on the probability of postoperative intravesical recurrence.

2. Patients and methods

Of consecutive patients who were treated with TUR of newly diagnosed primary bladder cancer between April 2000 and December 2007, this study included a total of 115 patients fulfilling the following criteria: (1) superficial pure urothelial carcinoma of the bladder (i.e., pTa or pT1) was pathologically confirmed; (2) concomitant carcinoma in situ (CIS) was not detected; (3) intravesical instillation therapy was not performed postoperatively. In this series, complete resection of all visible tumors was carried out, and several deep muscular samples were taken using the resection instrument. Irrespective of the findings of preoperative urinary cytology, random bladder biopsy specimens, targeting the trigone, posterior wall, bilateral lateral walls, dome, anterior wall, prostatic urethra in men, and/or suspicious regions, were obtained before TUR in all patients. Histopathologic examinations were performed by a single pathologist according to the 2002 American Joint Committee on Cancer TNM classification system, and tumor grade was assigned based on the 1973 World Health Organization (WHO) grading system.

Follow-up of patients after TUR was conducted as follows: cystoscopy and urinary cytologic examination were performed every 3 months for 3 years after TUR, then every 6 months from 3 to 5 years, and then annually after 5 years, and intravenous pyelography was performed every 6 months until 3 years after TUR and then annually until 5 years after TUR. On detection of tumors or hyperemic mucosa by cystoscopy and/or positive findings on urinary cytology, transurethral biopsy of the abnormal region and/or TUR of the tumor were performed. Informed consent for performing this study was obtained from each of these patients, and the study design was approved by the Research Ethics Committee of our institution.

Immunohistochemical staining of TUR specimens was performed as previously described [14]. Briefly, sections from formaldehyde-fixed, paraffin-embedded tissue from 115 specimens were deparaffinized by xylene and rehydrated in decreasing concentrations of ethanol. After blocking endogenous peroxidase with 3% hydrogen peroxidase in methanol, sections were boiled in 0.01 M citrate buffer for 10 min and incubated with 5% normal blocking serum in Tris-buffered saline for 20 min. The sections were then incubated with anti-human E-cadherin mouse monoclonal antibody and N-cadherin mouse monoclonal antibody (DAKO, Carpinteria, CA) for 60 minutes, followed by incubation with biotinylated rabbit anti-mouse IgG (Vector Laboratories, Burlingame, CA) for 30 minutes. After incubation in an avidin-biotin peroxidase complex for 30 minutes, the sections were exposed to diaminobenzidine tetrahydrochloride solution and counterstained with methyl green.

Staining results were interpreted by 2 independent observers who were blinded to the clinicopathologic data. Immunoreactivities of E-cadherin and N-cadherin in tissue sections were scored as previously reported [6,10]; that is, positive staining of E-cadherin was defined as the proportion of tumor cells with membranous staining $> 90\%$, while the definition of positive staining for N-cadherin was the presence of tumor cells showing immunoreactivity with membranous staining irrespective of the proportion of positively stained tumor cells due to the lack of expression of N-cadherin in normal bladder.

All statistical analyses were performed using Statview 5.0 software (Abacus Concepts, Berkeley, CA), and P values < 0.05 were considered significant. Fisher's exact test was used to analyze associations between intravesical recurrence and several parameters. The intravesical recurrence-free survival rates were calculated by the Kaplan-Meier method, and differences were determined by log-rank test. The prognostic significance of certain factors was assessed by the Cox proportional hazards regression model.

3. Results

The median age of 115 patients (male, 95; female 20) included in this study was 69 years (range, 43–84 years). Distribution of T stage and tumor grade in TUR specimens from these 115 patients were Ta in 59 (51.3%), T1 in 56 (48.7%), G1 in 36 (31.3%), G2 in 66 (57.4%), and G3 in 13 (11.3%). In addition, multiple tumors were detected in 59 patients (51.7%). During the observation period of this study (median, 34 months; range, 6–94 months), intravesical recurrence occurred in 35 patients (30.4%).

