Reprinted from
Jpn J Clin Oncol 2006;36(5)301–308
doi:10.1093/jjco/hyl022

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Received September 7, 2005; accepted February 12, 2006; published online May 30, 2006

Background: The Research Center for Cancer Prevention and Screening program is a one-arm prospective study designed to evaluate the effect of multiple modalities for cancer screening. Basic programs consist of screening tests for cancer of the lung, esophagus, stomach, colon, rectum, liver, gall bladder, pancreas and kidneys, in addition to prostate cancer screening for males and breast, cervical, endometrial and ovarian cancer screenings for females.
Objective: To investigate the possibility of overdiagnosis, we compared the observed numbers with expected numbers based on the model.
Methods: We calculated the expected number of cancers on the basis of negative or positive history of screening tests within the previous year, based on assumed sensitivity and sojourn time. Observed numbers of screen-detected cases for stomach, colorectal, lung, prostate and breast cancer were compared with expected numbers.
Results: From February 2004 to January 2005, 3786 participants were enrolled in our study. The overall cancer detection rate was 5.8% (119/2061) for males and 4.1% (71/1725) for females. No statistically significant difference was found between observed and expected cases for colorectal cancer screening, gastric cancer screening for females and lung cancer screening for males. Observed numbers of breast, prostate and lung cancer for females exceeded those expected ($P < 0.05$).
Conclusions: Although cancer screening programs in the present study increased the detection of potentially curable cancers, these modalities, particularly lung, breast and prostate screening, might detect cancers which would not necessarily be clinically significant. We should therefore weigh up benefit and harm for such cancer screening programs.

Key words: cancer screening – detection rate – sensitivity – sojourn time – overdiagnosis

INTRODUCTION

In an attempt to prevent premature death, the Health Service Law for the Aged introduced cancer screening programs in Japan for all residents over the age of 40 in 1983. Screening for gastric and cervical cancer was introduced initially, and colorectal, lung and breast cancer screening programs followed. At present, five cancer screening programs are conducted nationwide, and over 25 million people are screened annually (1). Although the research group for cancer screening in Japan recommended six cancer screening programs (2) in 2001, new modalities for cancer screening have been introduced in several local municipalities without evaluation by reliable studies. To reduce mortality from a specific cancer, effective, evidence-based screening should be conducted and appropriate management of quality assurance is required.

In 2004, the Japanese Government initiated the Third-Term Comprehensive 10-Year Strategy for Cancer Control, aimed at reducing the incidence and mortality of cancer in Japan. The Research Center for Cancer Prevention and Screening (RCCPS) was established at the campus of the National Cancer Center, Tokyo, in the same year. Although development of the new modalities is worthwhile, a systematic approach for the evaluation of cancer screening programs is required. In order to investigate the efficacy of cancer screening, programs using new modalities have been conducted. Variable cancers were detected in the past year, but might consist of overdiagnosis

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cases. To investigate its possibility, we compared the observed numbers with expected numbers based on the model.

SUBJECTS AND METHODS

Cancer Screening Programs

The RCCPS Cancer Screening Program is a one-arm prospective study designed to evaluate the effect of multiple cancer screening modalities. This is a hospital-based program and participants are enrolled on a voluntary basis. Age for the target group was 50 years and over for males and 40 years and over for females. Exclusion criteria were previous diagnosis of cancer and followed-up for pre-cancerous disease based on self-reporting. The research and screening methods were explained to all participants using written materials and face-to-face presentations by health-care professionals. In addition, participants signed informed consent documents approved by the National Cancer Center. All participants responded to a questionnaire concerning life style, smoking, alcohol intake, nutrition, past history of disease including cancer, family history and previous investigations within a year. These participants will be followed using a questionnaire survey after the baseline screening year. Follow-up studies include a hospital survey to investigate medical records of cancer patients detected by cancer screening and interval cancer rates based on the participant's response. In addition, these participants are asked to attend repeat screening 5 years after the baseline.

Basic programs consisted of screenings for esophageal, gastric, colon, rectal, lung, hepatic, gall bladder, pancreatic and renal cancer. Cancer screening modalities were as follows: gastrofiberscopy (GFS) for the esophagus and stomach; total colonofiberscopy (TCF) or barium enema (BE) for the colon and rectum; computed tomography (CT) and sputum cytology for the lung; and abdominal ultrasonography (US) for the liver, gall bladder, pancreas and kidneys. The participants could choose TCF or BE based on their preferences. For males, prostate cancer screening was performed using an assay of prostate specific antigen (PSA) serum levels with a cut-off value of 2.7 ng/ml. For females, a combination of modalities was performed: two-view mammography (MMG), US and physical examination (PE) for the breasts, Pap smear for the cervix, and magnetic resonance imaging (MRI) for the endometrium and ovaries. Moreover, whole body scanning using positron emission tomography (PET) with injection of 2.78 MBq/kg fluorine-18-FDG was provided as an optional investigation. This study was approved by the Institutional Review Board of the National Cancer Center.

Comparison of Observed and Expected Detection Numbers

Numbers of subjects recruited into the program from February 2004 to January 2005 and observed numbers of detected cancers were classified by 5-year age group and by gender. In the questionnaire survey, we collected information on the following investigations performed within the previous year as follows: photofluorography, GFS, fecal occult blood test (FOBT), TCF, BE, chest radiography and MMG. We could not obtain information regarding previous investigation of CT for lung and PSA because these indicators were lack of the questionnaire.

Since screening detects cancer in a large prevalence pool, detection rate is influenced by previous investigations. Sojourn time (ST) is the duration of the detectable, preclinical phase of cancer (Fig. 1). The ST depends both on the natural history of the cancer and performance of screening modalities. Maximum lead time would therefore be achieved if screening was performed at the beginning of the ST. Although ST and sensitivity (SE) vary with age on individual cases, we used estimated mean values obtained from literatures. For simplicity of the present study, we assumed the following conditions: (i) ST and SE were constant in all age groups and (ii) SE was constant throughout ST.

We calculated the expected numbers of gastric, colorectal, lung, prostate and breast cancers in patients. The subjects are divided into three groups based on the previous history as follows: (i) subjects with no history of screening, (ii) subjects with history by the same test and (iii) subjects with history by the different test. In the first group, given that I represents underlying incidence and P target population numbers, expected numbers (E) at prevalence screening, which corresponds screening without previous investigation, can be derived from the following formula: $E = I \times (P/100\,000) \times \mathrm{ST} \times \mathrm{SE}(3)$. PSA screening is applicable to this case because previous history cannot be obtained from the questionnaire. In the second group, the expected numbers (Ex) is the sum of incidence and false-negative cases of previous investigation (Fig. 2). The sensitivity of modality1 assumed SE1 and ST1 for its sojourn time. Ex is calculated as follows: $\mathrm{Ex} = I \times (P/100\,000) \times (\mathrm{ST1}\text{-}(\mathrm{ST1}\text{-}1) \times \mathrm{SE1}) \times \mathrm{SE1}$. The modality2 was previous investigation, which is different from the modality of RCCPS screening program. Similarly, the sensitivity of modality2 assumed SE2 and ST2 for its sojourn time. These cases are the participants who have a screening history using other modalities in colorectal, gastric and lung cancer screening. When participants had history of previous investigation

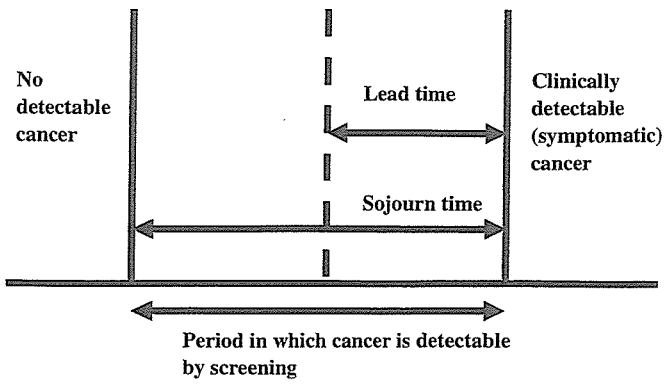

Figure 1. A graphical representation of the prognosis of clinical cancer and role of screening.

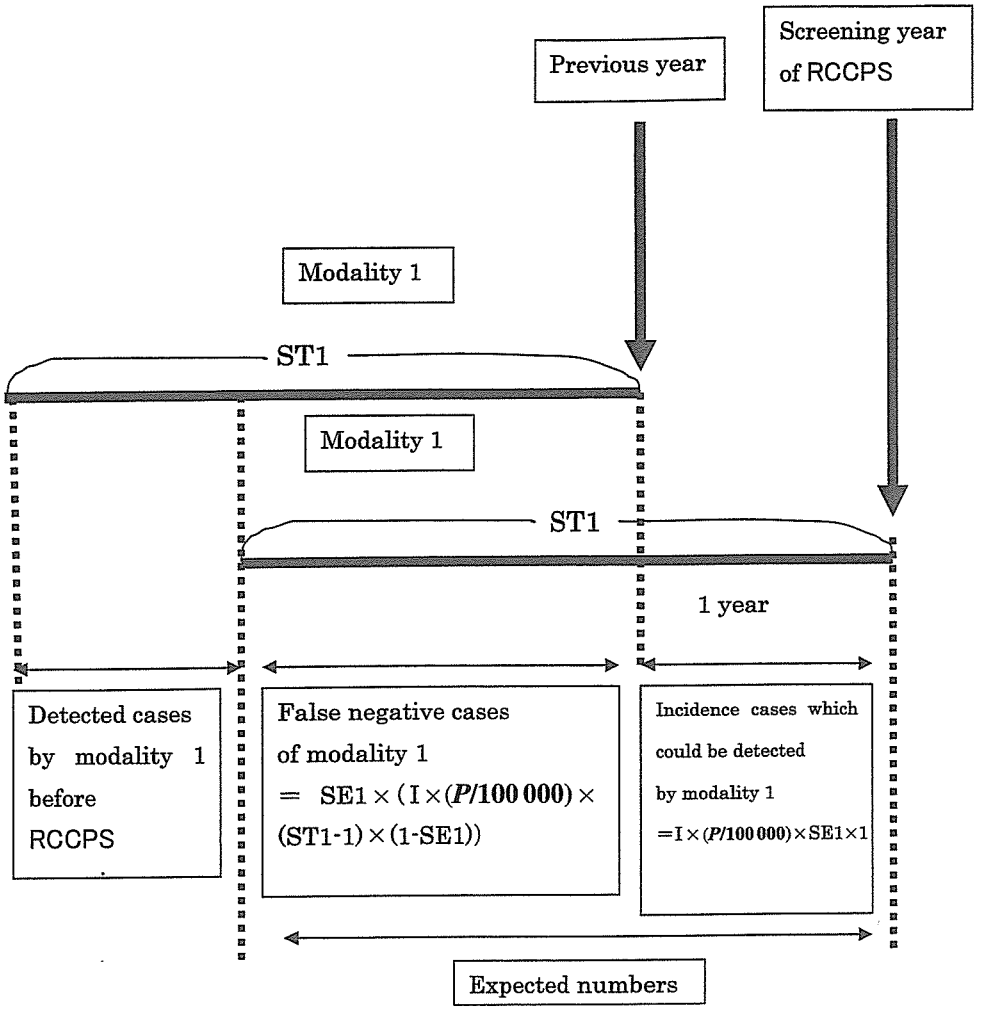

Figure 2. Calculation of expected numbers with previous examination using same modality. RCCPS: Research Center for Cancer Prevention and Screening; I: Incidence; P: Target population numbers; SE: Sensitivity; ST: Sojourn time.

using modality2, the expected number (Ey) including false-negative cases of previous screening is as follows: $Ey = I \times (P/100\,000) \times (ST1\text{-}(ST2 \times SE2)) \times SE1$ (Fig. 3).

