JUA Cancer Registration Statistics

Oncological outcomes of the prostate cancer patients registered in 2004: Report from the Cancer Registration Committee of the JUA

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Objectives: In 2001, the Cancer Registration Committee of the Japanese Urological Association initiated a data collection of prostate cancer patients into a computer-based database. The aim of the present study is to report the clinical and pathological characteristics and outcomes of prostate cancer patients diagnosed in 2004 in Japan.
Methods: Overall, 11 385 patients from 239 institutions were registered into the database. After excluding 1105 patients because of insufficient data, duplication or insufficient follow up, 10 280 patients were eligible for the analysis. Most of them (10 198, 99.2%) were Japanese and 1195 (11.6%) had metastatic disease at the time of diagnosis. The mean and median follow up was 53.2 months and 61.5 months, respectively.
Results: The 5-year overall and prostate cancer-specific survival rate was 89.7% and 94.8%, respectively. The 5-year prostate cancer-specific survival rate of M0 and M1 disease was 98.4% and 61.1%, respectively. For 8424 cases of organ-confined or regional disease, Japanese urologists used as the initial treatment hormone ablation therapy alone (3360, 39.9%), radical prostatectomy (3140, 38.1%), radiation therapy (1530, 18.2%) and watchful waiting (394, 4.7%) including active surveillance or palliative observation.
Conclusions: This is the first large population report of survival data in Japanese prostate cancer patients. In Japan, the disease population, survival period with metastatic disease and ratio of patients having hormone ablation therapy differ from those in Western countries.

Key words: epidemiology, Japanese, prostate neoplasm, registration, survival.

Introduction

In the 1990s, prostate-specific antigen (PSA) testing became widespread in Japan, as in the USA and Europe. The incidence of prostate cancer in Japan also appears to be rising. There is no doubt that PSA screening contributes to earlier diagnosis of prostate cancer. Whether earlier detection of the prostate cancer in Japanese men helps reduce prostate cancer-specific mortality is unknown as a result of the lack of detailed information about Japanese prostate cancer patients.

In 2001, the Japanese Urological Association (JUA) initiated a study to estimate the etiology, diagnosis, initial treatment, pathological findings and final outcomes of prostate cancer using computer-based registration of prostate

cancer patients from institutions all over Japan. In 2005, we published the initial report on the registered 4529 prostate cancer patients diagnosed in 2000[1] and the estimated etiology, diagnosis and initial planned treatment were analyzed. In 2010, detailed information including the main treatment modality used, adjuvant therapies used and survival of prostate cancer patients diagnosed in 2004 was collected to assess the current situation of prostate cancer in Japan.

Methods

Patients and treatments

In 2010, data on patients diagnosed with prostate cancer in 2004 were collected, along with 5-year survival data and radical prostatectomy pathology results. Incidental cancer found within specimens removed during radical cystoprostatectomy for bladder cancer and transitional cell carcinoma of the prostate concomitant with bladder cancer were excluded from this registry. In all, 11 385 patients were

registered from 239 institutions. Excluded from the analysis were 37 duplications (only one record was removed and the patient remained in the registry), six patients because of insufficient data and 1062 patients with less than 180 days of follow up, leaving 10 280 patients included in the analysis.

Variables

Pathological staging was based on the fifth edition of the TNM classification and the third edition of the General Rule for Clinical and Pathological Studies on Prostate Cancer (2001).[2] For the PSA analysis, only cases measured with the Tandem-R kit PSA assay ($n = 4567$, 44.4%) were included to avoid statistical scatter. The definition of PSA failure was determined based on the clinician's judgement.

Survival data were analyzed according to the main treatment modality and the M stage. The initial main treatment modalities used were categorized into four groups: hormone ablation therapy alone (Hx), radical prostatectomy (RP) with or without neoadjuvant hormone treatment (NHT), radiation therapy (Rx) with or without NHT and watchful waiting (W/W) including active surveillance or palliative observation irrespective of the intent. Characteristics and outcomes from the four treatment groups were analyzed separately.

Analysis of progression-free survival was not possible as a result of difficulties in timing recurrence correctly. In some RP cases, adjuvant therapy was initiated just after the operation on the basis of the pathological findings. In addition, there were substantial differences in how post-Rx PSA failure was defined. For these reasons, the exact timing of recurrence was not able to be determined for a sizable number of patients, whom we consequently described as having "stable disease." Therefore, we had no other choice but to focus on the mortality rate, overall survival (OS) and prostate cancer-specific survival (PCSS).

