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Bladder Cancer Working Group Report

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Epidemiology of bladder cancer: Bladder cancer is the 7th most common cancer in men and the 17th most common in women in the world. The incidence of bladder cancer varies considerably among countries, with the highest incidence rates seen in Western countries and the lowest rates in Asian countries. In recent years, the mortality rate due to bladder cancer has been stable or decreased gradually. Lifestyle and urothelial carcinoma: Occupational risks, environmental risks, dietary habits and cigarette smoking are lifestyle factors known to influence the development of urothelial carcinoma. Although the relative risk of bladder cancer associated with occupations is small, the public health impact may be significant. The Western pattern of diet is associated with a significant increase in the risk of bladder cancer. It has been found that smoking accounts for more than 50% of bladder cancers in men and 30% in women. Urological patients' awareness of smoking as a risk factor for bladder cancer is lower than their awareness regarding other smoking-related disease entities. Counseling patients regarding the risk of tobacco is a role for urologists. Genetic susceptibility to urothelial carcinoma: Recent single-nucleotide polymorphism genetic studies in relation to bladder carcinogenesis have revealed several associated genetic polymorphisms of detoxification or DNA repair genes, such as NAT2, GST and OGG1. That information is important in relation to environmental risk factors and ethnic differences and will help predict the prognosis of patients with bladder cancer. Further studies are needed to confirm potential gene–gene and gene–environmental interactions leading to bladder carcinogenesis.

Key words: bladder cancer – epidemiology – risk factors

The Working Report Urological Cancer: Bladder cancer presentation was divided into three chapters: the epidemiology of bladder cancer, lifestyle and urothelial carcinoma, and genetic susceptibility to urothelial carcinoma.

EPIDEMIOLOGY OF BLADDER CANCER

Statistics regarding the epidemiology of bladder cancer show that about 357 000 new cases of bladder cancer were diagnosed in the world in 2002 (Table 1). This is the 7th most common cancer in men and the 17th most common in women. Bladder cancer is three to four times more common in men than in women. Bladder cancer has a higher incidence in the USA and Europe compared with Asian countries. Regarding the mortality of bladder cancer, in 2002, about 145 000 patients died in the world. The age-standardized mortality rates are 2–10 per 100 000 males and 0.5–4 per 100 000 females (1).

Looking at various countries, the age-standardized incidence rate for bladder cancer in 2005 was highest in Italy, whereas Japan, China and India had the lowest rates shown in Fig. 1 (1). American whites and blacks showed very different rates. In the most recent data, for 2008, the incidences had gone up, but not greatly. Spain became the top country, followed by Italy. African-Americans again had a lower incidence of bladder cancer than white Americans, but it is interesting that they had a higher mortality rate (2).

Table 1. Epidemiology of bladder cancer

357,000 new cases in the world (2002)
7th most common cancer in males
17th in females
3 to 4 times more common among males than among females
USA and European countries > Asian countries
Age-adjusted mortality
2-10 per 100,000 males
0.5-4 per 100,000 females

Regarding the distribution of tumor histology in black and white patients in the USA, almost all whites had pure urothelial carcinoma, whereas only about 80% of blacks did. The other histologies were about the same. The distribution of the T stage shows that 81% of whites had non-muscle invasive bladder cancer, compared with 62% of blacks. This is a very different distribution. A significant difference in survival was seen for T3 patients by race (3).

The incidence of bladder cancer in Japanese males in 2002 was about 20 per 100 000 population. Similarly, for Japanese women, the incidence of bladder cancer is <5 per 100 000 population. The trends in the crude cancer incidence rate per 100 000 Japanese population show that bladder cancer is increasing in both Japanese men and women. However, the age-standardized bladder cancer incidence rates per 100 000 world population have not been increasing much and are almost stable. Since 1980, malignant neoplasms have shown the highest mortality rate among diseases in Japan. The mortality rates for bladder cancer have increased in both Japanese males and females. However, the increase has not been very great in the case of the age-standardized bladder cancer death rates, and the rate actually decreased in females (Fig. 2). The mortality rate in Japanese males in 2006 was about 6 deaths per 100 000, whereas it was about 3 or 4 deaths per 100 000 females. The crude cancer mortality rates per 100 000 Japanese population for each sex have gradually increased. However, the age-standardized bladder cancer death rates per 100 000 world population have remained steady for both genders (http://www.ncc.go.jp/index.html).

In international comparison, the proportions of deaths of males due to bladder cancer are about 5% in Japan and Korea, and about 6.5% in the USA. On the other hand, the rates for females are about 2% in Japan and Korea, and about 3% in the USA. The age-standardized bladder cancer death rates per 100 000 Japanese 1985 standard population show that there has generally been stability during the last 40 years in both males and females. The age-standardized bladder cancer mortality rates for elderly males have increased, but they have been stable for middle-aged male patients. Females showed the same tendency (http://www.ncc.go.jp/index.html).

About 6000 bladder cancer patients were registered by the Japanese Urological Association during the 3 years from 1999 through 2001 (Fig. 3). On the basis of the T staging, about 73% of those patients had non-muscle invasive bladder carcinoma and 23% had invasive disease, for a ratio of about 3 to 1. In the clinical N stage and M stage findings, 81% were N0 and 83% were M0. The predominant histology was urothelial carcinoma, i.e. transitional cell carcinoma, seen in 94% of patients. The highest grade and predominant grade data showed that close to half of the Japanese bladder cancer patients were G2. The pathological stage data showed that 75% were pTis, pTa or pT1, and 18% were muscle-invasive pT2 through pT4. Regarding the mode of treatment of those patients, nearly 80% underwent transurethral surgery (TUR/TUC), and close to 20% underwent total cystectomy (4).

In conclusion, the incidence of bladder cancer varies considerably among countries, with the highest incidence rates seen in Western countries. In recent years, the mortality rate due to bladder cancer has been stable or decreased gradually.

LIFESTYLE AND UROTHELIAL CARCINOMA

Occupational risks, environmental risks, dietary habits and cigarette smoking are lifestyle factors known to influence the development of urothelial carcinoma. As occupations at risk for urothelial and bladder cancers, the recent literature points out bitumen (asphalt, tar) workers, automobile industry workers, hairdressers using colorants, sewing machine workers, painters, printers and paperhangers, and truck and bus drivers (Table 2). Regarding exposure to colorants, or dyes, occupational exposure to aromatic amines is a known bladder cancer risk factor. However, the impact of exposure to azo dyes, which may release aromatic amines in humans, is at present controversial.

A German case-controlled study was published in 2008 that investigated 156 bladder cancer patients and 336 control subjects (5). The odds ratio for painters was reported to be 1.98, with a 95% confidence interval (CI) of 0.64–6.11. For hairdressers, the odds ratio was 4.9 (95% CI: 0.85–28.39). For the wood processing occupation, the odds ratio was 1.19 (95% CI: 0.58–2.41), and for chronic exposure to colorants, the odds ratio was 1.84 (95% CI: 0.68–4.95). Individuals exposed to colorants showed an elevated risk for bladder cancer, but the significance was borderline.

The International Agency for Research on Cancer submitted a report in 2008 in Lyons, France. They reported that 80% of modern hair dyes are permanent (oxidative) hair dyes and consist of colorless primary intermediates and couplers. They also noted that current epidemiological studies have shown a small but consistent increase in bladder cancer in male hairdressers and barbers. They concluded that hair dyes are probably a Grade 2A carcinogen in humans.