The incidences of gastric, colorectal, lung, prostate and breast cancer were obtained from estimations calculated by cancer registries (4), while the ST and SE of breast cancer screening were assumed based on published reports (3,5–9). The ST or lead time of prostate cancer screening was determined from published articles and it ranged from 5 to 15 years (10–17). In other modalities, SE has been reported without adjustment for ST (18–20). In the baseline analysis, SE was assumed as follows: 70% for GFS; 70% for BE; 70% for TCF; 80% for CT; 80% for the combination of MMG, US and PE; 70% for MMG; 70% for PSA; 50% for chest radiography; 50% for FOBT; and 60% for photofluorography. ST was assumed as follows: 5 years for GFS; 5 years for BE; 10 years for TCF; 5 years for CT; 5 years for a combination of MMG, US and PE; 4 years for MMG only; and 10 years for PSA screening. In colorectal cancer screening, ST of immunological FOBT was assumed to be 2 years [published reports which reported the range from 2 to 4.70 years using various estimation models (21–23)]. The ST of chest radiography is 1 year based on previous reports (24,25). No references to ST of photofluorography could be found; this was assumed to be 3 years in the present study. We estimated E of detected cancers and compared these with observed numbers (O) to calculate the ratio O/E. The observed and expected numbers of detected cancer were compared using the chi-squared test. A sensitivity analysis was used to assess the effect of varying individual model parameters during the construction and testing of the models; this was performed to assess the effects of changes in our assumptions regarding ST and SE. We conducted a sensitivity analysis in the cases in which difference of the ratio O/E was significant.

RESULTS

Table 1 presents the distribution of all participants by 5-year age group and by gender. From establishment of the study in February 2003 to January 2004, 3786 participants were enrolled: 2061 males and 1725 females. In both genders, most participants (over 25%) were in the 60- to 64-year age

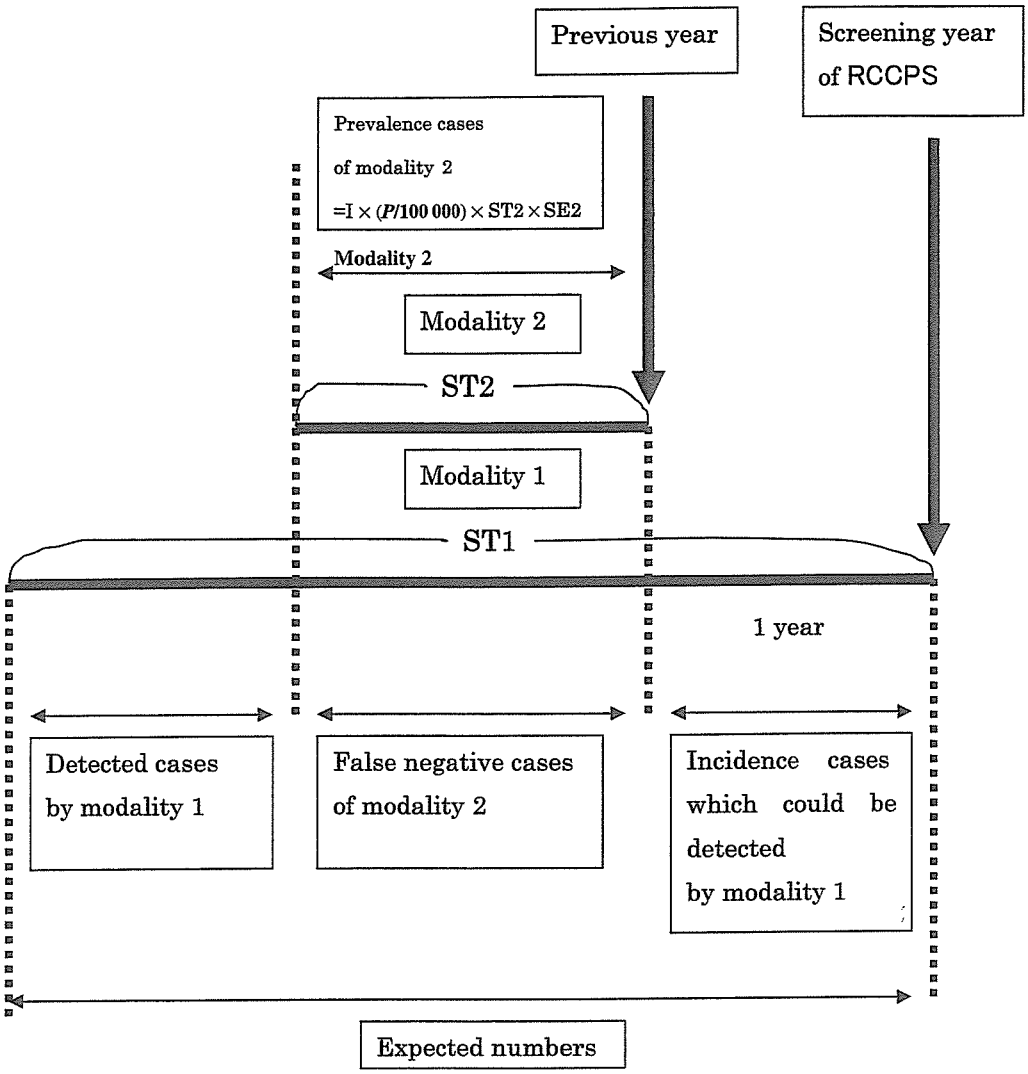

Figure 3. Calculation of expected numbers with previous examination using different modalities. RCCPS: Research Center for Cancer Prevention and Screening; I: Incidence; P: Target population numbers; SE: Sensitivity; ST: Sojourn time.

groups. Of participants over 70 years of age, 5.5% (114/2061) were males and 3.9% (67/1725) were females. Almost 90% of participants came from the Tokyo metropolitan area and the seven surrounding prefectures. Regarding colorectal cancer screening, TCF was performed in 83.6% (1723/2061) of male participants and 77.8% (1342/1725) of female participants, and the remaining 15.4% (317/2061) of male and 19.9% (343/1725) of female participants had BE. PET scans were performed for 79.0% (1629/2061) of males and 74.3% (1282/1725) of females. In the first year of the RCCPS programs, 190 cancers were detected (Table 2). The detection rate for all cancers was 5.8% (119/2061) for males and 4.1% (71/1725) for females. Approximately twice as many males as females had undergone TCF within the previous year (Table 3). In contrast, GFS had been performed in similar numbers of males and females. The frequency of MMG within the previous year was 18.5% (317/1712).

Expected numbers of detected cancers were calculated by classifying participants into groups by screening modalities for gastric, colorectal, lung, prostate and breast cancer (Table 4). In males, expected numbers of cancers were as follows: gastric cancer, 15.3 cases; colorectal cancer, 2.3 cases for BE and 21.9 cases for TCF; lung cancer, 10.9 cases; and prostate cancer, 7.0 cases. In females, expected numbers were as follows: gastric cancer, 3.7 cases; colorectal cancer, 1.1 cases for BE and 7.6 cases for TCF; lung cancer, 2.4 cases; and breast cancer, 6.2 cases. For TCF screening, observed numbers were almost equal. The observed numbers for gastric cancer were almost two times than expected numbers. But, in females, it was not significantly different. On the other hand, lung cancer was observed seven times more often in females but nearly equal in males. Prostate cancer and breast cancer were both detected over two times more frequently than expected. On the sensitivity analysis of prostate, breast and lung cancer screening for females, expected numbers of prostate and lung cancer increased in accordance with ST and SE. For prostate cancer screening, *O/E* ratio ranged between 5.36 and 16.07 according to SE values from 30 to 90% when ST was set at 5 years;

Table 1. Distribution of participants in RCCPS (February 2004–January 2005)

All participants	Sex	Age									
		40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80 years over	All
	Male	0	0	311	500	552	554	89	23	2	2061
	(%)	0.0	0.0	15.1	24.2	26.8	26.9	4.3	1.1	0.1	100.00
	Female	126	156	260	375	429	312	51	14	2	1725
	(%)	7.3	9.0	15.1	21.7	24.9	18.1	3.0	0.8	0.1	100.00
Examinees within participants											
BE	Male	0	0	48	69	91	92	13	3	1	317
	Female	25	31	46	66	86	77	7	4	1	343
TCF	Male	0	0	257	427	488	457	73	20	1	1723
	Female	97	121	208	298	337	230	40	10	1	1342
PET	Male	0	0	250	405	450	423	78	21	2	1629
	Female	78	114	196	276	334	228	41	13	2	1282

BE: barium enema; TCF: total colonoscopy; PET: positron emission tomography.

Table 2. Age distribution of screen-detected cancer and detection rate by screening modality among the participants in the RCCPS (February 2004–January 2005)

Cancer	Modality	Sex	Examinees	Detected numbers (years)										Detection rate (%)
				40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	Above 80	All	
Esophagus	GFS	Male	2040	0	0	0	0	2	5	1	0	0	8	0.39
		Female	1684	0	0	0	0	0	0	0	0	0	0	0.00
Stomach	GFS	Male	2042	0	0	0	5	11	10	2	0	0	28	1.37
		Female	1684	0	1	2	0	1	3	0	0	0	7	0.42
Colon and rectum	BE	Male	317	0	0	0	1	1	1	1	0	0	4	1.26
		Female	342	0	0	1	1	0	2	0	0	0	4	1.17
Colon and rectum	TCF	Male	1723	0	0	3	1	9	10	3	0	0	26	1.51
		Female	1342	0	0	4	1	3	6	0	1	0	15	1.12
Lung	CT	Male	2061	0	0	1	2	3	7	0	1	0	14	0.68
		Female	1697	2	1	5	5	1	3	1	0	0	18	1.06
Prostate	PSA	Male	2042	0	0	1	3	5	12	2	1	0	24	1.18
Breast	MMG + US + PE	Female	1712	2	3	2	3	2	0	2	0	1	15	0.88
Others		Male	2061	0	0	4	0	4	7	0	0	0	15	0.73
		Female	1725	1	2	2	2	1	2	1	1	0	12	0.70
All cancer		Male	2061	0	0	9	12	35	52	9	2	0	119	5.77
		Female	1725	5	7	16	12	8	16	4	2	1	71	4.12

GFS: gastrofiberscopy; BE: barium enema; TCF: total colonoscopy; CT: computed tomography;PSA: prostate specific antigen; MMG: mammography; US: ultrasonography; PE: physical examination.
Detected cancers included these cases: multiple cancers at the same organ (5 persons, 11 cancers) and multiple cancers at multiple organs (6 persons, 13 cancers).

observed numbers of prostate cancer always exceeded expected numbers at any cases if ST was changed from 5 to 15 years. For lung cancer screening for females, *O/E* ratio ranged between 6.72 and 12.10 according to SE values from 50 to 90% when ST was set at 5 years; observed numbers of breast cancer were three times more than expected at any cases if ST was changed from 5 to 10 years.