Statistical methods

For statistical analysis, Student's t-test was used for analysis of intergroup differences in means and the χ^2-test was used for intergroup comparisons. Survival data was analyzed by the Kaplan–Meier method.

Results

Overall data

The registered patients' characteristics including age, PSA, Gleason score and TNM classification were summarized according to the main initial treatment modality (see Table S1, supporting information). In the 10 280 patients, the number of the patients treated by Hx, RP, Rx and W/W was 4934 (49.8%), 3212 (31.5%), 1605 (10.4%) and 485 (4.7%), respectively. The 44 patients were treated by other modalities.

There were statistically significant differences among patients in different treatment groups. Patients treated with RP were the youngest (median age 68.0 years), with patients treated with Hx on average approximately 8.5 years older (median age 76.0 years). Overall, median PSA at diagnosis was 13.0 ng/mL, but the median PSA within the W/W group was 7.3 ng/mL, which was the lowest. Median Gleason score was 7 among Hx, RP and Rx groups, and 6 in W/W patients. Approximately 50–60% of each group was staged as T1c or T2 disease. In contrast, 11.5% of patients presented with metastatic disease at the time of diagnosis.

The 5-year OS and PCSS of all 10 280 patients was 98.7% and 94.8%, respectively. Figure 1 shows the Kaplan–Meier curves according to M stage. Bony disease (M1b) comprised the majority of M1 patients. The 5-year OS and PCSS was 61.8% and 66.7%, respectively. In M1 disease, there was a significant correlation between survival and Gleason score ($P < 0.001$).

T1-4N0M0 prostate cancer

There were 8424 patients with T1-4N0M0 prostate cancer. The distribution and proportion of clinical T (cT) stage and age by treatment group are shown in Figure 2. Interestingly, in Japan more than 30% of patients received Hx as the main treatment modality across all cT stages. Even for cT1 or cT2 disease, RP, Hx and Rx were carried out in approximately 50%, 30% and 20% of the cases, respectively. The age distribution differed dramatically across treatment groups. For patients less than 75 years-of-age, RP was widely used. Rx was carried out at similar rates (approximately 20%) in patients up to 80 years-of-age. Hx was the major treatment in patients over 80 years-of-age.

OS and PCSS in T1-4N0M0 disease by treatment group were shown to be 97.6% and 99.6% in RP, 95.6% and 98.5% in Rx, 96.4% and 99.7% in W/W and 88.9% and 97.7% in Hx. Five-year PCSS for patients without metastatic disease was excellent (98.4%).

Distribution of age and PSA in patients with T1-4N0M0 prostate cancer according to treatment was shown in Figure S1. Figure S2 shows cT distribution and the main treatment adopted in these patients. Figure S3 shows overall and prostate cancer-specific survival by main treatment adopted in these patients.