A 2009 report dealt with a study of the risk of bladder cancer in the automobile industry that was conducted as a

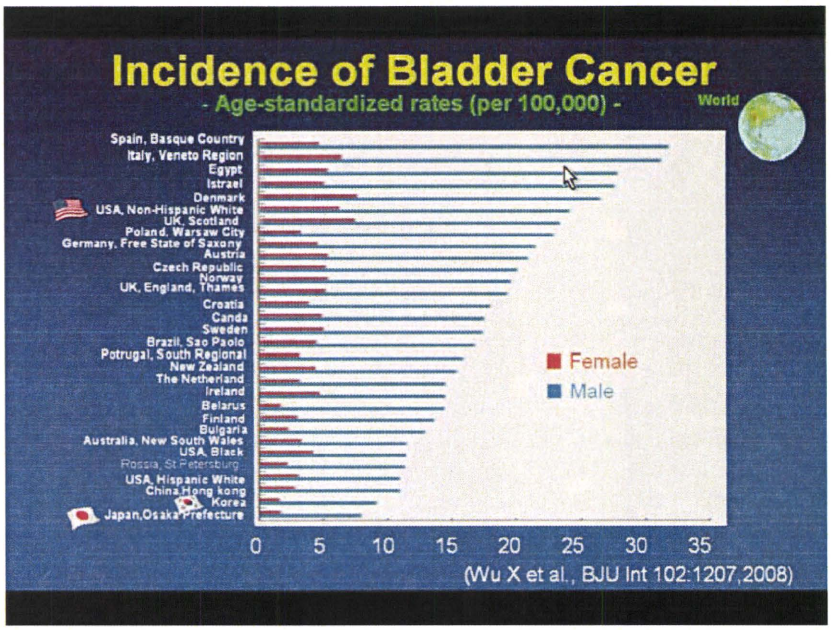

Figure 1. Incidence of bladder cancer: age-standardized rates (per 100 000).

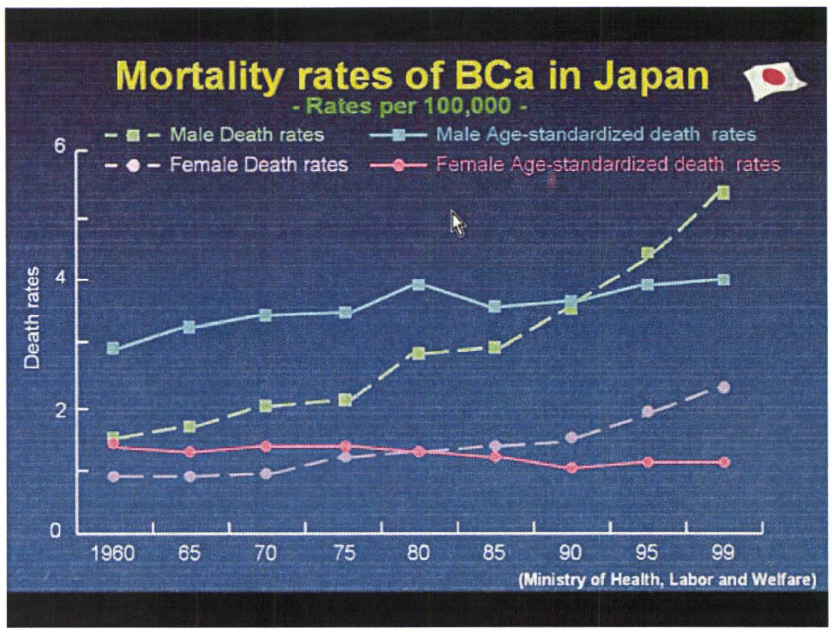

Figure 2. Mortality rates of bladder cancer in Japan.

case-controlled, population-based study in Michigan, USA (6). The results showed a higher risk of bladder cancer for those who worked for 20 or more years on the assembly line, with an odds ratio of 2.10 (95% CI: 1.15–3.80). Moreover, statistical interaction was shown between usual employment on the assembly line and smoking status, and the odds ratio was very high for smokers who had worked for a long time. It was concluded that further research is necessary to identify the exposures that might be contributing to bladder cancer on the assembly line and to examine whether those exposures continue to exist in today's workplace.

The environmental risks for bladder cancer include drinking water. Arsenic exposure is a well-known risk factor, and a very recent publication reported that chronic arsenic exposure at levels found in US drinking water was associated with bladder cancer (7). A population-based, case-controlled study conducted in New Hampshire in the USA consisted of

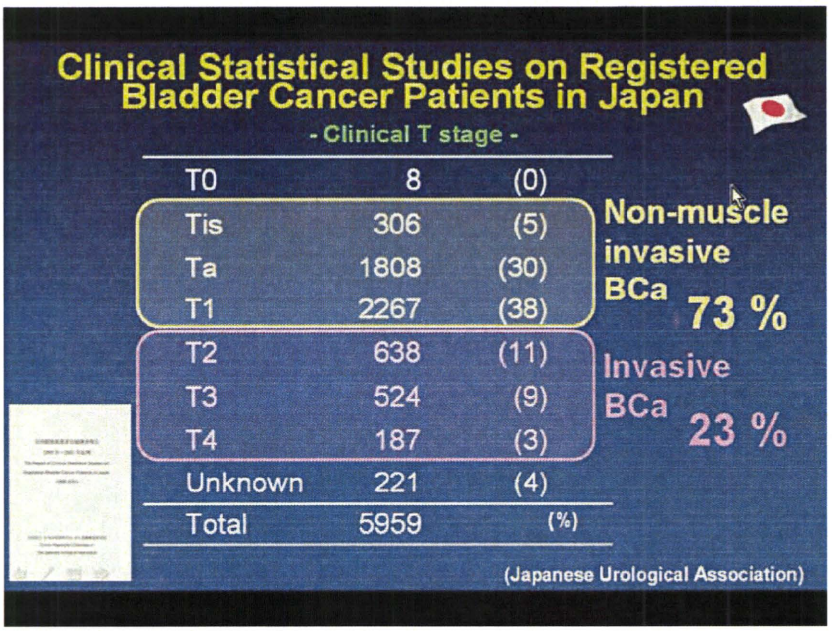

Figure 3. Clinical statistical studies on registered bladder cancer patients in Japan.

Table 2. Occupational high risk for blader cancer

Bitumen workers (asphalt, tar)
Automobile industries
Hair dressers exposed to colorants
Sewing machinests
Painters
Printers and paper hangers
Truck drivers
Etc.

832 cases of bladder cancer. Cases experienced a de-escalated survival hazard ratio versus a low (meaning less than the 25th percentile) toenail arsenic overall survival hazard ratio of 0.5. The population with a lower arsenic concentration was at lower risk. The bladder cancer cause-specific survival showed a similar trend, but did not reach statistical significance.

With regard to trihalomethanes (THMs), a 2007 report dealt with bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering and swimming in pools (8). More than 1200 bladder cancer cases and more than 1200 control subjects were included in a case-controlled study in Spain in 1998–2001. The results showed that long-term exposure to THMs was associated with a 2-fold bladder cancer risk, with an odds ratio of 2.10, for average household THM levels of >49 versus <8 μg/l. The odds ratio for showering/bathing was 1.83, whereas the odds ratio for swimming in a pool was 1.57 (Fig. 4).

The impact of dietary habits on the risk of bladder cancer was investigated by means of a principal components analysis case-controlled study conducted in Uruguay and reported in 2008 (9). The study subjects were 255 cases of newly diagnosed urothelial cancer and 501 hospitalized control subjects. In the case of a sweet beverage pattern characterized by high loading with coffee or tea with added sugar, the odds ratio was 3.27 and the 95% CI was 1.96–5.45. For a Western dietary pattern of high loading with red meat, fried eggs, potatoes and red wine, the odds ratio was significantly high at 2.35.

A 2008 study conducted in Canada investigated for associations of meat and fish consumption with various cancers (10). Nearly 20 000 patients with cancer of the stomach, colon, rectum, pancreas, lung, breast, prostate, bladder, etc., completed a questionnaire. The study included over 5000 population controls between 1994 and 1997 in eight Canadian provinces. The results indicated that total meat and processed meat consumption were directly related to the risk of various cancers: stomach, colon, rectum, pancreas, lung, breast, prostate, testis, kidney, bladder and leukemia. Also, red meat was significantly associated with colon, lung (mainly in men) and bladder cancer (Fig. 5). A second report, published in 2009, investigated meat intake and bladder cancer risk in a Swedish prospective cohort. In 1997, 82 002 Swedish women and men who were free of cancer completed a food-frequency questionnaire and were then prospectively observed (11). During a mean follow-up of 9.4 years, 485 cases of bladder cancer were diagnosed, but there was no association between the intake of total or any specific type of meat. This is thus a negative report as to the impact of meat intake.