DISCUSSION

The efficacy of reducing mortality rates from cancer has been established for several cancer screening programs. Based on these studies, the research group for cancer screening in Japan recommended the following six cancer screening programs (2): photofluorography for gastric cancer, fecal occult blood

Table 3. Proportion of having previous investigations within a year by screening modalities

Examination	Modality	Previous examination within a year	
		Male (%)	Female (%)
Stomach	XP	43.5 (887/2040)	30.0 (505/1684)
	GFS	28.7 (586/2040)	23.3 (393/1684)
Colon and rectum	FOBT	52.7 (1074/2040)	40.7 (685/1684)
	BE	4.9 (99/2040)	3.0 (50/1684)
	TCF	15.4 (315/2040)	8.3 (139/1684)
Lung	Chest X-ray	73.9 (1524/2061)	62.0 (1052/1697)
Breast	MMG	–	18.5 (317/1712)

The percentage of previous examination compared males and females using the chi-squared test.
XP: gastrophotofluorography; FOBT: fecal occult blood test; GFS: gastrofiberscopy; BE: barium enema; TCF: total colonoscopy.
PSA: prostate specific antigen; MMG: mammography; US: ultrasonography; PE: physical examination.

test for colorectal cancer, chest radiography and sputum cytology for lung cancer, Pap smear for cervical cancer, a combination of physical examination and mammography for breast cancer, and hepatitis virus markers for hepatocellular carcinoma. Recently, the guideline for colorectal cancer screening has been revised, and chemical and immunological fecal occult blood tests have been recommended as population-based screening (20). Both TCF and BE could be introduced in opportunistic screening as long as well-controlled risk management is performed. Although these guidelines follow evidence-based cancer screening programs, new modalities which show no evidence of mortality reduction have rapidly been disseminated. These new modalities, such PET, CT and GFS, possess high sensitivity and are therefore anticipated to detect early cancer; however, while they are useful for cancer detection, their effectiveness in cancer screening is unclear.

The detection rates in our study were higher than those of population-based screening (20). There are two possibilities for this difference. First, for over 70% of participants, it was the first experience that they were examined by GFS, TCF, BE, CT and MMG. When screening is initiated, an apparent excess of diagnosed cancers is inevitable, because in the first round of screening a large number of cancers that would have occurred in future are diagnosed earlier. Second, the sensitivity of the modalities in our study was superior to those of population-based screening (18–20). Population-based screening programs have been conducted using chest radiography and sputum cytology for individuals at high risk of lung cancer, while similar programs using photofluorography for gastric cancer and immunological fecal occult blood testing for colorectal cancer have also been performed. Considered these conditions, we calculated the expected numbers of detected cancers in our cohort based on assumptions of sensitivity and sojourn time in several modalities. The difference of observed and expected numbers could be changed according to use of the data. We conducted a sensitivity analysis to investigate the robustness because it was possible that our conclusion would be changed according to the data used for the analysis. For example, we used incidence rates obtained from population-based cancer registries. The incidence rate from cancer registries is the weighed average of incidence among the population with and without previous history of screening. These assumptions might introduce under- or overestimation.

In the cases of prostate, breast and gastric cancer for males and lung cancer for females, the observed numbers exceeded expectation and were similar to those expected in the other cases. High detection rate is a consequence of the screening itself, i.e. overdiagnosis, especially in prostate and lung cancer for females. Overdiagnosis has been pointed out and was a major harm in both screening programs (26). Although the test was conducted using the same modality for lung cancer screening, the results were different between males and females in our study. The difference of two groups might be explained by the difference of the history of chest radiography. Strauss et al. (27) state that the overdiagnosis hypothesis is counter to virtually all known data on the natural history and biological behavior of lung cancer. In recent screening studies, both detection rate and stage I cancer by CT exceeded that of chest radiography (28,29). For the very reason, overdiagnosis could be a more serious problem for CT screening. On the other hand, the cut-off point for prostate cancer screening is controversial. PSA value of 4.0 ng/ml is a popular cut-off point for prostate cancer screening; 2.7 ng/ml was used in the present study. However, only two cases (8.3%) of the detected prostate cancers exhibited PSA levels below 4.0 ng/ml. In the European Randomized Study of Screening for Prostate Cancer, the cut-off PSA level was changed from 4.0 to 3.0 ng/ml (30). Krumholtz and colleagues (31) found a prostate cancer incidence rate of 22% in patients with 2.6–4.0 ng/ml PSA based on biopsies of 94 patients with clinical stage T1c. Recently, the prevalence of prostate cancer was reported to be 14.9% for those with PSA values below 4.0 ng/ml (32). Of these tumors, 15% contained Gleason pattern 4, indicating that high-grade cancer occasionally occurs in the presence of low PSA. Disagreement exists as to the best cut-off value for PSA. Greater detection of prostate cancer increases the risk of overdiagnosis and overtreatment, which can cause erectile dysfunction and urinary incontinence. The risk of overdiagnosis has been reported as more than 48% within a screening population with a 4-year screening interval (13). Etzioni and colleagues calculated the overdiagnosis rates of prostate cancer screening as 29% for whites and 44% for blacks, based on SEER-Medicare database (14). Men with low-grade prostate cancer (Gleason score of 2–4) have minimal risk of dying from prostate cancer during 20 years of follow-up compared with men with high-grade prostate cancer (Gleason score of 8–10) (33). On the other hand, Bill-Axelson et al. (34) reported that radical prostatectomy reduces disease-specific mortality and overall mortality compared with watchful waiting. Including selection of therapy, the efficacy of prostate

Table 4. Comparison of the observed and expected numbers of cancer by screening modality

Cancer screening	Modality	Baseline analysis		Male				Female			
		Sensitivity (%)	Sojourn time (years)	Observed numbers	Expected numbers	*O/E*	*P*-value	Observed numbers	Expected numbers	*O/E*	*P*-value
Stomach	GFS	70	5	28	15.31	1.83	0.0463	7	3.69	1.90	0.3649
Colon and rectum	BE	70	5	4	2.25	1.78	0.4120	4	1.08	3.70	0.1781
	TCF	70	10	26	21.90	1.19	0.5610	15	7.64	1.96	0.1427
Lung	CT	80	5	14	10.86	1.29	0.5473	18	2.38	7.56	0.0021
Prostate	PSA	70	10	24	7.00	3.43	0.0022	–	–	–	–
Breast	MMG+US+PE	80	5	–	–	–	–	15	6.22	2.41	0.0488

The observed and predicted numbers of detected cancer were compared using the paired *t*-test. XP: gastrophotofluorography; FOBT: fecal occult blood test; GFS: gastrofiberscopy; BE: barium enema; TCF: total colonoscopy; PSA: prostate specific antigen; MMG: mammography; US: ultrasonography; PE: physical examination. *O/E* = observed numbers/expected numbers.

cancer screening programs is still unclear. Although the cancer screening programs in the present study increased the detection of potentially curable cancers, these modalities might detect tumors that would not be clinically significant. We should accordingly weigh up the benefits and harms of cancer screening using these modalities, and such information should be given to the participants of our study.

The present study is the first report from the RCCPS and has several limitations. First, our cohort of around 4000 volunteers is insufficient to observe reduction of mortality rates from specific cancer and no comparable group was included. Second, participants were volunteers who were receptive to screening by the new modalities. Hence, a self-selection bias could not be excluded. In the present study, we estimated expected numbers using a simple model based on approximate assumptions. However, to estimate correct sojourn time accurately and to modify our model accordingly, lengthy follow-up is needed. We have started follow-up studies, which include an annual questionnaire survey of participants and a hospital survey to acquire information on cancer patients. Information concerning interval cancer can be obtained through this survey, and sensitivity and sojourn time of several cancers can be reinvestigated based on the new model. In addition, we aim to investigate all participants using the same modalities after 5 years and are planning further programs to evaluate the accuracy of the screening modalities.

Acknowledgments

This study was supported by a Grant-in-Aid for the Third-Term Comprehensive 10-Year Strategy for Cancer Control (H16-cancer control-016) from Ministry of Health, Labour and Welfare of Japan. The authors thank Ms Kanoko Matsushima and Ms Junko Asai for the manuscript preparations.

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Review Article

Development and progression of urothelial carcinoma

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(Received April 26, 2006/Accepted May 11, 2006/Online publication July 6, 2006)

Urothelial carcinomas are well known to feature multifocal development in the urinary tract, both synchronously and asynchronously. This phenomenon can be explained by either seeding of cancer cells in the urinary tract or field cancerization. As there are two characteristic morphological patterns of urothelial carinomas, papillary and nodular, published papers were here reviewed to understand the development and progression of urothelial carcinoma regarding multifocality due to seeding or field changes with reference to the type of urothelial carcinoma. From animal experiments using rats, mice and dogs treated with *N*-butyl-*N*-(4-hydoroxybutyl) nitrosamine, and from pathological observation of human cystectomy specimens on step-sectioning and molecular analysis, nodular carcinomas appear to either develop via papillary carcinomas or *de novo*. Clinical aspects of multifocal tumor development are outside of the scope of this review, although an understanding of the mechanisms underlying multifocality and the papillary/nodular morphological relationship is important to determine follow-up strategies for patients treated for primary urothelial carcinomas and for reconstruction of the urinary tract after cystectomy. (*Cancer Sci* 2006; 97: 821–828)

The urothelium (also known as the transitional cell epithelium) covers the luminal surface of almost the entire urinary tract, extending from the renal pelvis, through the ureter and bladder, to the proximal urethra. Typically it is composed of three to seven layers of cells, that is, basal cells, intermediate cells and superficial cells. The superficial cells are large and binucleated, with a flat characteristic shape leading to the term 'umbrella cell'. Their luminal surfaces are covered with an asymmetric unit membrane, which functions as the permeability barrier between the urine and blood vessels in the urothelium.[1]

Worldwide, there are approximately 336 000 new cases of urothelial carcinoma and 132 000 deaths annually.[2] The majority of lesions are bladder carcinomas, and urothelial carcinomas of the renal pelvis and ureter account for only approximately 7% of the total.[3] In Japan and western countries, more than 90% of bladder carcinomas are urothelial carcinomas, and squamous cell carcinomas and adenocarcinomas are rare. Risk factors include tobacco smoking, occupational exposure to aromatic amines, consumption of arsenic-laced water, radiation therapy of neighboring organs and chemotherapeutic drugs such as alkylating agents. In contrast, in Egypt, where the dominant etiology is schistosomiasis infection, squamous cell carcinomas are the most prevalent bladder carcinoma. Here the focus of attention is urothelial carcinoma in its papillary and nodular forms. Squamous cell carcinoma and the clinical relevance of metaplasia will not be covered.

Multifocal development of urothelial carcinomas

The clinical aspects of multiple urothelial carcinomas need to be emphasized:[4]

1 There are patients in which lesions in the renal pelvis, ureter and bladder are observed simultaneously (Figs 1a, 2).

2 When standard nephrectomy is performed for renal pelvic and ureteral carcinomas, approximately one-third of the lower ureter is left intact. In this remaining ureter, urothelial carcinomas develop subsequently in 20–50% of the patients (Fig. 1b). Consequently, at the present time, the state of the art surgery for renal pelvic and/or ureteral carcinomas is total nephroureterectomy, including removal of a small portion of the bladder with the ureteral orifice (bladder cuff) in the affected side.