As shown in Table 1, positive staining of E-cadherin and N-cadherin were detected in 62 (53.9%) and 48 (41.7%) patients, respectively. Representative findings of immunohistochemical study for detecting E-cadherin and N-cadherin expression are presented in Fig. 1. The incidence of intravesical recurrence in patients with negative E-cadherin expression was significantly greater than that in those with positive expression, whereas patients with positive N-cad-

Table 1
Incidence of intravesical recurrence according to expression of E-cadherin and N-cadherin

	No. pts (%)	No. pts with intreavesical recurrence (%)	*P* value
E-cadherin expression			0.0004
Negative	53 (46.1)	28 (52.8)	
Positive	62 (53.9)	7 (11.3)	
N-cadherin expression			<0.0001
Negative	67 (58.3)	2 (3.0)	
Positive	48 (41.7)	33 (68.8)	

herin expression were significantly more likely to develop intravesical recurrence than those with negative expression (Table 1). Furthermore, there were significant differences in intravesical recurrence-free survival based on positivities of both E-cadherin and N-cadherin in TUR specimens (Fig. 2).

We then divided the 115 patients into 4 groups according to the expression pattern of E-cadherin and N-cadherin as follows: group A, E-cadherin positive and N-cadherin positive; group B, E-cadherin positive and N-cadherin negative; group C, E-cadherin negative and N-cadherin positive; and group D, E-cadherin negative and N-cadherin negative. Intravesical recurrence was most likely to occur in group C, and there were significant differences in the incidence of intravesical recurrence between group C and the remaining 3 groups (Table 2). In addition, intravesical recurrence-free survival in group C was significantly poorer than those in the remaining 3 groups (Fig. 3).

As shown in Table 3, univariate and multivariate analyses were performed to evaluate the significance of several parameters, including the expression patterns of E-cadherin and N-cadherin, as predictors of intravesical recurrence. Multiple tumors, negative E-cadherin expression, positive N-cadherin expression, and classification into group C were identified by univariate analysis as factors significantly associated with intravesical recurrence. Multivariate analysis of these 4 factors identified as significant on univariate analysis showed that all 4 factors were independently associated with intravesical recurrence; however, classification into group C, which was characterized by negative E-cadherin as well as positive N-cadherin expression, appeared to be the most powerful independent predictor of intravesical recurrence following TUR.

4. Discussion

Cadherins are a family of calcium-dependent, cell–cell adhesion molecules implicated in the progression of malignant tumors [3]. E-cadherin, the most intensively investi-