Radical prostatectomy

RP was carried out in 3212 patients (see Table S2, supporting information). Overall, 96.2% of RP patients had radical prostatectomy through the retropubic approach, and 89% had an open procedure. Concerning neurovascular bundle preservation, 70.4% of the patients received RP without nerve preservation. Lymph node dissection was carried out in 91% of the patients with mainly limited obturator lymph node dissection (71.6%).
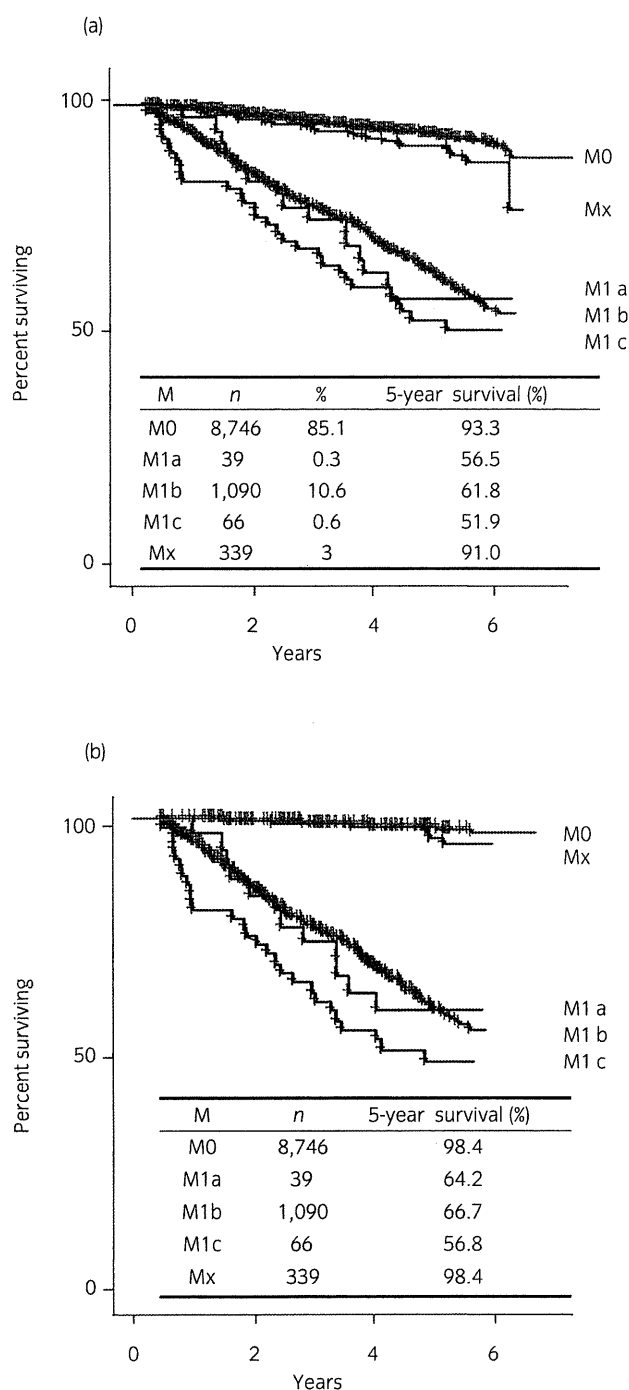
Fig. 1 Kaplan–Meier curves of (a) overall survival and (b) prostate cancer-specific survival according to M stage (*n* = 10 280).

The outcomes of 3200 RP patients according to NHT duration are summarized (see Table S3, supporting information). Because of uncertain NHT status, 12 patients were excluded. In the RP with NHT group (*n* = 1164), most pathological parameters including node metastasis (pN) and surgical margin status (ew) were better than in those patients without NHT (*n* = 2045; *P* < 0.001), except for seminal vesicle invasion (sv). However, the survival status of RP with NHT group did not differ from the RP without NHT group. The disease-free rate and prostate cancer death rate in the RP group within this observation period of approximately 5 years was approximately 70–75% and less than 1%, respectively.

Hormonal therapy alone

In this registration series, 4934 patients were treated with Hx alone (see Table S4, supporting information). In these patients, 3582 patients (72.6%) had non-metastatic disease (M0) and 1061 patients (21.5%) had bony metastasis (M1b). The combination of luteinizing hormone-releasing hormone (LH-RH) analogs with non-steroidal anti-androgen drugs were used in the majority of the Hx patients (67.4%). In M0 disease, 25% of patients received monotherapy with LH-RH analogs or surgical castration, and 67.4% patients were treated with maximum androgen blockade (MAB). Estrogen or estramustine phosphate therapy as the initial Hx was rare for M0 disease. For M1b disease, 82% of patients received MAB and 14.4% of patients received estrogen or estramustine phosphate as the initial treatment. The 5-year PCSS in patients with M0 disease was 93.3% and in M1b patients, it was 71.2%. In M0 patients, 8.4% of the patients died of other causes, which seemed to be higher when compared with patients treated with other modalities.

Curative radiation for prostate cancer

Rx as a radical treatment was used for 1554 patients. There were 28 patients who received particle radiotherapy and 27 patients were treated by uncertain modality. Excluding these patients, the characteristics of the 1499 patients are summarized (see Table S5, supporting information). Radiation therapy was classified as external beam radiation therapy with Liniac (EBRT; *n* = 1241), brachytherapy (BT; *n* = 210) or a combination (BT + EBRT; *n* = 48). Median age in EBRT was 72.9 years and median PSA was 15.0 ng/mL. In contrast, that in BT was 70.0 years and median PSA was 7.30 ng/mL. When compared with EBRT patients, BT patients were younger and had lower PSA, Gleason scores and earlier stage disease. The median PSA level in patients who received EBRT was 15.0 ng/mL, higher than in RP patients. In 1241 EBRT patients, 88.6% received radiation to the prostate only and the median dose in EBRT was 70 Gy. No cancer deaths were observed in patients who received BT and BT + EBRT. In the EBRT group, 5-year PCSS was 98.3% (see Table S6, supporting information).