3 Even if total nephroureterectomy is carried out, however, 15–50% of patients exhibit subsequent carcinomas in the bladder (Fig. 1c).

4 When superficial papillary urothelial carcinomas of the bladder are resected transurethrally (TUR), the rate for subsequent development of urothelial carcinomas of a similar biological nature in the normal-appearing bladder mucosa is reported to be 50–80% (Fig. 1d).

5 When cystoprostatectomy (i.e. removal of the bladder and prostate) is carried out for male bladder cancer patients, 4–17% incidences of urothelial carcinomas in the remaining urethra have been described[5] (Fig. 1e). In female bladder cancer patients, involvement of the urethra is reported to occur in 1.4–36% of cases.[6]

After surgical treatment of bladder carcinomas, the incidence of subsequent upper urinary tract carcinomas (of the renal pelvis or ureter) ranges from 0.7 to 4%.[7] Most of these carcinomas are diagnosed 4–6 years after the initial appearance

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doi: 10.1111/j.1349-7006.2006.00264.x
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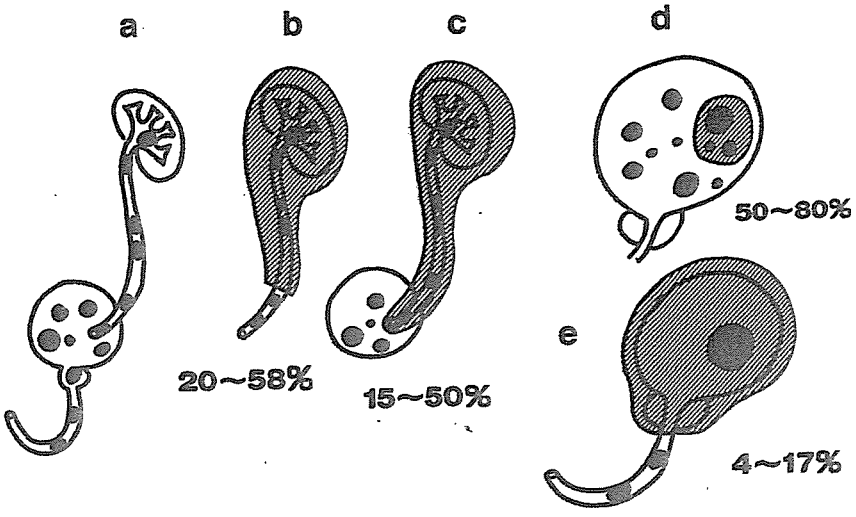

Fig. 1. Clinical findings indicating multifocal tumor development in the urinary tract. Black lesions are original tumors and red lesions are recurrent tumors.

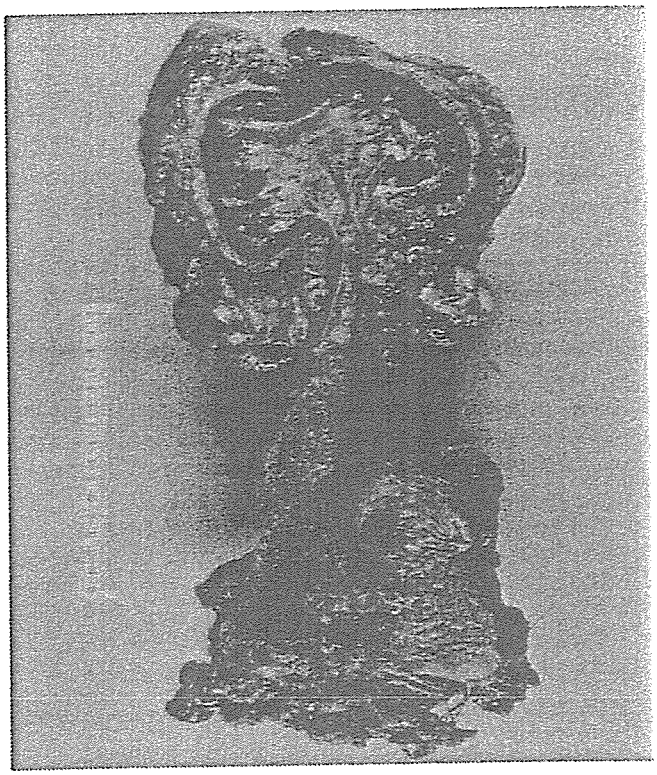

Fig. 2. A case of renal pelvic, ureteral and vesical carcinomas who underwent right nephroureterocystectomy.

of the bladder carcinoma.[8,9] However, the risk is reported to increase to the level of 6–20% (15–22-fold) if the patients suffer from vesico-ureteral reflux.[10]

With upper urinary tract carcinomas, simultaneous bilateral lesions are rare, the estimated incidence being 1–5%.[11] Approximately 2–3% of patients with unilateral upper urinary tract carcinoma experience subsequent contralateral upper urinary tract carcinoma.[12] These clinical data should be taken into serious consideration when we decide on nephroureterectomy or cystectomy and plans for follow up after surgery, and the upper and lower urinary tract and the contralateral upper urinary tract should be assumed to constitute a single clinical unit from the renal pelvis to the urethra.

Field cancerization versus clonal expansion

To explain multifocal carcinoma development in the urinary tract, two theories have been proposed.[13,14] The first is field cancerization, proposed in 1953 by Slaughter *et al.*, which was based on observations of the multicentric development of cancers in the oral cavity, with the high impact of carcinogens and promoting agents being associated with some lifestyle factors.[15] A similar understanding is possible for the urinary tract as the entire urothelium is exposed to carcinogens contaminating the urine. The second hypothesis is that multiple carcinomas in the urinary tract are the result of intraluminal spread from a single lesion, originating from a single transformed cell, namely seeding or implantation of cancer cells at different sites. This phenomenon is also called clonal expansion of multifocal carcinomas. Debates on multiple cancer development have been similar for cancers of the oral cavity,[16] respiratory tract,[17] head and neck,[18] breast,[19] ovary[20] and cervix.[21] Recently, strong molecular evidence has been presented in support of clonal expansion in the epithelium of oral cavity and respiratory tract cases.[16,22]

Many urologists and pathologists have supported the field cancerization hypothesis in the urinary tract, but recent molecular studies have also pointed to a clonal origin for most multifocal urothelial carcinomas. Various molecular analyses have been applied. Sidransky *et al.* investigated X-chromosome inactivation in multiple bladder carcinomas of four female patients and proved that the same allele of the X-chromosome was inactivated in all lesions within the single bladder.[23] Subsequently, Lunec *et al.*[24] and Habuchi *et al.*[25] examined patients having heterotopic synchronous or recurrent urothelial carcinomas in the bladder or upper urinary tract. These carcinomas had identical mutation sites and patterns of p53 gene alteration, indicating the metachronous carcinomas to be derived from the original carcinoma cells due to clonal expansion. Habuchi also reviewed the origin of

doi: 10.1111/j.1349-7006.2006.00264.x
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multifocal carcinomas of the bladder and upper urinary tract.[26] Other genetic features that can be used to assess clonal origin are loss of heterozygosity (LOH) and microsatellite alteration patterns, both commonly used as markers of neoplasia.

Stoehr *et al.* analyzed primary carcinomas in 14 cystectomy specimens for p53 protein overexpression by immunohistochemistry and p53 gene mutation by genomic sequencing.[27] They reported detection of p53-mutant cells in histologically normal adjacent or remote mucosa and in preneoplastic urothelial areas in four patients with invasive bladder carcinoma, concluding extensive intraurothelial tumor cell spread.

Evidence of a monoclonal origin and intraepithelial spread has also been provided by Simon *et al.* from comparative genomic hybridization in 32 bladder tumors originating from six cystectomy specimens.[28] Identical *TR53* mutations and protein overexpression were found in tumors from the same individual, as well as in mucosal samples from the continuous areas. The sequence of genomic changes apparently acquired during progression of bladder carcinomas was highly complex and varied within each patient and from tumor to tumor. Early changes included alterations in −17p, +20p, −9p, −9q, +2q34-qter, +12q14-q21, +1q22-q25, −8p22-pter, −5q31-qter and +17q. Subsequent tumor progression was characterized by accumulation of changes in +11q14, −21q, −5q13-q14, +8q22, +10p, −10q22qter and −11p. Cytogenetic variety in multifocal tumors has also been described in support of intraluminal tumor seeding.[29]

It is conceivable that widespread p53-mutated cells in the normal urothelium are generated in the bladder due to carcinogen exposure, and that from these, new tumors later develop with surrounding normal-appearing mucosa having p53 mutations. However, there is no mechanistic explanation for the intraepithelial spread of carcinoma cells to remote normal-appearing mucosa, and it is unrealistic to consider a mechanism due to cell motility.

Although the clonal theory now dominates in explanations of multifocality of urothelial carcinomas, there are also conflicting observations. Cheng *et al.* collected cancer cells by microdissection from 18 cystectomy specimens from female patients and analyzed the X-chromosome-linked human androgen receptor gene.[30] Only 11 of the 18 specimens were informative, with nine exhibiting non-random inactivation of the target locus and seven showing different patterns, indicating field change in these cases. Paiss *et al.* examined X-chromosome inactivation in 45 archival or fresh frozen bladder tumors obtained from 27 female patients using a polymerase chain reaction-based procedure.[31] Polyclonal patterns were observed in 16 of the 45 tumors. Stoehr *et al.* examined multiple samples from four cystectomy specimens for LOH at chromosomes 8p, 9p, 9q and 17p and they observed oligoclonality in two patients.[27] Thus, both hypotheses continue to be discussed, although clonal expansion by intraluminal spread of primary carcinoma appears the dominant explanation for multifocality.

Relationship between papillary carcinoma and nodular carcinoma

Urothelial carcinogenesis has been investigated in various species of animal. A particular focus has been on the histogenesis of lesions in rats treated with the carcinogen *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BHBN).[32] Normal urothelium of rats is composed of two to three layers of urothelial cells and when BHBN is given in the drinking water, the mucosal layer becomes hyperplastic at 4 weeks. If BHBN administration is stopped at this point, mild hyperplastic mucosal lesion regresses to the normal state. However, if BHBN treatment is continued, mucosal hyperplasia progresses to papillary growth and papillary carcinomas develop via papillomas (Fig. 3). Large papillary carcinomas may occasionally invade the bladder wall. Usually, papillary carcinomas are multifocal but superficial, indicating bladder carcinoma in rats to be a good model for human papillary bladder carcinoma. With progression, urinary bladders become filled with multifocal urothelial carcinomas and rats die due to massive bleeding. Papillary carcinomas are always induced in rats, irrespective of the strain of animal, the concentration of BHBN, or the carcinogen, with similar findings being reported with *N*-(4-[5-nitro-2-furyl]-2-thizolyl)formamide and *N*-methyl-*N*-nitrosourea.

Urinary bladder carcinogenesis in mice treated with BHBN in the drinking water originates in the normal mucosa (composed of two to three layers of urothelium) and progresses through mild hyperplasia, dysplasia and carcinoma *in situ*, to form large nodular invasive carcinomas[33] (Fig. 4). Bilateral ureters are frequently obstructed due to invasion of carcinomas into the bladder wall, and when advanced, mice die because of renal insufficiency due to hydronephrosis. Because of these features, the bladder carcinomas induced by BHBN in mice offer good models for human nodular invasive bladder carcinoma. Of interest, it has proven impossible to induce multiple papillary carcinomas in any strain of mouse, not with any concentration of BHBN nor any other carcinogen. Thus, there is a clear contrast between the biological and morphological characteristics of bladder carcinomas in rats and mice.