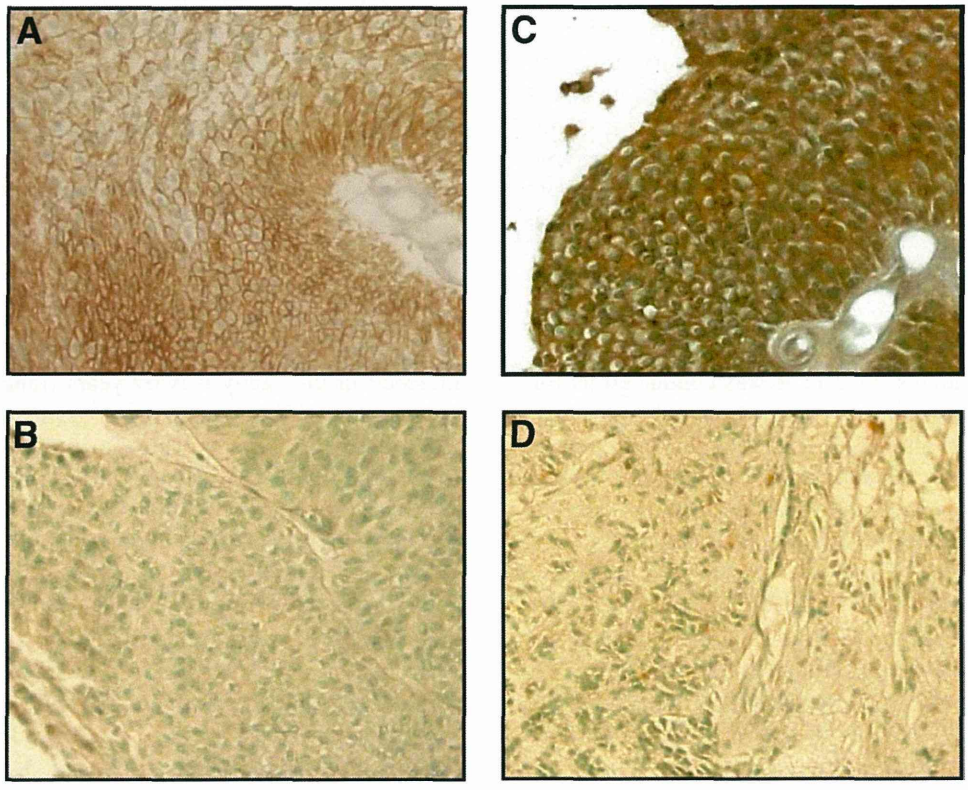

Fig. 1. Typical outcomes of immunohistochemical staining of primary superficial bladder cancer with E-cadherin or N-cadherin antibody. (A) Positive expression of E-cadherin. (B) Negative expression of E-cadherin. (C) Positive expression of N-cadherin. (D) Negative expression of N-cadherin. (Color version of figure is available online.)

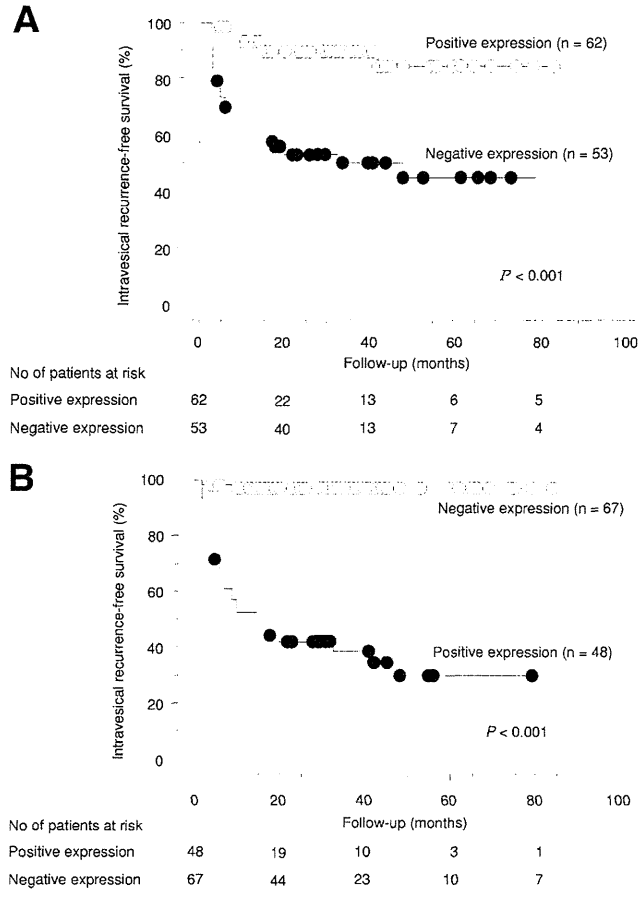

Fig. 2. Intravesical recurrence-free survival of patients with primary superficial bladder cancer who underwent transurethral resection according to the expression profiles of E-cadherin (A) and N-cadherin (B).

gated member of the cadherin family, plays a potential role in the suppression of tumor cell invasion, and loss or decreased expression of E-cadherin was observed in various kinds of cancer specimens [4,5]. In recent years, the importance of EMT, a process in which epithelial cells lose their characteristic polarity, disassemble cell–cell junctions, and become more migratory, has been recognized in several pathophysiologic processes [12]. During EMT, cadherin switching, characterized by the down-regulation of E-cadherin as well as up-regulation of N-cadherin, has been shown, resulting in the extensive reorganization of cell–cell junctions; therefore, the significance of N-cadherin has also been intensively studied, and it has been well documented that aberrant expression of N-cadherin in cancer cells helps to promote their aggressiveness through enhanced invasive potential [8–11]. In bladder cancer, the involvement of E-cadherin and N-cadherin in the progression of invasive disease has been described [6–11]; however, it remains largely unknown whether the expression profile of E-cadherin and N-cadherin is associated with the probability of intravesical recurrence in NMIBC. Considering these findings, we assessed the impact of E-cadherin and N-cadherin expression in TUR specimens obtained from 115 patients with NMIBC on their postoperative intravesical recurrence.