Watchful waiting

In this registry, W/W included active surveillance, deferred treatment and palliative observation. At the time of registration, 72.4% of patients were maintained on watchful
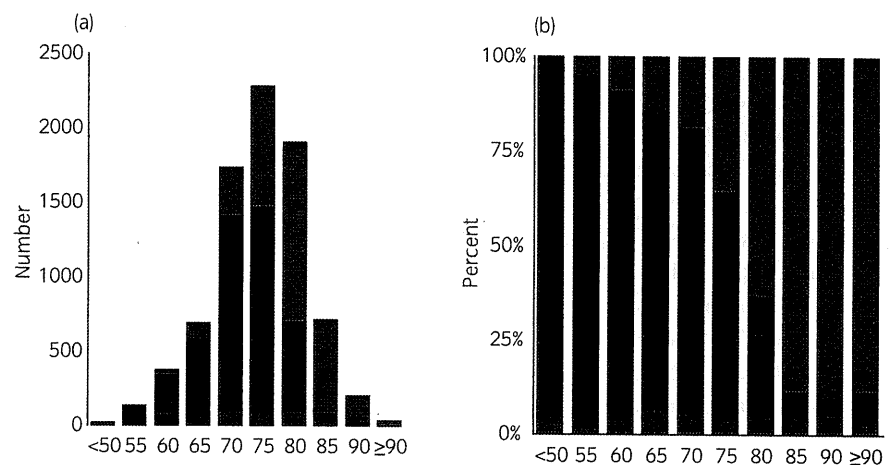
Fig. 2 Age distribution by main treatment modality in patients with T1-4N0M0 prostate cancer (*n* = 8424). (a) Totals and numbers of patients who underwent each treatment modality. (b) Percentages of each treatment by age. Hx, hormone ablation therapy; RP, radical prostatectomy; Rx, radiation therapy; W/W, watchful waiting.

Number of the patients by age and main treatments

	<50	50–55<	55–60<	60–65<	65–70<	70–75<	75–80<	80–85<	85–90<	≥90
Hx	0	7	34	108	329	815	1207	637	184	31
RP	18	94	269	675	982	899	198	4	0	0
Rx	2	30	58	166	326	485	409	41	9	3
W/W	1	2	15	45	100	86	96	37	11	1

waiting. In the W/W group, 0.62% of the patients died of prostate cancer. The incidence was similar to that in the RP patients (see Table S7, supporting information).

Discussion

The present report is the first large-scale study of the characteristics and survival of prostate cancer patients in Japan based on multi-institutional registry data. The estimated number of newly diagnosed prostate cancer patients in Japan in 2005 was 42 997.[3] This registry seems to cover approximately one-quarter of newly diagnosed prostate cancer in Japan. With regard to prostate cancer incidence and mortality, ethnic differences between American or European and Asian men are well known. Understanding the actual situation of Japanese prostate cancer patients is indispensable to addressing many clinical issues regarding prostate cancer treatment.

The incidence of metastatic prostate cancer at the initial registration was 11.6% in the present study. In the USA, 6.5% were distant stage according to the report from the 1990–2000 database of the Surveillance, Epidemiology and End Results (SEER) Program[4], suggesting the incidence of metastatic disease is higher in Japan than in the USA. However, the incidence was 21.3% from the Japanese registration data in 2000.[1] Compared with the data from 2000, the ratio of distant disease in 2004 was reduced by half. However, the number of the distant diseases in 2000 (*n* = 964) was almost the same as that in 2004 (*n* = 1195).

In the report derived from the 1973–2000 database of the SEER Program[4], 5- and 10-year PCSS were approximately 99% and 95%, respectively. Two-thirds of patients were

diagnosed with well or moderately differentiated localized or regional prostate cancer. Among these patients, 5- and 10-year PCSS were approximately 100%. In the present study, 5-year PCSS was 94.8%, which resembles the SEER data from 1995. The PCSS of localized or regional prostate cancer was 98.4%, similar to the SEER data. Five-year PCSS of patients with bony metastasis in Japan was 66.7%, which was better than the 27–37% 5-year PCSS in the USA[4]. The reason why Japanese patients with bony metastasis showed a longer survival period than American patients is uncertain.