Bladder carcinogenesis in female dogs has been studied extensively by Okajima *et al.* who used these animals to periodically observe the surface of the bladder epithelium directly by cystoscopy.[34] They made capsules of BHBN (80–500 mg/capsule), which were administered once a day. After 4–5 years, papillary superficial bladder carcinomas were induced (Fig. 5), and when these were examined by cystoscopy without further BHBN treatment after more than 10 years, the bladders of the dogs were full of multifocal papillary carcinomas. When dogs were given 500 mg of BHBN daily, nodular invasive carcinomas were induced after approximately 1 year. These findings indicate that bladder carcinogenesis in dogs can be controlled by the concentration and period of BHBN administration in terms of the type of carcinoma (i.e. papillary superficial and nodular invasive bladder carcinoma). Thus, in the various animal species, rats, mice and dogs, the relationship between papillary and nodular carcinomas in the bladder appears to differ.

Morphological and pathological characteristics of human urothelial carcinomas, mainly bladder carcinomas, are a combination of papillary (P), papillonodular (PN), nodular (N) and carcinoma *in situ* (C). On careful analysis of cancerous lesions and normal-looking mucosa of 186 cystectomized specimens by step-sectioning,[35] we classified 17 as C and 80

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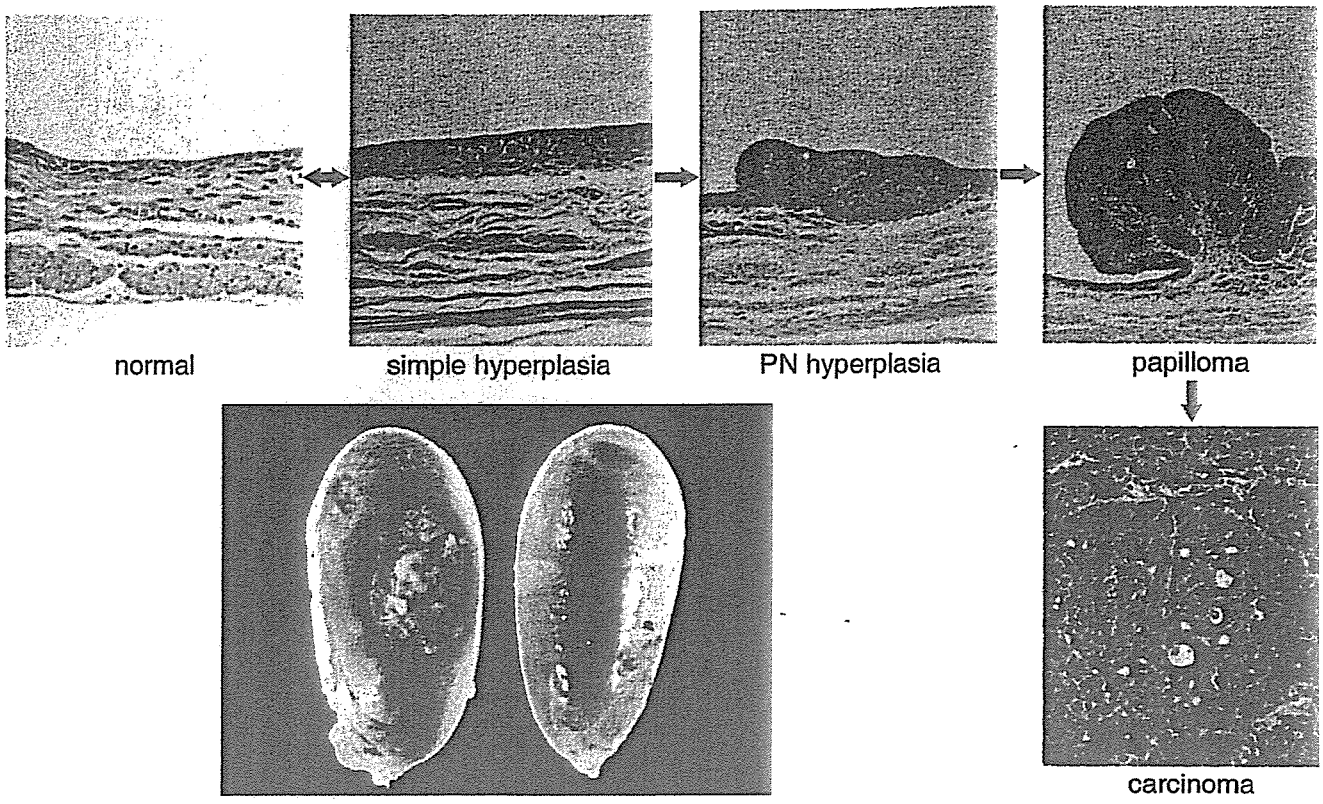

Fig. 3. Histogenesis and progession of papillary carcinomas in rats. (Reproduced with permission from Medical view Co., T. Kakizoe, Development and Progression of Bladder Cancer, 1995.)

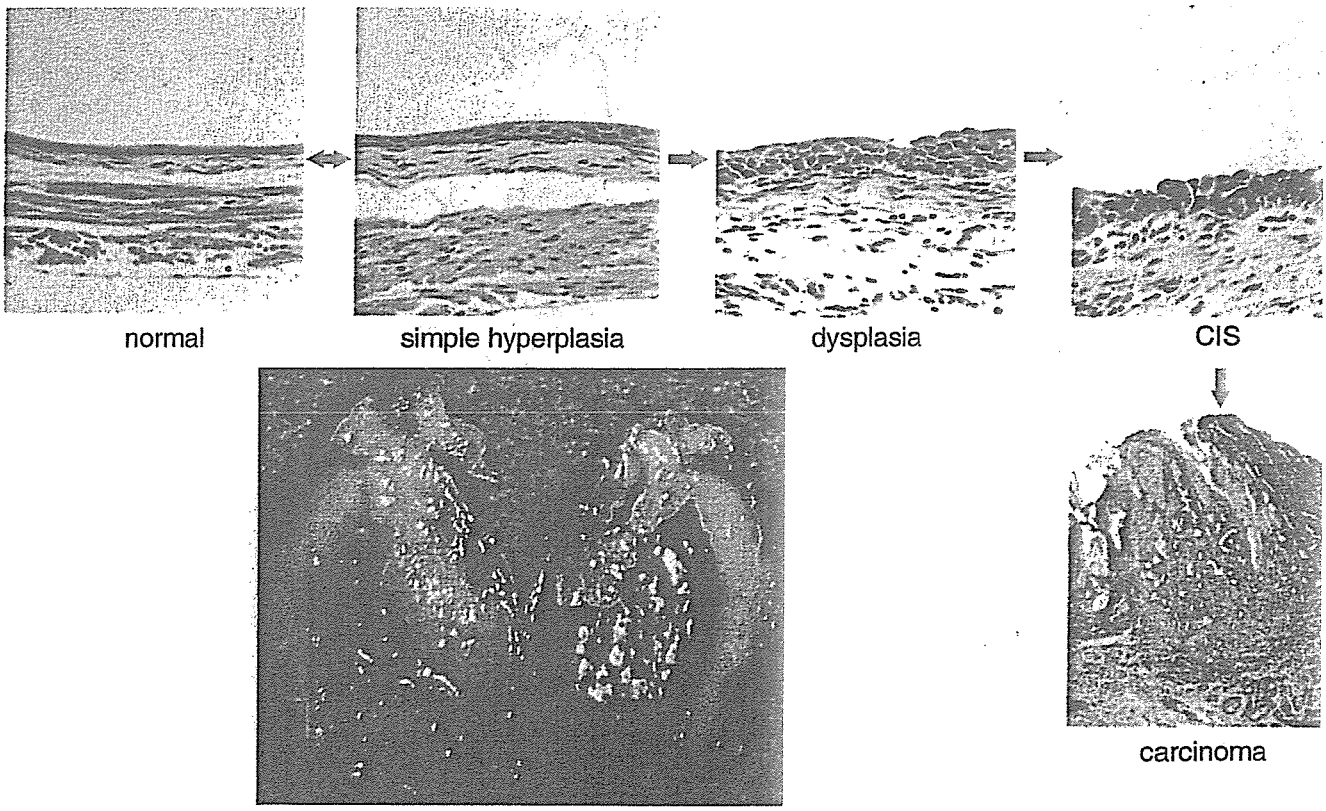

Fig. 4. Histogenesis and progression of nodular invasive carcinoma in mice. (Reproduced with permission from Medical view Co., T. Kakizoe, Development and Progression of Bladder Cancer, 1995.)

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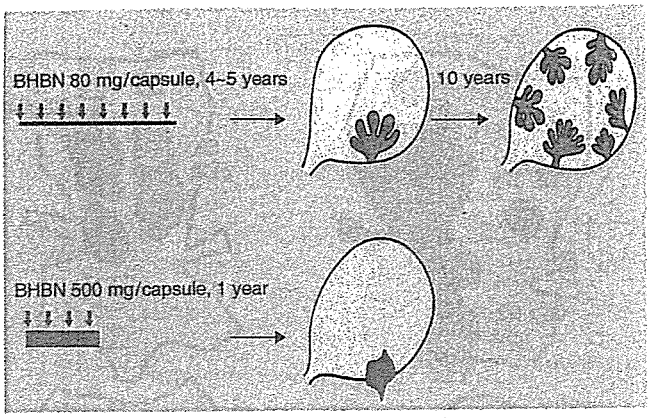

Fig. 5. Development and progression of papillary and nodular carcinomas depending on the concentration and period of administration of *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BHBN) in female dogs.[34] (Reproduced with permission from Medical view Co., T. Kakizoe, Development and Progression of Bladder Cancer, 1995.)

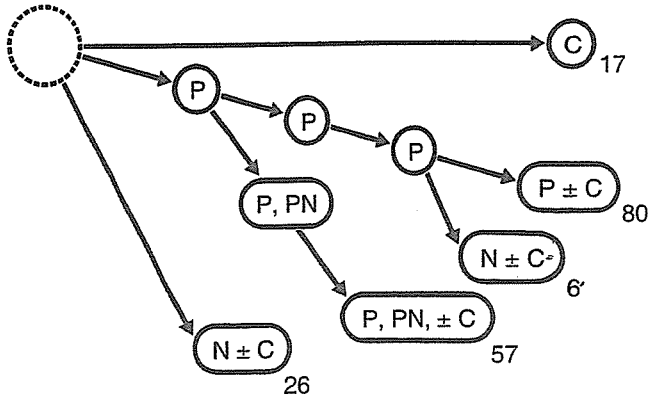

Fig. 6. Conceptual progression routes of papillary (P), papillonodular (PN) and nodular (N) carcinoma, and carcinoma *in situ* (C), in 186 cystectomized specimens examined by step-sectioning.[35]

as P and P + C. Fifty-seven cases featured apparent early changes from P to a mixture of P and N, whereas six showed late development of N with repeated recurrence of P. The findings thus indicated some N to have developed from P as more anaplastic cell populations within a pre-existing low-grade lesion, whereas others arose directly *de novo* from C (Fig. 6). Topographic relationships between P and N in the pT3 group are illustrated in Fig. 7. Figure 8 demonstrates findings for patients having a previous history of repeated recurrence of papillary carcinomas treated by TUR. At the time of cystectomy, all the cystectomized specimens showed a variable degree of coexistence of P, N and C.