In this series, 53.9% and 41.7% of NMIBC specimens were judged positive for E-cadherin and N-cadherin expression, respectively. Furthermore, the incidence of intravesical recurrence was significantly associated with both negative expression of E-cadherin and positive expression of N-cadherin, and there were significant differences in intravesical recurrence-free survival following TUR according to the positivities of these 2 adhesion molecules. To date, despite limited data concerning the prognostic significance of E-cadherin and N-cadherin in NMIBC, the present study clearly showed a significant association of these 2 molecules with intravesical recurrence of NMIBC.

To more precisely predict the probability of intravesical recurrence of NMIBC based on the expression of either E-cadherin or N-cadherin, it would be of interest to consider the expression patterns of these 2 adhesion molecules simultaneously. Accordingly, we divided the included patients into 4 groups as described above, and compared the incidence of intravesical recurrence according to this classification. As expected, the incidence of intravesical recurrence in group C, characterized by negative E-cadherin and positive N-cadherin expression, was extremely high, and this proportion was significantly greater than those in the other 3 groups. Intravesical recurrence-free survival in group C was also significantly unfavorable compared with those in the other 3 groups. These findings suggest that it would be particularly useful to perform a combined assessment of E-cadherin and N-cadherin expression in TUR specimens for predicting the prognosis of patients with NMIBC.

Table 2
Incidence of intravesical recurrence according to classification based on expression patterns of E-cadherin and N-cadherin

Classification*	No pts (%)	No pts with intreavesical recurrence (%)	*P* value			
			Group A vs. others	Group B vs. others	Group C vs. others	Group D vs. others
Group A	19 (16.5)	6 (31.6)	—	0.0022	<0.0001	0.036
Group B	44 (38.3)	1 (2.3)	<0.0022	—	<0.0001	0.99
Group C	30 (26.1)	27 (90.0)	<0.0001	<0.0001	—	<0.0001
Group D	22 (19.1)	1 (4.5)	0.0036	0.99	<0.0001	—

* Group A, E-cadherin positive and N-cadherin positive; Group B, E-cadherin positive and N-cadherin negative; Group C, E-cadherin negative and N-cadherin positive; Group D, E-cadherin negative and N-cadherin negative.

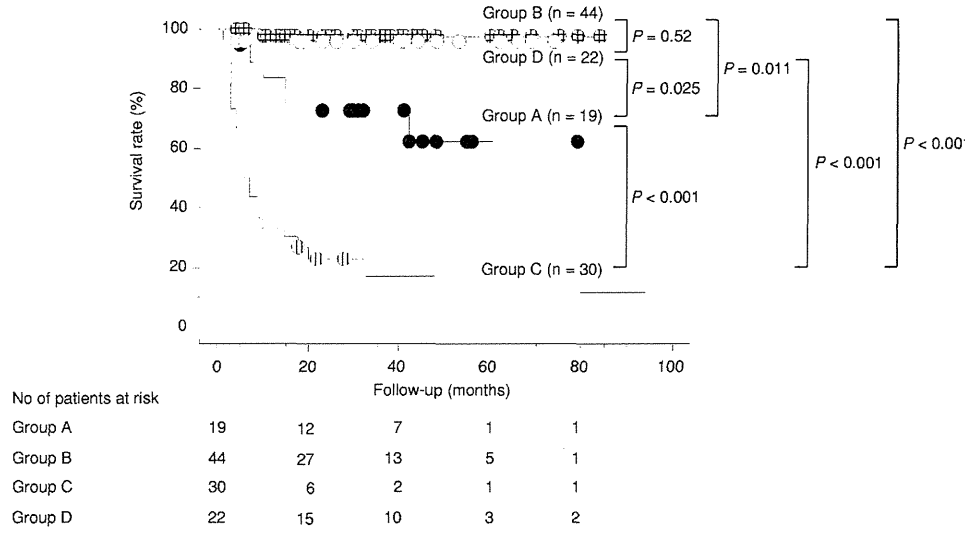

Fig. 3. Intravesical recurrence-free survival of patients with primary superficial bladder cancer who underwent transurethral resection according to the classification based on expression patterns of E-cadherin and N-cadherin. Group A, E-cadherin positive and N-cadherin positive; group B, E-cadherin positive and N-cadherin negative; group C, E-cadherin negative and N-cadherin positive; and group D, E-cadherin negative and N-cadherin negative.