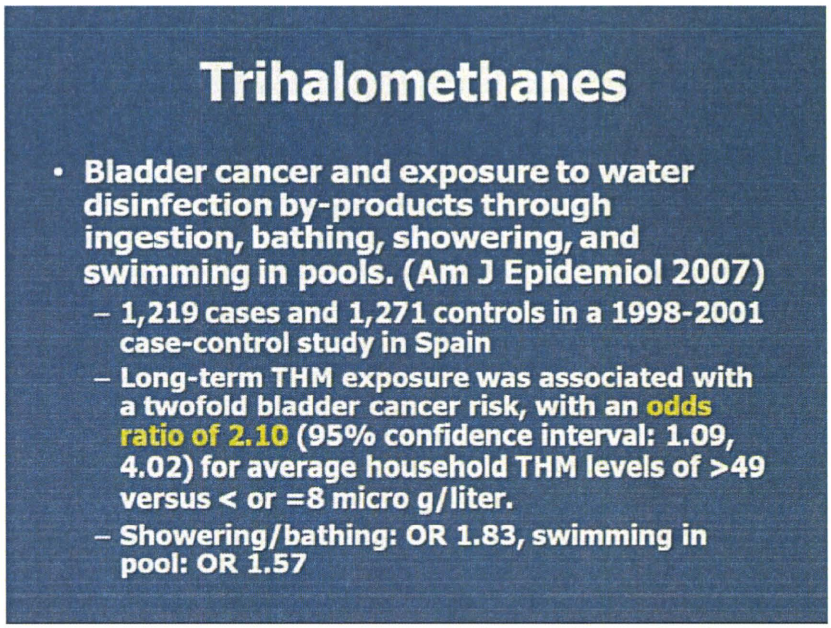

Figure 4. Trihalomethanes.

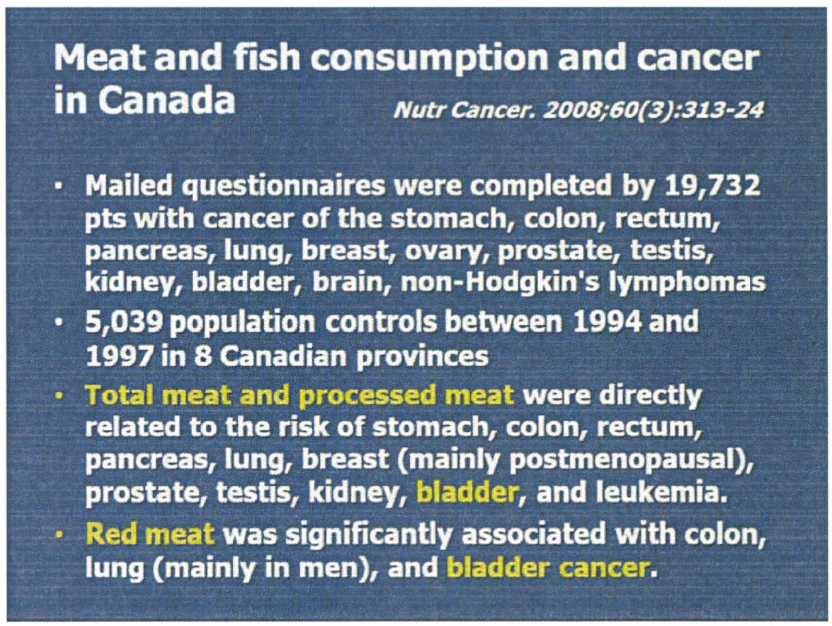

Figure 5. Meat and fish consumption and cancer in Canada.

Finally, with regard to cigarette smoking, it has been found that smoking accounts for more than 50% of bladder cancers in men and 30% in women. Patient awareness of smoking as a risk factor for bladder cancer was addressed in a 2009 published report (12). A prospective observational study investigated 202 consecutive urological inpatients by the use of a structured questionnaire (Fig. 6). Only 118 of the patients (58.4%) stated that they were aware of smoking as a risk factor for bladder cancer, which was strikingly lower than the 94.6% awareness with regard to chronic obstructive pulmonary disease, the 91.6% awareness with regard to heart and vascular problems and the 92.1% awareness with regard to lung cancer.

In conclusion, although the relative risk of bladder cancer associated with occupations is small, the public health impact may be significant. The Western pattern of diet was associated with a significant increase in the risk of bladder cancer. Urological patients' awareness of smoking as a risk

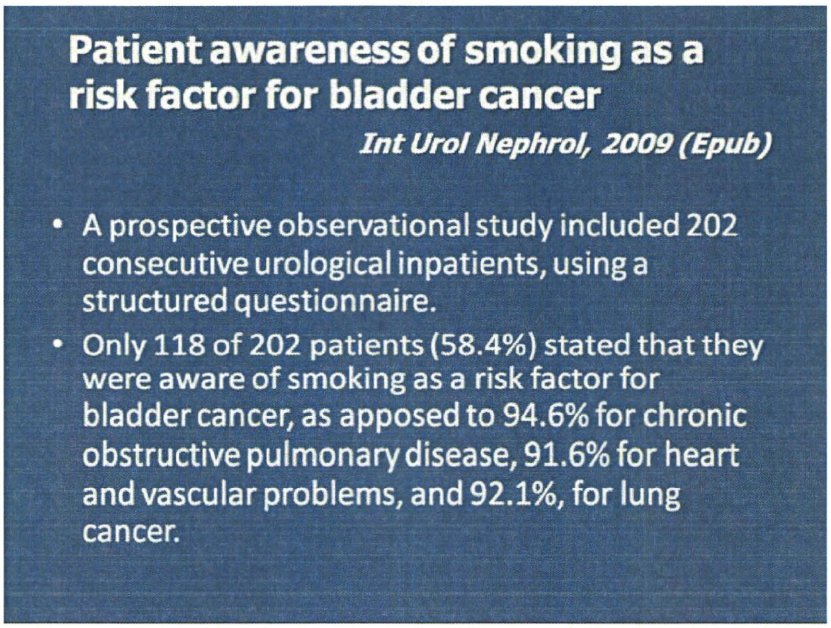

Figure 6. Patient awareness of smoking as a risk factor bladder cancer [Anastasiou et al. (12)].

factor for bladder cancer is lower than their awareness regarding other smoking-related disease entities. Counseling patients regarding the risk of tobacco is a role for urologists.

GENETIC SUSCEPTIBILITY TO UROTHELIAL CARCINOMA

Genetic variation in the human genome is an emerging resource for studying cancers, and it was reported that most population-attributable cancer heritability is related to polymorphic variation in the DNA sequence (13). An important point is that many of the genes encoding cytokines and enzymes are polymorphic.

N-acetyltransferase 2 is considered to be an important enzyme for detoxification of carcinogens, especially in bladder cancer. Slow and rapid acetylators have been shown to have variable association with bladder cancer (14–16). Racial differences have been shown for the prevalence of the slow acetylator genotype, with <20% in Asians and >55% in Caucasians (17,18). In Korean subjects, 7.5% of bladder cancer patients had the slow acetylator genotype (19). The lower incidence of this genotype in Asians may result in a lower incidence of bladder cancer.

A Korean study found that the risk of bladder cancer was increased in patients with tuberculosis and bronchial asthma (19). The reasons for this are unclear, but since patients with these chronic diseases undergo long-term drug treatment, perhaps the drugs or the disease processes are also influencing factors. On the other hand, the glutathione *S*-transferase T1 genotype is significantly associated with bladder cancer, and GSTM1 and GSTT1 appear to be important in detoxifying the products of oxidative stress (20,21). The frequencies of the GSTM1 and GSTT1 genotypes vary according to ethnicity (22,23). Western data suggest that null type GSTM1-negative and GSTT1-negative genotypes are significant risk factors for bladder cancer. In a Korean case-controlled study, a smoking history and a GSTT1 null genotype were significantly associated with bladder cancer risk, whereas in another population, the GSTM1 null genotype was not associated with bladder cancer risk (23). In uni- and multivariate analyses of environmental data from various countries and ethnic groups, a smoking history was possibly associated with bladder cancer, but the frequency of the GSTT1 null genotype was negatively associated with bladder cancer risk.