Molecular pathways of urothelial carcinogenesis

With papillary superficial and nodular invasive carcinomas, there appear to be differences in molecular pathways as well

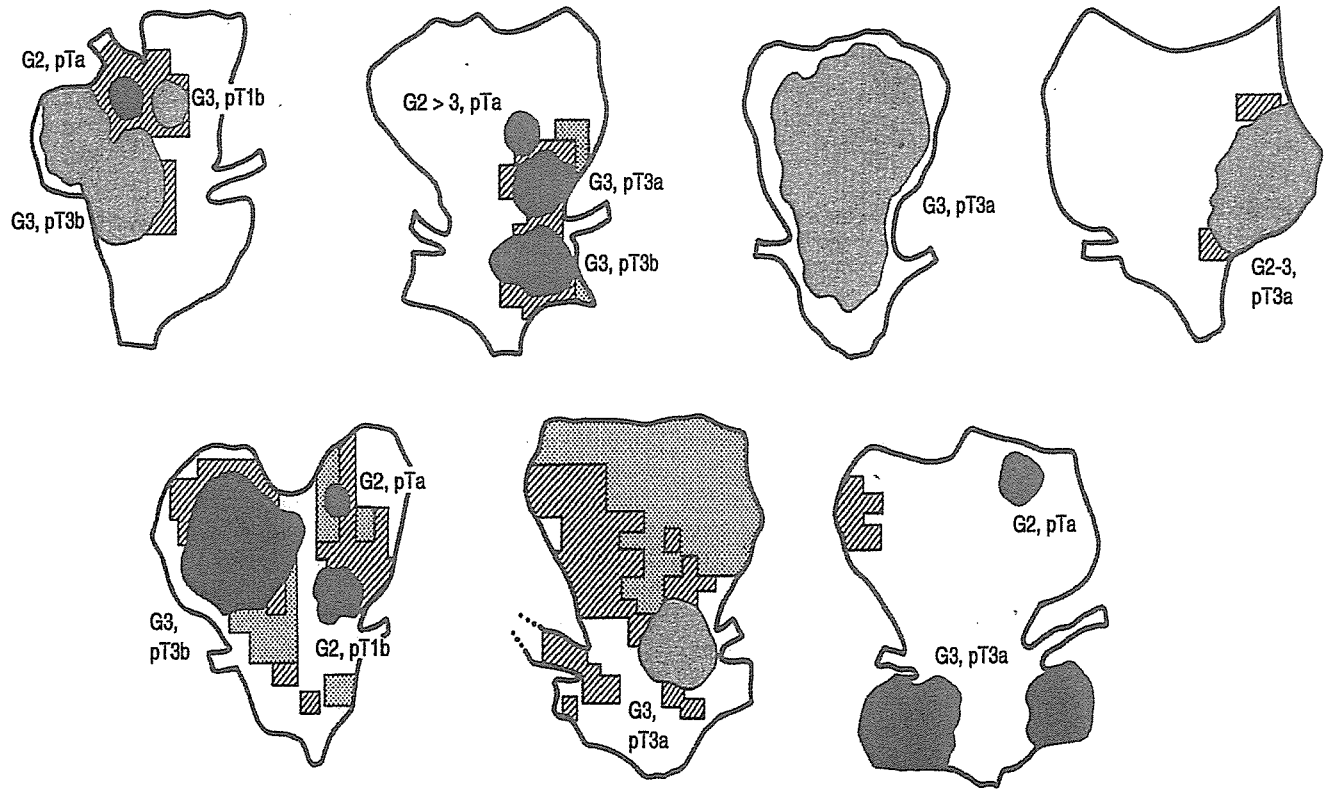

Fig. 7. Cases of pT3 cystectomized specimens indicating coexistence of papillary (P; blue), papillonodular (PN; yellow) and nodular (N; red) carcinoma together with oblique line area (C) and shaded area (dysplasia).[35]

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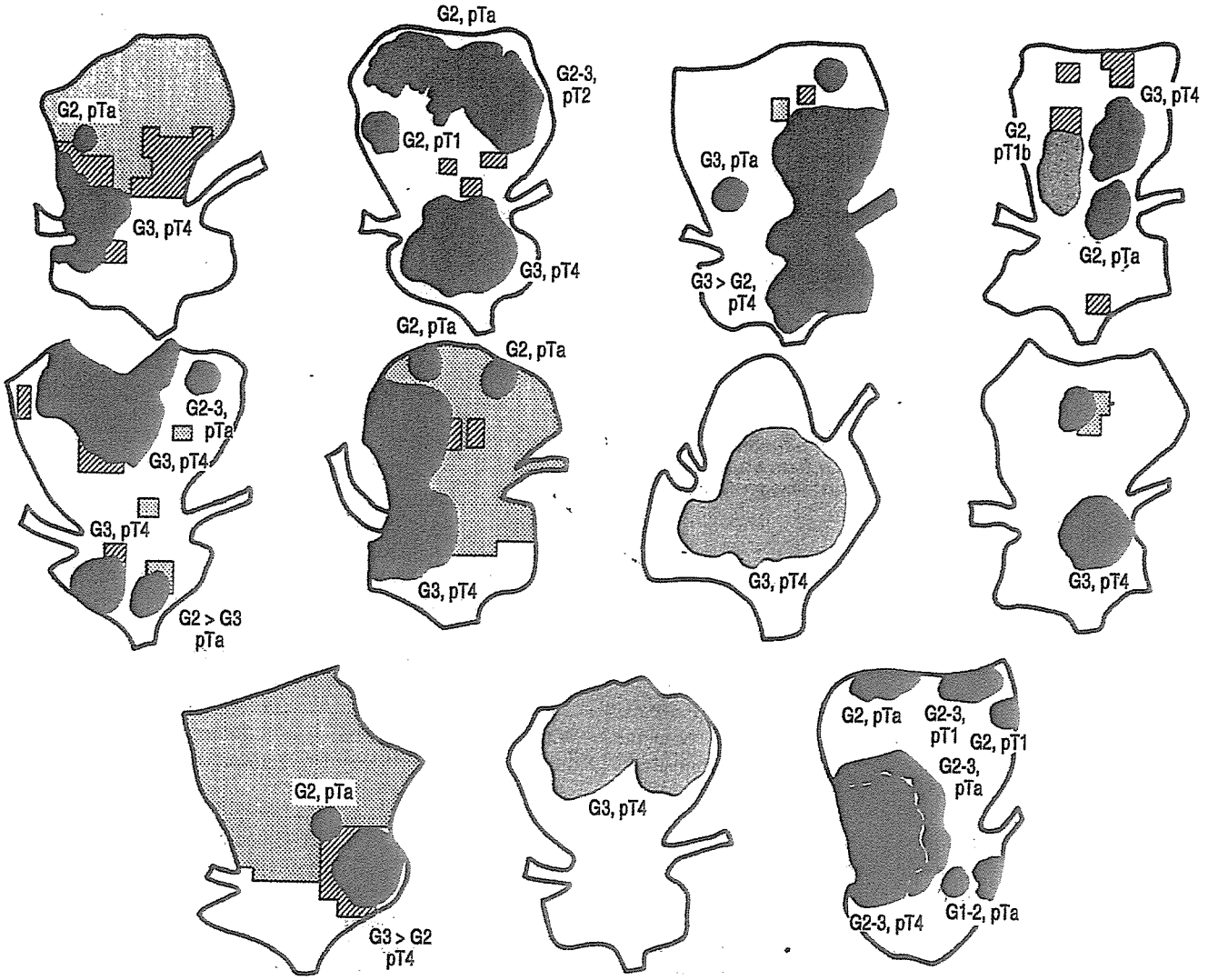

Fig. 8. Eleven cases of cystectomized specimens having histories of multiple transurethral resection for papillary recurrent carcinomas showing variety of stages and morphology in a single bladder.[35]

as morphology[36–38] (Fig. 9). The most common genetic alterations in low-grade papillary urothelial carcinomas are LOH of chromosome 9 and activating mutations of fibroblast growth factor receptor 3 (FGFR3).[39,40] Over 70% of low-grade papillary carcinoma exhibit FGFR3 mutations, but only 10–20% of high-grade invasive carcinomas have FGFR3 mutations, implying a key role for FGFR3 together with mutations of 9p and 9q, specifically for the induction of low-grade papillary carcinomas. Invasive carcinoma is frequently associated with p53 mutations.[41–43]

In addition to the above-mentioned genomic abnormalities associated with urothelial carcinoma, epigenetic alterations also occur during urothelial carcinogenesis. Almost all cells in the human body contain the same sequence of DNA, but cells in different organs during different developmental stages express different genes by epigenetic control of cellular function. This expression control of DNA is achieved by DNA methylation, chromatin structure and transcription factors. DNA can be methylated at cytosine residues adjacent to guanine residues (CpG) and CpG sites are distributed non-randomly throughout the genome, being found as islands in the promoter and exonic regions. Inactivation of tumor suppressor genes is known to occur via promoter hypermethylation, frequently due to DNA methyltransferase 1 (DNMT1). Expression of DNMT1 is increased in tumors and even during the precancerous stages of the urothelium with the development of flat carcinomas *in situ*.[44] Hypermethylation in urothelial carcinogenesis has also been observed in the promoter region of the E-cadherin gene, indicating an association with carcinoma *in situ* and detachment of cells or clusters of carcinoma cells in the urine.[45]

Development and progression of urothelial carcinoma

As is shown in Fig. 1, multifocal transitional cell carcinomas may develop in any region of the urinary tract, from the renal pelvis/ureter to the bladder/urethra.[4,46] Whereas upper urinary

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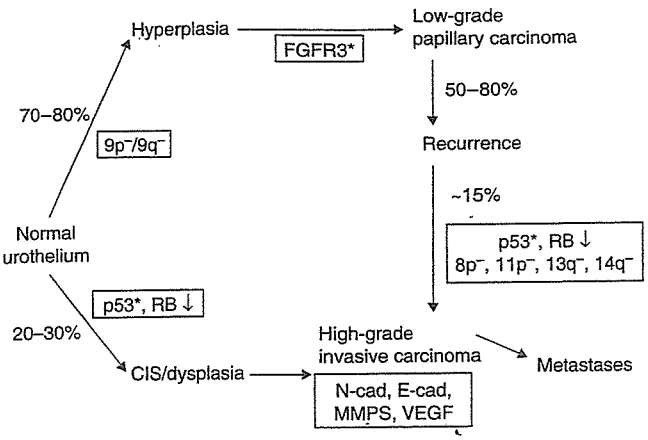

Fig. 9. Presumed molecular pathways of development of papillary and nodular carcinoma. (Modified from Wu.[38]) CIS, carcinoma *in situ*; FGFR3, fibroblast growth factor receptor 3; MMPS, matrix metal proteinases; RB, retinoblastoma gene; VEGF, vascular endothelial growth factor.