The main treatment used for non-metastatic prostate cancer patients in Japan was quite different from that in the USA. In the USA, approximately half of prostate cancer patients received surgery and more than one-third underwent Rx.[5] In Japan, Hx comprised of 39.9% of the initial main treatment, even for non-metastatic prostate cancer. One of the reasons for the high rate of Hx might be the relatively advanced age at diagnosis. Another reason might be the high rate of health insurance coverage and indifference about erectile dysfunction. In the present study, the most frequent treatment for non-metastatic prostate cancer in patients less than 70-years-old was RP (62.5%). Essentially, for patients younger than 70-years-old, Japanese urologists might choose treatments in agreement with major guidelines published by the National Comprehensive Cancer Network and the European Association of Urology, among others.

Concerning the administration of Hx medications, MAB therapy was recommended for stage D2 prostate cancer.[6] However, in Japan, 65% of patients with non-metastatic disease received MAB therapy and 25% of them received

LH-RH analogs or surgical castration as monotherapy. The 5-year PCSS of non-metastatic prostate cancer patients in Japan showed excellent results, even in the W/W group. The OS of patients with Hx seemed to be lower than that with other modalities. The patients undergoing Hx are relatively older.

In the present series, detailed data on RP was analyzed. In 2004, open retropubic RP (89.6%) with obturator lymph node dissection (71.6%) was the most common procedure. Interestingly, just 20% of patients received nerve-sparing operations in Japan. In high-volume hospitals in the USA, most radical prostatectomy seems to be carried out using the nerve-sparing technique. For most Japanese men, there might be less concern about sexual function when compared with American men.

The pathological results were sorted by NHT duration, because they might be affected by NHT status. Similar to the data from many randomized controlled studies of NHT[7,8] most pathological findings were improved by longer NHT, except for seminal sv and pN. However, there was no remarkable improvement in prognosis despite longer NHT as previously reported. However, these data came from non-randomized, non-historically controlled patients.

Additionally, the present study might be the largest population study of Rx in Japan. In past years, the trends and patterns of Rx in Japan were reported by the patterns of care study (PCS).[9,10] The age, PSA, Gleason score and radiation dose in the EBRT group of the present study were similar to PCS data. The median PSA of 15.0 ng/mL in the EBRT patients was higher than that of the patients treated with RP. Japanese urologists seemed to select EBRT for treating localized advanced disease. The EBRT group in the registry had a disease-free rate of 58% and a stable disease rate of 22.7%. Recently, higher dose radiation has been recognized to contribute to better cancer control. In 2004, 11.0% of the patients received 72 Gy and 11.4% patients received 76 Gy EBRT. Nearly 50% of patients underwent 68 Gy EBRT. Recently, relatively high dose EBRT in combination with NHT was attempted using the intensity modulated radiotherapy technique.

In conclusion, this is the first report of survival data involving one-quarter of newly diagnosed prostate cancer patients in Japan. In Japan, the patient population, survival period with metastatic disease and the ratio of patients receiving Hx differ from Western countries. Also noteworthy is the reduction in the ratio of metastatic prostate cancer at diagnosis, which was 11.6% in 2004, approximately half the rate in 2000. However, the total number of newly diagnosed patients with metastatic prostate cancer in 2004 was almost same as that in 2000. In terms of localized (cT2 or earlier stage) prostate cancer, Hx was used as the main treatment in 36.7% of Japanese patients. The 5-year survival of patients with localized prostate cancer was excellent irrespective of the main treatment used. Five-year OS and PCSS

of patients with M1b disease were superior to that in the USA.

Acknowledgments

Conflict of interest

None declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Distribution of age (A) and PSA (B) in patients with T1-4N0M0 prostate cancer ($n = 8424$) according to treatment. RP, radical prostatectomy; Rx, radiation therapy; Hx, hormone ablation therapy; W/W, watchful waiting.

Fig. S2 cT distribution and the main treatment adopted in patients with T1-4N0M0 prostate cancer ($n = 8424$). The graph A shows totals and numbers of patients who underwent each treatment modality. The graph B shows percentages of each treatment by clinical stage. RP, radical prostatectomy; Rx, radiation therapy; Hx, hormone ablation therapy; W/W, watchful waiting.