It would be potentially useful to develop a prediction system for intravesical recurrence following TUR of NMIBC for determining postoperative follow-up as well as treatment schedules; hence, the impact of conventional prognostic parameters and the expression profile of E-cadherin and N-cadherin on intravesical recurrence-free survival following TUR were compared. Of several factors examined in this series, multiple tumor, negative E-cadherin expression, positive N-cadherin expression, and classification into group C appeared to be significantly associated with intravesical recurrence on univariate analysis. Furthermore, these 4 factors were also identified as independent predictors of intravesical recurrence following TUR on multivariate analysis. To date, a number of studies have reported several predictive factors for intravesical recurrence, of which tumor multiplicity is regarded as one of the most useful indicators of clinical outcome in patients with NMIBC undergoing TUR [15–17]. However, it may not be sufficient to analyze clinicopathologic parameters alone for stratifying patients with superficial bladder cancer into risk groups with respect to intravesical recurrence following TUR, considering the diverse genetic as well as biological features of NMIBC [18]. Collectively, these findings suggest that consideration of expression profile of both E-cadherin and N-cadherin in addition to conventional prognostic parameters, such as tumor multiplicity, may allow more accurate individualization of risk for postoperative intravesical recurrence in patients with NMIBC.

Here, we would like to emphasize the limitations of this study. A series of 115 patients with a disease as common as NMIBC treated with TUR is not a sufficient sample size. Furthermore, this study excluded patients with concomitant CIS and/or those receiving adjuvant intravesical instillation therapy, in order to avoid simultaneous assessment of pa-

Table 3
Univariate and multivariate analyses of association between various parameters and intravesical recurrence-free survival in 115 patients with non-muscle-invasive bladder cancer who underwent transurethral resection

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age (years) (<70 vs. 70 or older)	1.02	0.54–2.11	0.57	—	—	—
Gender (male vs. female)	1.21	0.47–3.99	0.42	—	—	—
Multiplicity (single vs. multiple)	2.20	1.73–5.65	0.024	1.91	1.14–4.11	0.042
T stage (Ta versus T1)	1.42	0.31–2.54	0.070	—	—	—
Grade (G1 or 2 vs. G3)	1.33	0.37–4.74	0.65	—	—	—
E-cadherin (negative vs. positive)	4.97	1.12–10.82	0.036	4.15	1.22–10.43	0.020
N-cadherin (negative vs. positive)	5.35	1.86–12.90	0.022	5.88	2.63–18.96	0.018
Classification* (Group C vs. Group A, B or D)	8.81	1.34–19.68	0.010	9.61	1.31–21.33	0.0010

CI = confidence interval.

* Group A, E-cadherin positive and N-cadherin positive; Group B, E-cadherin positive and N-cadherin negative; Group C, E-cadherin negative and N-cadherin positive; and Group D, E-cadherin negative and N-cadherin negative.

tients with varied characteristics; therefore, it would be difficult to apply the findings obtained from this study to an entire cohort of NMIBC, particularly those showing high grade disease. In addition, the outcomes presented in this study strongly suggest the involvement of a switch from E-cadherin to N-cadherin in the molecular mechanism mediating intravesical recurrence; however, it would apparently be warranted to perform experimental studies using human NMIBC model systems to address the functional role of cadherin switching in intravesical recurrence following TUR.

In conclusion, we analyzed the expression profiles of E-cadherin and N-cadherin in NMIBC as predictors of intravesical recurrence following TUR, and demonstrated that patients with NMIBC characterized by negative E-cadherin as well as positive N-cadherin expression are significantly more likely to develop postoperative intravesical recurrence. These findings suggest that switching from E-cadherin to N-cadherin expression might be involved in the mechanism underlying intravesical recurrence of NMIBC.

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