tract carcinomas occur infrequently after transurethral management of bladder carcinomas, the incidence is much greater in patients with vesico-ureteral reflux,[10] suggesting seeding or implantation of primary urothelial carcinoma cells after spread via the urine rather than field cancerization. In addition, recent molecular analyses using X-chromosome inactivation,[23] p53 mutation,[24–28] LOH[27,28] and comparative genomic hybridization[29] have provided compelling evidence that multifocal urothelial carcinomas are monoclonal in origin, despite some discrepancies.[30,31]

Comparing the basic morphological patterns of urothelial carcinomas, namely low-grade, superficial papillary carcinomas and high-grade, invasive nodular carcinomas, these two patterns of urothelial carcinomas are clearly separated in rats[32] and mice.[33] However, in dogs,[34] papillary carcinomas and nodular carcinomas can both be induced, depending on the concentration and period of carcinogen administration. In humans, papillary carcinomas and nodular carcinomas may originally develop separately, but coexistence of the two types is occasionally observed in a single bladder together with dysplasia and carcinoma *in situ*.[35] During the process of repeated recurrence, progression from papillary carcinoma to nodular carcinoma may be observed and molecular analysis of bladder carcinogenesis indicates the presence of two pathways: LOH of 9p/9q loss and FGFR3 mutation resulting in papillary carcinoma, and if p53 mutation occurs, nodular carcinoma. If p53 mutations occur initially, nodular carcinoma develops via dysplasia and carcinoma *in situ*. The available data clearly indicate that multiple genetic alterations are associated with the development and progression of bladder cancer.[36–38]

In the normal-appearing mucosa of the renal pelvis, ureter and bladder, dysplasia and carcinoma *in situ* may be frequently observed.[46] As mucosal dysplasia is not malignant, a derivation by implantation from primary carcinomas is not conceivable. In normal-appearing mucosa in remote areas from tumors, p53 mutation may be observed.[27] Intraepithelial spread[27,28] has been proposed as an explanation but this would appear unlikely. The phenomenon of coexistence of dysplasia, carcinoma *in situ* and p53 mutation in normal-appearing mucosa can be far more readily explained by the field cancerization theory. Differences in growth patterns of papillary and nodular carcinomas, and carcinoma *in situ*, as well as in cellular polarity and grade of malignancy make a single origin by seeding unreasonable. Finally, on detailed analysis of recurrent patterns of papillary carcinomas after TUR with or without intravesical instillation therapy, Akaza *et al.* concluded that recurrence patterns are biphasic, with an initial peak due to seeding or implantation of cancer cells during therapy and a second peak due to a new tumor occurrence from a background of field changes.[47] Thus, multifocal bladder recurrence of urothelial carcinomas is due to a combination of seeding and field changes. This is directly relevant to the condition for reconstruction of the urinary tract after cystectomy, inhibition and control of multiple recurrences after TUR, and the frequency and timing of follow-up for upper tract malignancies after treatment of bladder carcinoma. However, such clinical issues will be covered elsewhere.

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doi: 10.1111/j.1349-7006.2006.00264.x
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Staging performance of carbon-11 choline positron emission tomography/computed tomography in patients with bone and soft tissue sarcoma: Comparison with conventional imaging

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(Received May 17, 2006/Revised June 21, 2006/Accepted June 26, 2006/Online publication August 14, 2006)

The present study was conducted to compare the diagnostic accuracy between carbon-11 choline (^{11}C-choline) positron emission tomography (PET)/computed tomography (CT) and conventional imaging for the staging of bone and soft tissue sarcomas. Sixteen patients who underwent ^{11}C-choline PET/CT prior to treatment were evaluated retrospectively for staging accuracy. Conventional imaging methods consisted of ^{99m}Tc-hydroxymethylene diphosphonate bone scintigraphy, chest CT and magnetic resonance imaging of the primary site. The images were reviewed and a consensus was reached by two board-certified radiologists who were unaware of any clinical or radiological information using hard-copy films and multimodality computer platform. Tumor stage was confirmed by histological examination and/or by an obvious progression in number and/or size of the lesions on follow-up examinations. Reviewers examining both ^{11}C-choline PET/CT and conventional imaging classified T stage in all patients. Interpretation based on ^{11}C-choline PET/CT, the Node (N) stage was correctly diagnosed in all patients, whereas the accuracy of conventional imaging in N stage was 63%. Tumor Node Metastasis (TNM) stage was assessed correctly with ^{11}C-choline PET/CT in 15 of 16 patients (94%) and with conventional imaging in eight of 16 patients (50%). The overall TNM staging and N staging accuracy of ^{11}C-choline PET/CT were significantly higher than that of conventional imaging ($P < 0.05$). ^{11}C-choline PET/CT is more accurate than conventional imaging regarding clinical staging of patients with bone and soft tissue sarcomas. A whole body ^{11}C-choline PET/CT might be acceptable for imaging studies of tumor staging prior to treatment. (*Cancer Sci* 2006; 97: 1125–1128)

The general diagnostic tools for staging bone and soft tissue sarcomas are clinical examination, magnetic resonance imaging (MRI) and X-ray of the primary tumor site, chest X-ray or computed tomography (CT), and bone scintigraphy.[1]

Positron emission tomography (PET) with [18F]-fluoro-2-deoxy-D-glucose (FDG) has been used in the evaluation of patients with bone and soft tissue sarcomas for grading and therapy monitoring.[2–7] Most of these studies reveal that ^{18}FDG-PET is superior in the assessment of grading and therapy monitoring compared with conventional imaging.

Recently, carbon-11 choline (^{11}C-choline) has been introduced as a new oncological positron-emitting radiopharmaceutical for evaluation of a variety of malignant tumors.[8–11] Choline is an essential component of the cell membrane, and choline uptake may be via a choline-specific transporter protein.[12] Choline kinase, which catalyzes the phosphorylation of choline, is upregulated in malignant cells. Some studies have demonstrated additional gains in diagnostic accuracy using ^{11}C-choline.[13] ^{11}C-choline uptake is significantly higher in malignant tumors than in benign tumors and correlates well with the degree of ^{18}FDG accumulation with the lesion, while the high background activity owing to excretion via urinary tract interferes with evaluation on ^{18}FDG-PET.[14,15] However, the role of ^{11}C-choline PET scan in the staging of bone and soft tissue sarcomas has not been clarified. To fully elucidate the role of ^{11}C-choline PET, the comparison with ^{18}FDG-PET and conventional imaging modalities are needed.

A new-modality PET/CT can improve the localization of tumors and accuracy of staging in patients because anatomic and molecular information can be coregistered precisely.[16] The aim of the current study was to compare the diagnostic accuracy between ^{11}C-choline PET/CT and conventional imaging for the staging of bone and soft tissue sarcomas.

Materials and Methods

Patient. We retrospectively reviewed ^{11}C-choline PET/CT results from September 2005 to March 2006 for patients with bone and soft tissue sarcomas, who subsequently underwent surgical resection, chemotherapy and/or radiotherapy within 2 weeks. ^{11}C-choline PET/CT was performed for initial staging in 12 patients and for restaging of recurrent disease in four patients. The study population consisted of 13 men and three women with a mean age of 44 years (range, 13–75 years). The clinical records of all of the patients were available for review. This study was conducted in accordance with the amended Helsinki declaration and the protocol was approved by the Institutional Review Board (National Cancer Center, Research Center for Cancer Prevention and Screening). All of the patients provided their written informed consent to participate in the present study and to review their records and images.

Radiopharmaceuticals. Carbon-11 choline was synthesized with a commercial module essentially using the method described by Hara and Yuasa.[17] $^{11}CO_2$ was converted to ^{11}C-methyl iodide by $LiAlH_4$/HI reaction. ^{11}C-methyl iodide was trapped in dimethylaminoethanol. After a washing step with ethanol and water, ^{11}C-choline retained on a cation exchange resin was eluted with saline. Radiochemical purity of the solution was evaluated by liquid chromatography radiodetector. The organic solvents were analyzed by gas chromatography. Endotoxin was assayed by the lysosomal acid lipase method.

PET/CT. Scans were acquired with a PET/CT device (Aquiduo; Toshiba Medical Systems, Tokyo, Japan) that consisted of a PET scanner (ECAT HR+; CTI, Knoxville, TN, USA) and 16-section CT scanner (Aquilion V-detector; Toshiba Medical Systems) with a whole-body mode implemented as the standard software. Prior to the ^{11}C-choline PET/CT study, the patients fasted for at least

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doi: 10.1111/j.1349-7006.2006.00288.x
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Table 1. Summary of patients and confirmed staging

Patient no.	Diagnosis	SUV	Size (mm)	Staging type	Location	TNM	Metastasis	Grade	Stage
1	Leiomyosarcoma	4.63	110	Initial	Retroperitoneum	T2bN0M1	Soft tissue	High	IV
2	Rhabdomyosarcoma	3.03	60	Initial	Perineum	T2bN1M0	Lymph node	High	IV
3	Pleomorphic malignant Fibrous histiocytoma	15.05	133	Initial	Chest wall	T2bN0M1	Bone, pleura, lymph node	High	IV
4	Leiomyosarcoma	4.10	80	Initial	Retroperitoneum	T2bN0M,P	Lung	Low	IV
5	Osteosarcoma	6.70	110	Initial	Iliac bone	T2N0M1b	Bone, lung	High	IVB
6	Clear cell sarcoma	13.03	80	Initial	Chest wall	T2bN0M1	Bone, lung, pleura, lymph node	High	IV
7	Myxoid liposarcoma	2.15	50	Initial	Leg	T1aN1M0	Lymph node	Low	IVB
8	Osteosarcoma	5.31	110	Initial	Tibia	T2N1M0	Lymph node	High	IV
9	Ewing sarcoma	3.46	95	Initial	Leg	T2bN0M0	N/A	High	III
10	Ewing sarcoma	9.86	102	Initial	Shoulder	T2N0M0	N/A	High	IIB
11	Ewing sarcoma	6.14	16	Initial	Spine	T1N0M0	N/A	High	IA
12	Chondrosarcoma	5.99	110	Initial	Iliac bone	T2N0M1b	Bone	High	IVB
13	Leiomyosarcoma	3.18	50	Restaging	Thigh	T1bN1M1	Bone, soft tissue, lymph node	High	IV
14	Osteosarcoma	4.95	75	Restaging	Jaw	T1N0M1a	Lung	High	IVA
15	Osteosarcoma	3.60	50	Restaging	Femur	T1N0M1b	Lung, bone	High	IVB
16	Alveolar soft part sarcoma	3.60	25	Restaging	Shoulder	T2N0M1	Bone	High	IV

N/A, not applicable; SUV, standardized uptake value; TNM, Tumor Node Metastasis.

6 h. CT was performed from the head to the mid-thigh according to a standardized protocol with the following setting: axial 3.0-mm collimation × 16 modes; 120 kVp; 100 mAs; and a 0.5-second tube rotation, pitch 11.0. Patients maintained normal shallow respiration during the three-dimensional acquisition of CT scans. No iodinated contrast material was administered. Emission scans from the base of the skull to the leg were obtained starting 5 min after the intravenous administration of 350–573 MBq of ^{11}C-choline. The acquisition time for PET was 2 min per table position. Images were reconstructed with attenuation-corrected ordered-subset expectation maximization with two iterations and eight subsets using emission scans and CT data.