OGG1 8-oxoguanine DNA glycosylase is a DNA repair enzyme, and research has identified two polymorphic sites (24,25). One site was at codon 326 in exon 7, and the other was at codon 324 in exon 6. The association between bladder cancer risk and the codon 326 genotype in OGG1 was examined by age- and sex-adjusted analyses. The distribution of the codon 326 genotype in bladder cancer patients was significantly different from the control subjects (26). In particular, the bladder cancer risk in Korean males younger than 40 years old was approximately six times higher than in men older than 40 (27). Frequent mutation at codon 326 in OGG1 was found in bladder tumor tissues: ~24–25% of tumor tissues.

Tumor necrosis factor-α (TNF-α) is a very well-known cytokine. GA polymorphism at the −308 nucleotide of the TNF-α promoter was frequently seen in several cancers (28,29). Several studies reported that the cancer stage and grade were significantly associated with the GA genotype in the TNF-α promoter region (26,30). Examination of GA polymorphism in the TNF-α promoter region did not reveal

any difference between bladder cancer patients and control subjects. However, the relationship between high-grade bladder cancer and the −308 genotype, especially the GA genotype, was statistically significant. The GA genotype at this site had a statistically significant impact on TNF-α production and was also associated with a statistically significant increase in gene transcription. Moreover, the serum concentration of TNF-α was significantly higher in bladder cancer patients than in the control subjects.

Patients and controls were analyzed to determine whether the genetic polymorphisms in these five genes are risk factors for bladder cancer, and OGG1, GSTM1 and GSTT1 were found to be significantly associated with increased bladder cancer risk (26). Also, in uni- and multivariate analyses, the OGG1 and GSTMI genotypes were significantly associated with recurrence and progression, respectively, of non-bladder cancer. Collectively, data show that these single-nucleotide polymorphism (SNP) markers may be good indicators of the prognosis of bladder cancer in the clinical setting.

Recently, a Chinese group investigated the risk of bladder cancer in relation to the CYP, NAT2, GSTM1 and GSTT1 genotypes by stratifying the patients on the basis of their smoking history (31). The data showed that, in smokers, the NAT2 acetylation, GSTM1 null genotype and GSTM1/GSTT1 double null genotype were significant risk factors for bladder cancer. Subtype analysis showed that the NAT2, GSTM1 and GSTM1/GSTT1 null genotypes were associated with the tumor stage or grade. Another paper published in 2009 provided the best evidence that the GSTM1 null genotype and the NAT2 slow-acetylator genotype are associated with genetic susceptibility to bladder cancer (32).

In conclusion, recent SNP genetic studies in relation to bladder carcinogenesis have revealed several genetic polymorphisms of detoxification or DNA repair genes, such as NAT2, GST and OGG1. That information is important in relation to environmental risk factors and ethnic differences and will help predict the prognosis of patients with bladder cancer. Further studies are needed to confirm potential gene–gene and gene–environmental interactions leading to bladder carcinogenesis. Treatment of bladder cancer is completely dependent on the nature of the bladder cancer cells. If it is non-invasive, it can be treated by bladder preservation, using cystoscopic resection. On the other hand, if it is muscle-invasive, it may have high malignant potential for invasion or metastasis. Such patients should be treated by total cystectomy.

Conflict of interest statement

None declared.

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Expression of integrin proteins in non-muscle-invasive bladder cancer: significance of intravesical recurrence after transurethral resection

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Study Type – Aetiology (case control)
Level of Evidence 3b

What's known on the subject? and What does the study add?
A number of studies have reported several clinicopathological factors closely associated with intravesical recurrence of non-muscle invasive bladder cancer (NMIBC). In addition, various types of molecular markers have been shown to be useful for predicting intravesical recurrence of NMIBC following transurethral resection (TUR).

Of six subunits of integrin proteins, including α2, α3, α5, α6, β1 and β4, the expression level of the β4 subunit in NMIBC, in addition to pathological T stage and concomitant carcinoma *in situ* appeared to be independently related to intravesical recurrence. Therefore, consideration of the expression levels of integrins, particularly that of the β4 subunit, in TUR specimens would contribute to further accurate prediction of intravesical recurrence of NMIBC.

OBJECTIVES

- To evaluate the expression of integrin proteins, a family of transmembrane heterodimers, in non-muscle-invasive bladder cancer (NMIBC).
- To assess the significance of these proteins as prognostic indicators in patients undergoing transurethral resection (TUR).

PATIENTS AND METHODS

- The present study comprised 161 patients diagnosed as having NMIBC after TUR.
- Expression levels of six subunits of integrin proteins, including α2, α3, α5, α6, β1 and β4, were measured in TUR specimens by immunohistochemical staining.

RESULTS

- Of the six proteins, expression levels of α2-, α3-, α6- and β4-subunits were significantly associated with the incidence of intravesical recurrence. Univariate analysis identified expression levels of α3-, α6- and β4-subunits as important predictors of intravesical recurrence, while tumour size, pathological T stage and concomitant carcinoma *in situ* (CIS) were also important.
- Multivariate analysis showed that the expression level of the β4 subunit, pathological T stage and concomitant CIS are independently related to intravesical recurrence.
- There were significant differences in intravesical recurrence-free survival for patients who were positive for the three independent risk factors; intravesical recurrence occurred in 10 of 49 (20.4%) patients who were negative for all risk factors, 31 of 68 who were positive for one risk factor (45.6%), and 30 of 44 who were positive for two or three risk factors (68.2%).

CONCLUSIONS

- Consideration of the expression levels of integrins, particularly those of the β4 subunit, in TUR specimens, in addition to conventional variables, would contribute to accurate prediction of intravesical recurrence after TUR for NMIBC patients.

KEYWORDS

non-muscle-invasive bladder cancer, transurethral resection, intravesical recurrence, integrin

INTRODUCTION

Approximately 80% of newly diagnosed bladder cancers are non-muscle- invasive tumours that are limited to the urothelium or infiltrate no deeper than the lamina propia. Complete transurethral resection (TUR) of the visible tumour burden, which is the current standard of care for patients with non-muscle-invasive bladder cancer (NMIBC), generally achieves a favourable prognosis with 5-year survival rates >80% [1]. However, intravesical recurrence after TUR has been reported in 30 to 80% of patients with NMIBC [1–3]. Intensive efforts, therefore, have been paid to the development of systems that can accurately predict the outcome of TUR, and these systems are of great value for planning postoperative adjuvant therapy, as well as for follow-up schedules for individual patients with NMIBC.

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To date, a number of studies have reported risk factors closely associated with intravesical recurrence of NMIBC, including tumour multiplicity, maximal tumour size, grade, stage, growth pattern and microvascular invasion [3–7]; however, these outcomes are not consistent. Hence, in order to provide more reliable information regarding the probability of intravesical recurrence in patients with NMIBC after TUR, several investigators have evaluated the value of various types of molecular markers, and some of these molecules were shown to be significantly related to postoperative prognostic outcomes [8–18]. For example, Mhawech-Fauceglia *et al.* [9] identified fibroblast growth factor receptor-3 as an important factor in predicting the time to recurrence in NMIBC using a high-throughput tissue microarray. Miyake *et al.* [14] and Behnsawy *et al.* [18] also reported that strong clusterin and weak p21 expression in resected NMIBC specimens could be important predictive factors of intravesical recurrence in patients with NMIBC after TUR. However, no systems have been introduced into clinical practice for predicting outcomes in patients with NMIBC who were treated with TUR.

Integrins, a family of transmembrane heterodimers, each of which is composed of a single α- and β-subunit, function as receptors for extracellular matrix molecules and mediate signal transduction for the control of diverse cell functions, such as survival, proliferation, differentiation, angiogenesis and migration [19,20]. Furthermore, aberrant expression of integrin has been shown to play an important role in the acquisition of aggressive phenotype in cancer cells [21,22]. Few studies have, however, investigated the role of integrins in bladder cancer progression [23–26]. It is, therefore, not known whether integrins have a marked impact on the prognosis of patients with bladder cancer, and with NMIBC in particular. Considering this, we evaluated the expression levels of six subunits of integrin proteins, including α2, α3, α5, α6, β1 and β4, in TUR specimens using immunohistochemical staining, and analysed these outcomes according to several clinicopathological variables.