Fig. S3 Kaplan–Meier curves of overall survival (A) and prostate cancer-specific survival (B) by main treatment adopted in patients with T1-4N0M0 prostate cancer ($n = 8224$). RP, radical prostatectomy; Rx, radiation therapy; Hx, hormone ablation therapy; W/W, watchful waiting.

Table S1 Characteristics of the registered patients.

Table S2 Characteristics of 3212 radical prostatectomy patients.

Table S3 Outcome of 3200 radical prostatectomy cases with or without neoadjuvant hormonal therapy.

Table S4 Outcome of 4934 patients treated with hormone ablation therapy alone.

Table S5 Characteristics of patients treated with radiation therapy as the main treatment.

Table S6 Outcome of patients treated with radiation therapy as the main treatment.

Table S7 Outcome of 485 patients treated with watchful waiting.

Appendix I Statistics from various institutions in Japan.

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Original Article: Clinical Investigation

Prostate cancer gene 3 urine assay for prostate cancer in Japanese men undergoing prostate biopsy

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Objectives: To examine the clinical utility of the prostate cancer gene 3 (PCA3) urine test in predicting prostate cancer in Japanese men undergoing prostate biopsy.

Method: The study group included 105 men who underwent extended prostate biopsy based on an elevated serum prostate-specific antigen (PSA). In all cases, the patients' race was Asian. Urine specimens were collected after digital rectal examination, and PCA3 score (PCA3/PSA mRNA) was determined in the urine using the APTIMA PCA3 assay. PCA3 score was investigated for a correlation with serum PSA, prostate volume (PV), PSA density and biopsy outcome.

Results: All urine samples collected were successfully analyzed (i.e. the informative specimen rate was 100%). Biopsy showed prostate cancer in 38 men (36%). The PCA3 score was not associated with serum PSA nor PV. The median PCA3 score in prostate cancer was significantly higher than that in negative biopsy (59.5 vs 14.2 $P < 0.0001$). The probability of prostate cancer was 69% at a PCA3 score of more than 50 and 5% at a PCA3 score of less than 20. On multivariable logistic regression, PSA density ($P < 0.05$) and PCA3 score ($P < 0.0001$) were the independent predictors for prostate cancer. There was no significant difference in AUC between PCA3 score and PSA density. The combination of PCA3 score and PSA density increased the AUC from 0.72 for PSA alone to 0.88.

Conclusion: The specificity of the PCA3 urine assay for prostate cancer was excellent in Japanese men undergoing biopsy. PCA3 score could improve the prediction for prostate cancer and help to better select men who might benefit from prostate biopsy.

Key words: Japanese men, PCA3 urine assay, prostate cancer.

Introduction

Serum prostate-specific antigen (PSA) has been widely used as a screening test for prostate cancer, resulting in an increase of prostate cancer detection.[1] Serum PSA elevation is not specific for prostate cancer, as it can be associated with several conditions, such as benign prostatic hyperplasia (BPH) and prostatitis. Although elevated serum PSA is commonly used as an indication for prostate biopsy, the low specificity of the PSA test limits its use as a prostate cancer screening tool. Many efforts have been investigated, including age-specific PSA, volume referenced PSA, and PSA isoforms, aiming for more accurate assessments.[2–4] Recently, several studies reported that an association between serum PSA and prostate volume has been increasing during the past two decades as a result of the detection of small tumors.[5,6] New markers that correlate with the burden of prostate cancer are needed.

Bussemaker *et al.* reported that prostate cancer gene 3 (PCA3) is a non-coding gene expression, a prostate-specific mRNA that is highly overexpressed in prostate cancer with low expression levels in normal prostate tissue.[7] The possible use of PCA3 urine assays in urine after attentive digital rectal examination was shown using a first-generation research assay.[8,9] Groskopf *et al.* developed the APTIMA PCA3 assay for clinical use, with simplicity in specimen processing and a high informative rate.[10] This assay uses PSA expression to normalize the PCA3 expression level to generate a PCA3 score. Several studies using the APTIMA PCA3 assay have shown that the PCA3 score is superior to serum PSA for predicting prostate cancer with high specificity in North American and European men undergoing biopsy and is significantly synergistic with serum PSA[11–13]. In the present study, we examined the clinical utility of the PCA3 urine test for prostate cancer in Japanese men undergoing prostate biopsy.