Positron emission tomography, CT and coregistered PET/CT images were analyzed with dedicated software (e-soft; Siemens). The initial review of the attenuation-corrected PET images was performed using transaxial, coronal and sagittal planes. The images were reviewed and a consensus was reached by two board-certified radiologists who were unaware of any clinical or radiological information using a multimodality computer platform. ^{11}C-choline uptake was considered to be abnormal when it was substantially greater than the surrounding normal tissue. For ^{11}C-choline PET/CT, tumor sizes and T staging were determined by the CT part of PET/CT. ^{11}C-choline-avid lymph nodes or distant metastases on PET/CT were interpreted as positive for metastases regardless of size. Lymph nodes with abnormal uptake were deemed positive for metastases even when they were smaller than 10.0 mm in short axis nodal diameter. Lung nodules without abnormal uptake but highly suggestive of lung metastases on ^{11}C-choline PET/CT were considered to be positive for metastases. A pixel region of interest (ROI) was outlined within regions of increased ^{11}C-choline uptake and measured on each slice. For quantitative interpretations, standardized uptake value (SUV) was determined according to the standard formula, with activity in the ROI given in Bq per mL/injected dose in Bq per weight (kg). However, time decay correction for whole-body image acquisition was not conducted. A SUV of more than 2.5 was considered to characterize malignancy.

Conventional imaging. Conventional imaging methods, performed within 2 weeks of ^{11}C-choline PET/CT, either before or after, were ^{99,m}Tc-hydroxymethylene diphosphonate (HMDP) bone scintigraphy, chest CT and MRI of the primary site. ^{99,m}Tc-HMDP bone scintigraphy was performed 2 h after intravenous injection of 740 MBq of ^{99,m}Tc-HMDP. Both anterior and posterior whole-body planar images were obtained simultaneously with a dual-headed gamma camera (E.CAM; Siemens). Chest CT was performed using a multidetector scanner (Aquilion V-detector; Toshiba Medical Systems) with the following setting: axial 4.0-mm × 4 modes; 120 kVp, automated electric current; 0.5-second tube rotation; and pitch 5. Images were reconstructed with 10.0-mm slice thickness by means of a standard algorithm. MRI of the primary site was performed using a 1.5 Tesla system (Signa Horizon; GE Medical Systems, Milwaukee, WI, USA or Visart; MRI produced by Toshiba Medical Systems, Tokyo, Japan). Pulse sequences comprised T1-weighted spin echo (SE) images, T2-weighted fast spin echo (FSE) images, as well as post-contrast T1-weighted SE images with fat suppression after injection of contrast material. Pulse sequence parameters and slice orientation varied with the examined anatomic site. The images were reviewed and a consensus was reached by two board-certified radiologists who were unaware of any clinical or radiological information using hard-copy films and multimodality computer platform. The two readers for ^{11}C-choline PET/CT and those for conventional imaging were not the same persons.

Each tumor was staged according to the Tumor Node Metastasis (TNM) classification of the International Union Against Cancer for sarcoma of bone and the American Joint Committee staging protocol for sarcoma of the soft tissue.[18,19] T, N and M stages were assigned for both PET/CT and conventional imaging. T staging was confirmed by pathological evaluation using specimens obtained from surgical resection of the primary tumors. N staging was confirmed by pathological examinations in two patients using specimens obtained from sampling of regional nodes. In terms of extraregional nodes in two patients, nodal staging was confirmed by an obvious progression in number and/or size of the lesions on follow-up examinations. The mean follow-up period was 172 days (range, 44–322 days).

Statistical analysis. All valuables were assessed on a patient-by-patient basis. The McNemar test was used for paired comparisons between ^{11}C-choline PET/CT and conventional imaging. Statistical analysis was performed with the SPSS version 11 software program (SPSS, Chicago, IL, USA).

Results

There were eight bone sarcomas and eight soft tissue sarcomas (Table 1). The primary sites included shoulder ($n = 2$), chest wall

doi: 10.1111/j.1349-7006.2006.00288.x
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Table 2. **Staging of bone and soft tissue sarcoma**

Variables	^{11}C-choline PET/CT	Conventional imaging	*P*-value
Overall stage			0.023
Correct	15 (94)	8 (50)	
Understaged	1 (6)	8 (50)	
Overstaged	0	0	
N stage			0.041
Correct	16 (100)	10 (63)	
Understaged	0	6 (38)	
Overstaged	0	0	
M stage			0.617
Correct	15 (94)	13 (81)	
Understaged	1 (6)	3 (19)	
Overstaged	0	0	

Note: Data are presented as number (*n*). Numbers in parentheses are percentages. CT, computed tomography; PET, positron emission tomography.

($n = 2$), retroperitoneum ($n = 2$), iliac bone ($n = 2$), leg ($n = 2$), thigh ($n = 1$), perineum ($n = 1$), tibia ($n = 1$), femur ($n = 1$), mandible ($n = 1$) and spine ($n = 1$). Pathological diagnoses were osteosarcoma ($n = 4$), Ewing sarcoma ($n = 3$), leiomyosarcoma ($n = 3$), clear cell sarcoma ($n = 1$), chondrosarcoma ($n = 1$), pleomorphic malignant fibrous histiocytoma ($n = 1$), myxoid liposarcoma ($n = 1$), rhabdomyosarcoma ($n = 1$), and alveolar soft part sarcoma ($n = 1$). Histological grade of tumors was grade 1 ($n = 1$), grade 2 ($n = 1$), grade 3 ($n = 11$) and grade 4 ($n = 3$).

All patients of initial staging had increased ^{11}C-choline uptake of the primary lesion (average maximal SUV ± SD: 5.92 ± 3.68 [range, 2.15–15.05]). Pathological T stages available in patients with initial staging are as follows: T1 ($n = 1$), T1a ($n = 1$), T1b ($n = 1$), T2 ($n = 4$) and T2b ($n = 5$). T stages in patients with restaging were T1 ($n = 2$), T1b ($n = 1$) and T2 ($n = 1$). Tumor size of patients for initial staging was 78.5 ± 34.0 mm (mean ± SD [range, 16.0–133.0 mm]). Both ^{11}C-choline PET/CT and conventional imaging classified the T stage correctly in all patients. Twelve (75%) of the 16 patients had N0 disease. Using ^{11}C-choline PET/CT, the N stage was correctly assigned in all patients, whereas the accuracy of conventional imaging in N stage was 63% ($P = 0.041$, Table 2). Understaging occurred in six patients (38%). Three of these patients (19%) had metastasis of inguinal node whose largest diameter was less than 10.0 mm (Fig. 1). The incidence of distant metastases was high in our study population. Both ^{11}C-choline PET/CT and conventional imaging detected bone metastases in seven patients (44%), lung metastases in five (31%) and pleural dissemination in two (18%, Fig. 2). Using ^{11}C-choline PET/CT, the M stage was correctly assigned in 15 patients (94%), whereas the accuracy of conventional imaging in M stage was 81% ($P = 0.617$, Table 2).

The complete stages of all patients were stage IA ($n = 1$), stage IIB ($n = 1$), stage III ($n = 1$) and stage IV ($n = 13$). TNM stage was correctly assessed with ^{11}C-choline PET/CT in 15 of 16 patients (94%) and with conventional imaging in eight of 16 patients (50%, $P = 0.023$, Table 2). ^{11}C-choline PET/CT assigned an incorrect TNM stage in a patient. This patient was understaged due to small metastatic lung tumor which was not clearly visualized by CT part of ^{11}C-choline PET/CT. Eight patients were understaged by conventional imaging (50%). Of these, skip metastases of soft tissues were identified in two (25%) and small nodal metastases in six (75%). ^{11}C-choline PET/CT correctly determined TNM stage in seven patients (44%) in whom stage derived from conventional imaging was incorrect.

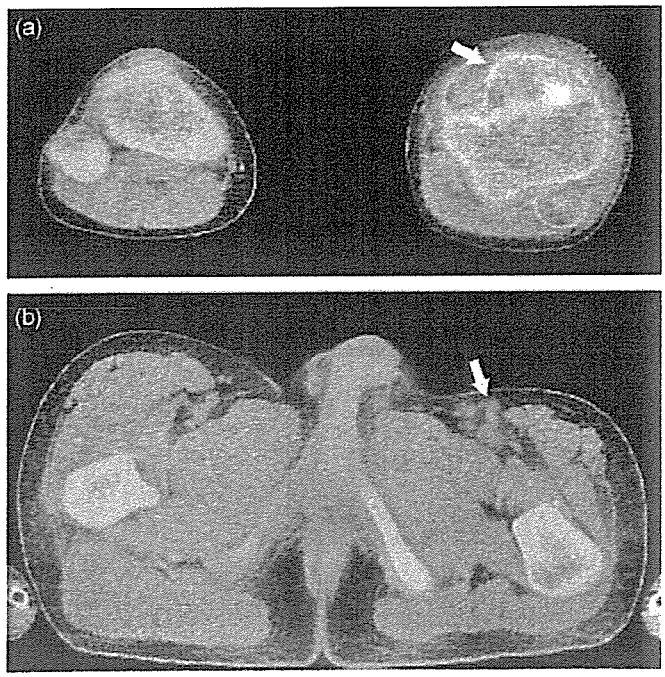

Fig. 1. A 13-year-old boy with osteosarcoma. (a) Transverse ^{11}C-choline positron emission tomography (PET)/computed tomography (CT) image revealed hypermetabolic focus in the proximal portion of the left tibia (arrow). PET/CT findings were verified at histopathological analysis. (b) Abnormal uptake of ^{11}C-choline was also noted in the left inguinal lymph node, which was interpreted as highly suspicious for malignancy (arrow). Subsequent resection revealed metastasis from osteosarcoma.

Discussion

The results of the present study show that ^{11}C-choline PET/CT improves the accuracy of staging in patients with bone and soft tissue sarcomas compared to conventional imaging. Specifically, ^{11}C-choline PET/CT has potentially significant implications for detecting nodal and distant metastases at overall staging. Reports about the efficacy of ^{11}C-choline in the localization and detection of bone and soft tissue sarcomas are still limited.[15] To our knowledge, no study regarding ^{11}C-choline PET/CT for staging bone and soft tissue sarcomas was found. In our study, seven of the 16 patients had skip metastases of soft tissue or nodal metastases detected by ^{11}C-choline PET/CT that were not identified by routine clinical and conventional radiological evaluation.

The ability of PET to depict increased metabolism in malignancies has greatly improved the accuracy in detecting neoplasms.[4] However, compared with conventional imaging studies, use of PET alone results in a lack of substantial detail.[20] The PET/CT device permits sequential acquisition of anatomic CT and functional PET images in a single scanning session. Morphological characterization of scintigraphic lesions by PET/CT resulted in a lower percentage of equivocal interpretations compared with that of conventional imaging. Tumor-detecting PET/CT technology is growing rapidly. However, there are only limited data available on staging of bone and soft tissue sarcomas with PET/CT.

Carbon-11 choline uptake was significantly higher in malignant soft tissue tumors and was due to the high utilization of cell membranes of these lesions. ^{11}C-choline uptake is observed physiologically in the liver, pancreas, kidney and duodenum. ^{11}C-choline is also secreted into phospholipid-rich pancreatic juice in a non-fasting state. A potential advantage of ^{11}C-choline PET/CT might be the assessment of tumors in the skull or retroperitoneum. Blood clearance of ^{11}C-choline is rapid and radioactive distribution

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