PATIENTS AND METHODS

The present study comprised 161 consecutive patients who were treated with TUR for newly diagnosed primary bladder cancer between January 2000 and December 2007 at Kobe University Hospital, and who were subsequently diagnosed as having NMIBC (i.e. stage Ta or T1 tumour). Informed consent was obtained from each patient, and the study design was approved by our institution's Research Ethics Committee. Growth pattern was macroscopically classified into either the papillary or non-papillary type. Tumour size was defined as the largest tumour measured using a 1-cm long resection loop. Complete resection of all visible tumours was done, and several deep muscular samples were obtained with the resection instrument. Irrespective of findings from preoperative urinary cytology, random bladder biopsy that targeted the trigone, posterior wall, bilateral lateral walls, dome, anterior wall, prostatic urethra in men and/or suspicious regions, was performed before TUR for all patients. Pathological examinations were carried out by a single pathologist according to the 2002 American Joint Committee on Cancer TNM classification system.

As a rule, the indication for adjuvant intravesical instillation therapy was defined as the presence of concomitant cancer *in situ* (CIS) and/or T1G3 disease. BCG was, therefore, administered for the majority of patients who received adjuvant intravesical instillation therapy in the present series. Follow-up of patients after TUR of NMIBC was carried out based on the relatively intensive schedule previously described [18], i.e. cystoscopy and urinary cytological examination were performed every 3 months for 3 years after TUR, and then every 6 months until 5 years after TUR, and intravenous pyelography was performed every 6 months until 3 years after TUR and then annually until 5 years after TUR. On detection of tumours or hyperemic mucosa by cystoscopy and/or positive findings from urinary cytology, transurethral biopsy of the abnormal region and/or TUR of the tumour were performed.

Immunohistochemical staining of TUR specimens was performed as previously described [27]. Briefly, sections from formaldehyde-fixed, paraffin-embedded tissue from 161 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 on 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 the following antibodies: anti-human α2 mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-human α3 mouse monoclonal antibody (Santa Cruz Biotechnology), anti-human α5 rabbit polyclonal antibody (Santa Cruz Biotechnology), anti-human α6 rabbit polyclonal antibody (Santa Cruz Biotechnology), anti-human β1 rabbit monoclonal antibody (Epitomics, Burlingame, CA, USA) and anti-human β4 rabbit polyclonal antibody (Santa Cruz Biotechnology). The sections were then incubated with biotinylated goat anti-mouse or rabbit IgG (Vector Laboratories, Burlingame, CA, USA). After incubation in avidin–biotin peroxidase complex for 30 min, the samples were exposed to diaminobenzidine tetrahydrochloride solution and counterstained with methyl green (Vector Laboratories).

Staining results were interpreted by two independent observers who were blinded to the clinicopathological data. If discordant interpretations were obtained, differences were resolved by joint review and/or consultation with a third observer familiar with immunohistochemical pathology. In this series, staining of each protein was characterized based on five staining levels (0, no staining; 1, 1% to 25% of cells showing membrane bound expression; 2, 25% to 50%; 3, 50% to 75%; and 4, 75% to 100%). For the purposes of presentation, the staining result was considered strong if more than 50% of cells expressed the antigen (staining levels 3 and 4) and weak if 50% or fewer of the cells showed a staining signal (levels 0 to 2) [28].

All statistical analyses were performed using Statview 5.0 software (Abacus Concepts, Inc., Berkeley, CA). The chi-squared test was used to analyse associations between intravesical recurrence and several variables. 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. $P < 0.05$ was considered significant.

RESULTS

During the median observation period of 47 months (range, 13–93), intravesical disease

TABLE 1 Characteristics of 161 patients with MIBC who underwent TUR with regard to intravesical recurrence

	Intravesical recurrence			Intravesical recurrence-free survival	
Variables	Negative (N = 90)	Positive (N = 71)	P value	3-year recurrence-free survival rate (%)	P value
Age (years)			0.71		0.94
<70	43	36		54.6	
	47	35		53.0	
Gender			0.28		0.26
M	79	58		53.9	
F	11	13		53.9	
Preoperative urinary cytology			0.97		0.79
Negative	53	42		53.8	
Positive	37	29		54.4	
Multiplicity of tumour			0.35		0.23
Single	46	31		59.5	
Multiple	44	40		48.3	
Maximal tumour size			<0.001		0.0011
≤3 cm	72	37		63.2	
>3 cm	18	34		36.8	
Tumour type			0.13		0.068
Papillary	80	57		57.6	
Non-papillary	10	14		34.7	
T stage			0.081		0.022
Ta	65	42		60.6	
T1	25	29		40.6	
Grade			0.37		0.37
G1	29	20		57.6	
G2	49	40		54.7	
G3	12	11		42.8	
CIS			0.0038		0.0010
Negative	76	46		61.6	
Positive	14	25		31.6	
Adjuvant intravesical instillation therapy			0.12		0.31
Yes	25	28		42.6	
No	65	43		59.2	
α2 integrin			0.036		0.056
Weak expression	65	40		60.1	
Strong expression	25	31		45.6	
α3 integrin			0.019		0.033
Weak expression	62	36		63.4	
Strong expression	28	35		42.5	
α5 integrin			0.91		0.92
Weak expression	41	33		54.4	
Strong expression	49	38		55.1	
α6 integrin			0.017		0.0089
Weak expression	40	45		43.2	
Strong expression	50	26		66.6	
β1 integrin			0.21		0.20
Weak expression	47	30		58.0	
Strong expression	43	41		52.1	
β4 integrin			0.0027		0.0058
Weak expression	26	37		38.8	
Strong expression	64	34		66.2	

TABLE 2 Univariate and multivariate analysis of factors associated with recurrence-free survival after TUR in 161 patients with NMIBC

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Age (<60 years vs ≥60 years)	1.02 (0.62–1.56)	0.93	0.99 (0.51–1.92)	0.97
Gender (M vs F)	1.41 (0.77–2.58)	0.27	0.71 (0.35–1.43)	0.34
Preoperative urinary cytology (negative vs positive)	1.06 (0.58–1.51)	0.79	0.87 (0.49–1.55)	0.63
Multiplicity of tumour (single vs multiple)	1.33 (0.47–1.21)	0.24	0.64 (0.38–1.09)	0.10
Maximal tumour size (≤3 cm vs >3 cm)	2.13 (1.33–3.45)	0.001	1.80 (1.09–2.98)	0.023
Tumour type (papillary vs non-papillary)	1.71 (0.95–3.08)	0.071	0.76 (0.31–1.84)	0.54
T stage (Ta vs T1)	1.72 (1.08–2.78)	0.024	1.80 (1.09–3.01)	0.026
Grade (G1 or G2 vs G3)	1.35 (0.39–1.42)	0.37	0.81 (0.33–1.96)	0.64
CIS (negative vs positive)	2.22 (1.37–3.57)	0.035	0.76 (0.39–1.50)	0.43
Adjuvant intravesical instillation therapy (yes vs no)	1.55 (0.32–1.36)	0.24	0.85 (0.39–1.51)	0.49
α2 integrin (weak expression vs strong expression)	1.56 (0.98–2.50)	0.058	0.70 (0.41–1.19)	0.19
α3 integrin (weak expression vs strong expression)	1.64 (1.03–2.63)	0.035	0.66 (0.39–1.12)	0.13
α5 integrin (weak expression vs strong expression)	0.97 (0.61–1.56)	0.92	0.82 (0.45–1.51)	0.53
α6 integrin (weak expression vs strong expression)	1.89 (1.16–3.06)	0.010	0.60 (0.33–1.08)	0.084
β1 integrin (weak expression vs strong expression)	0.73 (0.46–1.18)	0.20	0.71 (0.41–1.23)	0.22
β4 integrin (weak expression vs strong expression)	1.91 (1.20–3.05)	0.0067	1.85 (1.03–3.31)	0.040

FIG. 1. Intravesical recurrence-free survival of 161 patients with primary NMIBC who underwent TUR.