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Methods

This protocol was approved by the institutional review board (IRB) and all subjects provided written informed consent

Table 1 Characteristics of men with positive and negative biopsy

	Positive	Negative	P-value
No. patients	38	67	
Median (range) age (years)	68 (55–87)	64 (44–84)	0.038
Median (range) PSA ng/mL	10.7 (4.4–720.6)	6.3 (3.3–34.8)	<0.0001
Median (range) prostate volume (cc)	30.8 (13.5–105.0)	35.3 (18.0–96.2)	0.049
Median (range) PSAD	0.38 (0.14–15.5)	0.17 (0.06–0.77)	<0.0001
Median (range) PCA3 score	59.5 (12.8–498.1)	14.2 (0.5–157.1)	<0.0001

PCA3, prostate cancer gene 3; PSA, prostate-specific antigen; PSAD, PSA density.

before study enrolment. A total of 105 consecutive men with serum PSA 2.5 ng/mL or greater and/or an abnormal digital rectal examination (DRE) who underwent systematic extended prostate biopsy (8 core or more) at Kyoto Prefectural University of Medicine, Kyoto, Japan, from May 2007 to 2008 were enrolled in the present study. We excluded men who had a history of prostate cancer, men who took medicines that are known to affect serum PSA levels, who had a urinary tract infection and men who had undergone invasive treatment for BPH. The median (range) age was 66 years (44–87). The median (range) serum PSA was 7.2 ng/mL (3.3–720.6). A total of 85 cases had an initial biopsy and 20 cases had a repeated biopsy. Men with atypia/high-grade prostate intraepithelial neoplasm (HGPIN) were classified as negative in the present study. DRE was carried out by a single urologist (AO). After DRE, 20–30 mL of first voided urine sample was collected, stored at −70°C and tested within 90 days of collection. Enough pressure was applied on the prostate to depress the surface by approximately 1 cm, from the base to the apex, and from the lateral to the median line for each lobe. Exactly three strokes per lobe were carried out. All patients underwent transrectal ultrasound (TRUS) examination using a 7.5-MHz transducer and prostate volume was measured using the formula for elliptical volume ($\pi/6 \times$ height $\times$ width $\times$ length). PSA density (PSAD) was calculated as PSA divided by prostate volume. Urine specimens were examined using the APTIMA PCA3 assay.[10] On APTIMA PCA3 assay, PSA mRNA copy and PCA3 mRNA copy were determined according to a previously reported method, and PCA3 score was calculated using PCA3 mRNA copy divided by PSA mRNA copy.[10] The Mann–Whitney test was used to compare variables among the groups. The χ^2-test was used to assess for trends. Bivariable analysis (Pearson's correlation coefficient, r) was used to test the linearity of relationships among the variables. Area under receiver–operator curves (ROC; AUC) were compared by the ANOVA method together with the jackknife bias-correction method. Univariable and multivariable logistic regression analysis was used to determine the significant predictors of prostate cancer among the vari-

ables such as age, PSA, PV, PSAD, repeated biopsy or not and PCA3 score. In the multivariate analysis, variables were analyzed as categorical variables. Optimal cut-off of variables was determined at the point where the Younden index was maximum on the ROC analysis. The combined likelihood of PSAD and PCA3 score was calculated using the fitting principal of maximum likelihood test. These parameters are estimated by minimizing the sum of the negative logs of the probabilities attributed to the observations by the model (maximum likelihood). Analyses were carried out with Statview 5.0, JMP 8 (SAS Institute, Cary, NC, USA) and DBM MRMC Version 2.2 (C. E. Metz, The University of Chicago, Chicago, IL, USA). A P-value of <0.05 was considered statistically significant.