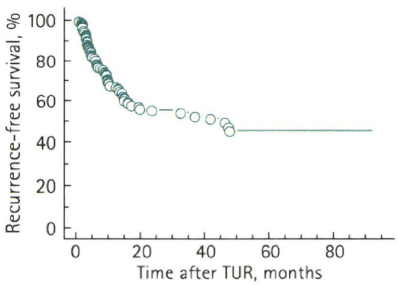
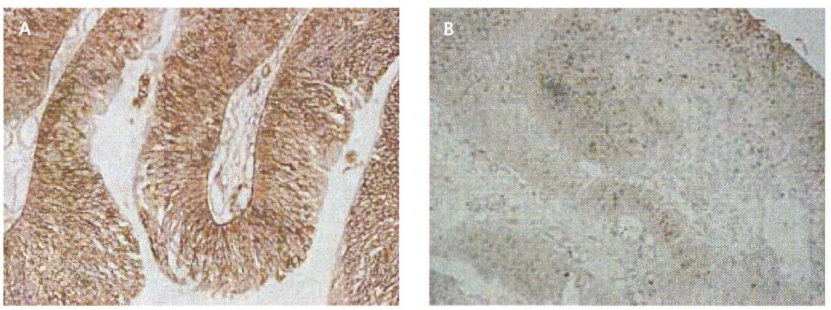


*FIG. 2. Typical outcomes of immunohistochemical staining of primary NMIBC with an antibody against β4 integrin subunit. **A**, NMIBC with weak expression of β4 integrin. **B**, NMIBC with strong expression of β4 integrin.*

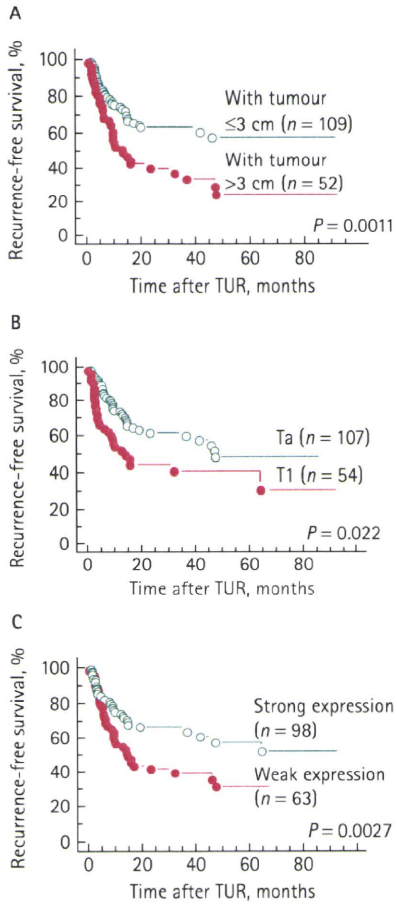


recurrence was detected in 71 of 161 patients (44.1%), and 1-, 3- and 5-year recurrence-free survival rates were 67.7%, 53.9% and 45.2%, respectively (Fig. 1). Table 1 presents the characteristics of 161 patients included in this study according to the presence or absence of intravesical recurrence. Of several variables examined in this study, maximal tumour size and concomitant CIS were significantly related to the incidence of intravesical recurrence. Furthermore, there was a significant association between the expression level of α2, α3, α6 or β4 integrin subunits in NMIBC and the incidence of intravesical recurrence after TUR, despite the lack of significant impact of the expression levels of α5 and β1 subunits on the incidence of intravesical recurrence.

We then evaluated the significance of several factors, including the expression patterns of integrin subunits, in the probability of intravesical recurrence. As shown in Table 2, univariate analysis using the Cox proportional hazards regression model showed that expression levels of the α3, α6 and β4 subunits were significant variables associated with intravesical recurrence-free survival, while maximal tumour size, pathological T stage and concomitant CIS were also significant. In addition, expression level of β4 integrin subunit, maximal tumour size and pathological T stage appeared to be independent predictive factors of intravesical recurrence on multivariate analysis (Table 2). Representative findings of immunohistochemical study for detecting β4 integrin expression are shown in Fig. 2, and recurrence-free survival curves according to maximal tumour size, pathological T stage and expression level of β4 integrin, are presented in Fig. 3. There were significant differences in recurrence-free survival with respect to all three of these factors.

To characterize more precisely the postoperative clinical features of NMIBC, we categorized patients who were positive for the three independent risk factors for intravesical recurrence (maximal tumour size, pathological T stage and β4 integrin expression level). During the observation period of this series, intravesical recurrence developed in 10 of 49 patients who were negative for all risk factors (20.4%), 31 of 68 positive for a single risk factor (45.6%), and 30

*FIG. 3. Intravesical recurrence-free survival of patients with primary NMIBC who underwent TUR with regard to maximal tumour size (**A**), pathological T stage (**B**) and expression level of β4 integrin subunit (**C**).*



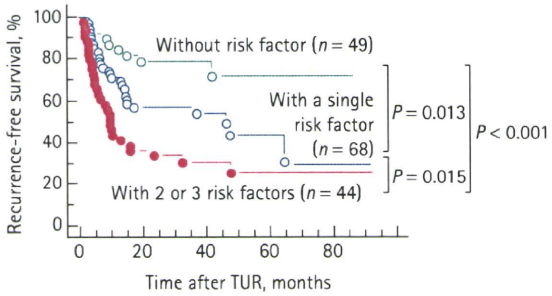


FIG. 4. Intravesical recurrence-free survival of patients with primary NMIBC with regard to the following independent risk factors for intravesical recurrence after TUR: maximal tumor size; pathological T stage; and expression level of β4 integrin subunit.

of 44 positive for two or three risk factors (68.2%). As shown in Fig. 4, there were significant differences in intravesical recurrence-free survival among these three subgroups.

DISCUSSION

To date, a number of investigators [3–7] have evaluated variables that precisely predict the probability of intravesical recurrence after TUR of NMIBC, however, the outcomes of these studies failed to provide consistent findings. Furthermore, considering the diverse genetic, as well as biological, characteristics of NMIBC [29], it may not be sufficient to assess clinicopathological factors alone for stratifying patients with NMIBC into risk groups with respect to intravesical recurrence after TUR. In the present study, therefore, we evaluated the values of integrins, a family of transmembrane heterodimers, which have been shown to be involved in a wide variety of pathophysiological cell functions [19–22], in addition to conventional clinicopathological variables for predicting prognosis in 161 consecutive patients undergoing TUR for NMIBC.

In the present series, 44.1% of the patients experienced intravesical disease recurrence, achieving the 5-year recurrence-free survival rate of 45.2%, which is comparable to those in previous studies [1–3]. Differences in characteristics, including the expression patterns of integrin subunits, between patients with and without intravesical recurrence, were then analysed. Among several conventional factors examined in this series, maximal tumour size and concomitant CIS were significantly associated with the incidence of intravesical recurrence. Several previous studies found results similar to those in the present study [3–7]. For example, Millan-Rodriguez *et al.* [7] reported a greater incidence of intravesical recurrence in patients with NMIBC who were diagnosed as having multiple tumours, tumours >3 cm and/or concomitant CIS. In addition, of the integrin subunits investigated in this series, the strong expression of α2 and α3 as well as the weak expression of α6 and β4 subunits were shown to have a significant impact on the incidence of intravesical recurrence. There have been only a few studies analysing the expression patterns of these subunits in NMIBC specimens [25,26,28] and the prognostic significance of integrin expression in NMIBC is still not known. It would be necessary to conduct a prospective assessment of a larger number of patients in order to draw a definitive conclusion on this issue.

To develop a reliable prediction system for intravesical recurrence after TUR of NMIBC, which is potentially useful for determining post-TUR follow-up as well as for treatment schedules, the effects of conventional prognostic variables and expression levels of integrin subunits on time to intravesical recurrence after TUR were investigated. Univariate analysis identified expression levels of α3, α6 and β4 subunits, in addition to tumour size, pathological T stage and concomitant CIS, as significant predictors of intravesical recurrence. Using multivariate analysis, however, only β4 integrin expression, tumour size and pathological T stage appeared to be independently associated with intravesical recurrence-free survival. As indicated above, tumour size and T stage are useful prognostic indicators in patients with NMIBC undergoing TUR [3–7]. To our knowledge, however, the present study is the first to demonstrate the independent significance of β4 integrin expression as a predictor of intravesical recurrence of NMIBC, although some studies [23,26] have suggested the important role of the β4 integrin subunit in disease progression of muscle-invasive bladder cancer. Furthermore, Clarke *et al.* [30] showed that forced expression of β4 integrin in a colon cancer cell line resulted in the induction of p21, which was shown to have a negative effect on intravesical recurrence of NMIBC in our previous study [18]. Collectively, these findings indicate that the linkage of β4 integrin to a signalling pathway involved in cell-cycle regulation through the induction of p21 expression may be involved in the molecular mechanism underlying the intravesical recurrence of NMIBC.

To allow more precise risk stratification for postoperative intravesical recurrence in an individual patient with NMIBC, combined assessment of the three independent predictors for intravesical recurrence

identified by multivariate analysis was performed. When the patients were classified into three subgroups, negative for all risk factors, positive for a single risk factor and positive for two or three risk factors, there were significant differences in recurrence-free survival among these three groups. These findings suggest that it would be useful to develop a novel system that can more accurately predict intravesical recurrence of NMIBC after TUR based on the consideration of the three risk factors (maximal tumour size, pathological T stage and β4 integrin expression). Moreover, the usefulness of the combined use of these three factors could contribute to further refinement of the system. However, a prospective study including additional information with more potential molecular markers involved in the intravesical recurrence of NMIBC will be required before a definitive conclusion can be drawn.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **NMIBC**, non-muscle-invasive bladder cancer; **TUR**, transurethral resection; **CIS**, carcinoma *in situ*.

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Original Article: Clinical Investigation

Maintenance intravesical bacillus Calmette-Guérin instillation for Ta, T1 cancer and carcinoma *in situ* of the bladder: Randomized controlled trial by the BCG Tokyo Strain Study Group

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Objectives: We carried out a prospective, randomized, controlled trial to investigate the efficacy and safety of both induction and maintenance therapy with intravesical instillation of bacillus Calmette-Guérin (BCG) for high-risk non-muscle invasive bladder cancer (NMIBC).

Methods: Intravesical instillation of 80 mg Tokyo strain was given to patients with high-risk NMIBC, including carcinoma *in situ* (CIS), once weekly for eight consecutive weeks as induction therapy. Patients who achieved complete response (CR) were randomly assigned to either the maintenance group or the observation group.

Results: A total of 90 patients were enrolled. After induction therapy, 75% of the patients achieved CR and 53 of them were enrolled in the randomized comparative phase. A total of four maintenance instillations were given. Median follow-up was 26.5 and 28.7 months after randomization in the maintenance and the observation group, respectively. Although it was not statistically significant, the 2-year recurrence-free survival rate in the maintenance group (95.8%) was higher than that in the observation group (74.1%, $P = 0.078$). Univariate analysis identified maintenance therapy as a significant factor influencing recurrence. During induction therapy, 82.2% of patients experienced urination-related adverse drug reactions, but most events were not serious. There were fewer adverse drug reactions with maintenance therapy than with induction therapy. Neither induction therapy nor maintenance therapy reduced patients' quality of life (QOL).

Conclusions: These findings show high levels of efficacy and safety of BCG induction treatment for high-risk NMIBC, and suggest that the number of maintenance instillations could probably be reduced without reducing treatment efficacy or influencing QOL.

Key words: Bacillus Calmette-Guérin, carcinoma *in situ*, maintenance instillation, non-muscle invasive bladder cancer, randomized trial.

Introduction

Intravesical instillation of bacillus Calmette-Guérin (BCG) has been reported to have an advantage over intravesical chemotherapy in terms of therapeutic benefit and prophylaxis of non-muscle invasive bladder cancer (NMIBC).[1,2] A meta-analysis study also showed that this treatment method reduced the risk of progression of NMIBC.[3] Intravesical instillation of BCG is currently regarded as a standard modality for the treatment of high-risk Ta, T1 bladder cancer and carcinoma *in situ* (CIS) of the bladder, as well as for the prophylaxis of recurrence after the resection of high-risk Ta, T1 bladder cancer.[4,5] The South West Oncology Group (SWOG) randomized clinical trial showed that 5-year recurrence-free survival and 5-year progression-free survival were higher in patients who received BCG induction therapy followed by maintenance therapy than those who received only BCG induction therapy.[6] A meta-analysis showed that only BCG instillation as maintenance therapy

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prevented the progression of NMIBC.[7] Thus, BCG maintenance therapy is recommended to prevent recurrence or progression and to prolong survival in patients with high-risk NMIBC.[4,5] In the SWOG randomized study, the maintenance period was longer than two years and a total number of 21 maintenance instillations were given, although a 3-week maintenance schedule seems to be regarded as the standard maintenance instillation method. This might be one of the reasons why only a small number of patients completed the maintenance BCG instillation. It shows that efforts should be made to develop another BCG maintenance schedule.

In this time, we carried out a prospective, randomized, comparative study to investigate the efficacy and safety of both induction and maintenance BCG therapy, as well as another schedule of maintenance instillation for high-risk Ta, T1 cancer and CIS of the bladder.

Methods

This is a multicenter, prospective, open-label, randomized, comparative study. The present clinical trial protocol was approved by the institutional review board of each participating institution. Patients with histologically-confirmed Ta, T1 transitional cell carcinoma or CIS of the bladder were eligible to participate in the present study. In the case of Ta and T1, the tumor had to be diagnosed as not completely resectable by transurethral resection.

Other inclusion criteria were: (i) performance status (PS) of 0 to 2; (ii) age between 20 and 79 years; (iii) able to undergo assessment, such as cystoscopy and urinary cytology, on a regular basis; (iv) presence of at least one cystoscopically measurable or evaluable lesion in the case of unresectable NMIBC; and (v) intact function of the main organs (i.e. bone marrow, liver, kidney, heart and lung) Exclusion criteria were: (i) strongly positive tuberculin reaction or active tuberculous lesion; (ii) history of BCG intravesical instillation therapy; (iii) severe bladder irritation before the start of drug administration; (iv) intravesical instillation therapy with an anticancer drug within 3 weeks before the start of BCG administration; (v) history of bladder-sparing treatment for invasive bladder cancer; (vi) history of upper urinary tract carcinoma; and (vii) active double cancer or serious medical complications. Before treatment, patients provided a history and underwent physical examination, urinalysis, urine cytology examination, complete blood count, blood urea nitrogen, serum creatinine, liver function tests and electrocardiography. Chest X-ray and excretory urography were also carried out.

All eligible patients had to receive a full explanation of this study and sign an informed consent form before registration by facsimile to the central registration center. Induction therapy with 80 mg of BCG Tokyo strain started within 4 weeks after bladder biopsy or TUR for histological diagnosis. Patients were required to hold their urine for approximately 2 h after instillation. BCG treatment was repeated weekly for eight consecutive weeks. In the case of adverse drug reactions (ADR), administration could be delayed, but not for longer than 3 weeks from the scheduled administration day. Efficacy was evaluated at 4–12 weeks after the completion of this induction therapy. Patients who achieved CR on the induction therapy were required to receive a full explanation of the randomized study before re-registration at the same central registration center as for the induction therapy. The registered patients were randomly assigned to either the maintenance or the observation group. Maintenance therapy involved a single intravesical instillation of BCG Tokyo strain 80 mg within 3 months after the randomization, followed by instillations at 3, 6 and 9 months after the initial dose (a total of 4 doses). The dosing interval of maintenance therapy was set as 3 months based on the approved regimen of a similar drug that is already on the market in the USA. The total number of maintenance doses was determined as follows; the maximum number of doses was considered to be 12 which is 1.5 times the approved regimen of BCG Tokyo strain in Japan and because eight doses would be given as induction therapy, an additional four doses were to be given as maintenance therapy.

Patients in both groups underwent urinalysis, urinary cytology and cystoscopy 2 months after randomization. These examinations were repeated for observing the presence or absence of recurrence every 3 months for the first

Table 1 Patient characteristics

	No. patients
Enrolled patients	90
Evaluable patients	84
Sex	
Male	68
Female	16
Age	
Younger than 70 years	39
70 years or older	45
Performance status	
0	80
1, 2	4
Tumor status	
Primary tumor	60
Recurrent tumor	24
Stage	
CIS	74
Ta,T1 tumor	10
Smoking habit	
No	28
Yes	56

CIS, carcinoma *in situ*.

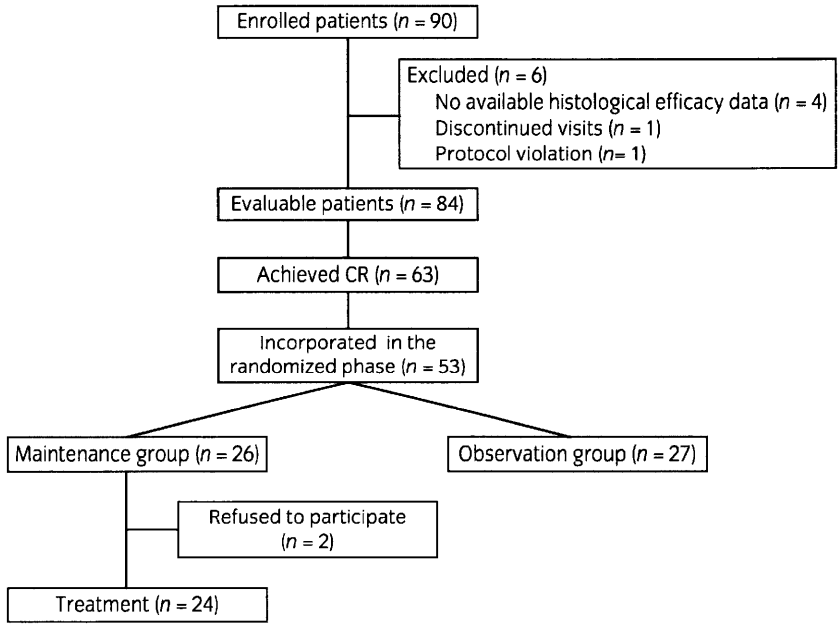

Fig. 1 CONSORT flow diagram. CR, complete response.

3 years, and thereafter every 6 months. Clinical chemistry examination was carried out before the induction therapy, and before the fifth instillation and 2 weeks after the eighth instillation of induction therapy. In the maintenance group, clinical laboratory tests were carried out at 2, 5, 8 and 11 months after randomization. ADR were monitored at every visit and assessed according to the Common Terminology Criteria of Adverse Events version 3.0 (CTC AE v3.0). ADR that were difficult to grade by CTC AE, such as pain on urination and difficulty to urinate, were classified into grade 1 (mild), grade 2 (moderate) and grade 3 (severe). Whenever disease progression was suspected, imaging tests such as CT were carried out. Quality of life (QOL) was assessed according to the Japanese version of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30) v2.0[8] before the induction therapy, after the fifth instillation of induction therapy, 4 weeks after the completion of induction therapy and 14 months after randomization. Before using the QOL scale, authorization was acquired by the prescribed procedure.

The primary endpoint was the efficacy of induction therapy, and the secondary endpoints were recurrence-free survival, progression-free survival, overall survival, QOL and the frequency and severity of ADR in both groups. The efficacy was assessed according to the third edition of the General Rules for Clinical and Pathological Studies on Bladder Cancer.[9] For Ta and T1 cancer, CR was defined as the complete clinical disappearance of target legions with negative biopsy and urine cytology. For CIS, CR was defined as negative biopsy and urine cytology. Recurrence was examined with cystoscopy and urine cytology, and then confirmed histologically. Disease progression was defined as the emergence of muscle invasive cancer or distant metastasis. The last observation day was 31 March 2007, when the present study was closed.

The sample size was determined as follows: CIS is the rarest type, representing just 4% of bladder cancer. Because there have been few reports of maintenance therapy given after BCG treatment for bladder cancer, as in the present study, it was considered difficult to determine the sample size from a statistical viewpoint. Therefore, the sample size was set as 30 patients in each group (a total of 60 patients) in the comparative phase of the study, taking feasibility into consideration. In a previous clinical study using BCG Tokyo strain, the CR rate in partly unresectable Ta, T1 bladder cancer and CIS of the bladder was 70.1%.[10] If the number of patients required for random assignment is 60, then 82.3 patients must be enrolled in induction therapy, taking an estimation error of 10% into account. Taking patients who withdrew from the study and those who refused to participate in the comparative phase of study into consideration, the number of patients enrolled was set as 90.

The significance of differences in patient background factors between the two groups was examined by the χ^2-square test. Recurrence-free, progression-free and overall survival curves were calculated by the Kaplan–Meier method and statistical significance was determined by the log–rank test. The Cox proportional hazard model was used to carry out univariate and multivariate analysis. QOL was evaluated using EORTC QOL-C30 scoring manual and examined by the paired *t*-test. Differences were considered to be significant at $P < 0.05$.

Table 2 Patient characteristics of the maintenance and observation groups

	Maintenance group	Observation group	*P*-value
Sex			
Male	19	21	0.8253
Female	5	6	
Age			
Younger than 70 years	9	14	0.3039
70 years or older	15	13	
Performance status			
0	23	25	0.9162
1, 2	1	2	
Tumor status			
Primary tumor	17	20	0.9558
Recurrent tumor	7	7	
Stage			
CIS	21	25	0.8897
Ta,T1 tumor	3	2	
Smoking habit			
No	9	7	0.5573
Yes	15	20	
Median follow-up period (range)	26.5 (13.9–40.7)	28.7 (14.9–51.7)	0.7199

CIS, carcinoma *in situ*.

Results

A total of 90 patients were enrolled between October 2002 and June 2005. The characteristics of enrolled patients are shown in Table 1. As shown in Figure 1, efficacy was evaluated for 84 patients. For the induction therapy, 6 of 10 patients with Ta and T1 cancer and 57 of 74 patients with CIS of the bladder achieved CR, resulting in CR rates of 60.0% and 77.0%, respectively. The overall CR rate was 75.0%. Among 63 patients who achieved CR, 53 patients were enrolled in the maintenance therapy phase and randomized: 26 to the maintenance group and 27 to the observation group. Two patients randomized to the maintenance group withdrew their consent before the start of maintenance instillation (Fig. 1). There were no significant differences in patient characteristics between the two groups (Table 2). The number of doses given to 24 patients randomized to the maintenance group was one in two patients (8.3%), two in three patients (12.5%), three in one patient (4.2%) and four in 18 patients (75.0%), showing a good completion rate of the maintenance regimen. There was not a significant difference in the follow-up period between the two groups. Recurrence was confirmed in one patient in the maintenance group and seven patients in the observation group, which showed 2-year recurrence-free survival rates of 95.8% and 74.1%, respectively. This indicates a lower recurrence rate in the maintenance therapy than in the observation group (Fig. 2), but the difference between the groups was not significant ($P = 0.078$). Disease progression was not seen in the maintenance group and was seen in one patient in the observation group. There was no significant difference in progression free survival between the groups ($P = 0.383$). Two patients each in the maintenance group and the observation group died during the study. One of the two

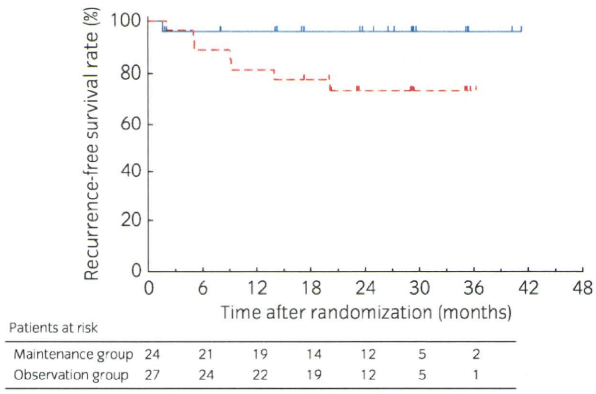

Fig. 2 Recurrence-free survival curves after randomization into two groups. A lower recurrence rate was observed in the maintenance group than in the observation group, but the difference between the two groups was not significant ($P = 0.078$). ——, Maintenance group ($n = 24$); - - - -, observation group ($n = 27$).