Results

All urine samples were successfully analyzed (i.e. the informative specimen rate was 100%). There was no significant interrelationship between PCA3 score and other variables, such as age, serum PSA and prostate volume. Biopsies were prostate cancer positive in 38 men (36%) and negative in 67 men, three of whom had atypia/HGPIN. A total of 14 (37%), 14 (37%) and 10 (26%) cases were diagnosed as having a biopsy Gleason score of 6, 7 and 8 or above, respectively. Characteristics of men with positive and negative biopsies are shown in Table 1. The median PCA3 score (range) was 59.5 (12.8–498.1) and 14.2 (0.5–157.1) in positive and negative biopsies, respectively. There was a significant difference in the PCA3 score between positive and negative biopsies ($P < 0.0001$). The distribution of PSA and PCA3 score based on biopsy results is shown in Figure 1. In the comparison of the diagnostic performance of PCA3 score, PSA and other variables, we excluded three men with PSA over 50 ng/mL. A total of 69% of men with PCA3 score >50 had prostate cancer compared with 4.9% of men with prostate cancer and a PCA3 score <20. Sensitivity and specificity at different PCA3 score cut-offs are shown in Table 2. Using a PCA3 cut-off value of 35.0, sensitivity, specificity and diagnostic accuracy were 74.3%, 74.6% and 74.5%,
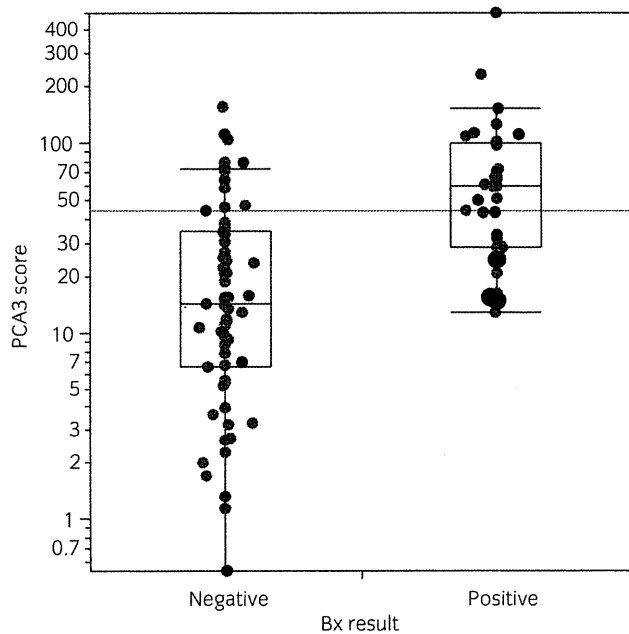
Fig. 1 The distribution of prostate-specific antigen (PSA) and prostate cancer gene 3 (PCA3) score based on biopsy result. ●, PSA >50 ng/mL; ●, PSA >20, ≤50 ng/mL; ●, PSA >10, ≤20 ng/mL; ●, PSA >4, ≤10 ng/mL; ●, PSA ≤4 ng/mL.

respectively ($P < 0.01$). AUC for PCA3 score was 0.8507 and AUC for serum PSA was 0.7243 in men with PSA less than 50 ng/mL($n = 102$). There was a significant difference in AUC between PCA3 score and serum PSA ($P < 0.05$), but not between PCA3 score and PSAD. In men with PSA between 4 and 10 ng/m ($n = 70$, median PSA 6.1 ng/mL, prostate cancer 17 cases), there was a significant difference in AUC between PCA3 score and serum PSA (0.8230 vs 0.5888, $P < 0.001$; Fig. 2). The specificity of PCA3 score, PSA, PV and PSAD at a fixed sensitivity of 88.6% in men with PSA less than 50 ng/mL were 70.1% (PCA3 score 26.0), 34.3% (PSA 5.8 ng/mL), 41.3% (PV 42.0 cc) and 62.7% (PSAD 0.1913), respectively. The specificity of PCA3 score, PSA, PV and PSAD at a fixed sensitivity of 88.2% in men with PSA between 4 and 10 ng/mL were 62.3% (PCA3 score 20.0), 20.8% (PSA 4.64 ng/mL), 58.5% (PV 32.6 cc) and 47.2% (PSAD 0.159), respectively. There was no significant difference in PCA3 score among subgroups (Gleason score of 6, 7 and 8 or more). Univariable logistic regression analysis showed age ($P < 0.05$), PSA ($P < 0.0005$), PV($P < 0.05$), PSAD ($P < 0.0001$) and PCA3 score ($P < 0.0001$) were significant factors for predicting prostate cancer. Multivariable logistic regression analysis showed that PSAD ($P < 0.05$) and PCA3 score ($P < 0.0001$) were the independent predictors for prostate cancer in men with PSA less than 50 ng/mL, and that only PCA3 score ($P < 0.0005$) was an independent predictor in men with PSA between 4 and 10 ng/mL (Tables 3,4). In men with PSA less than 50 ng/mL, we created the new combination parameter

with these independent predictors. The combination of PSAD and PCA3 score was calculated using the following formula: $1 / (1 + \mathrm{Exp} [3.12 - 4.28 \times \mathrm{PSAD} - 0.027 \times \mathrm{PCA3}$ score]). AUC for the combination of PSAD and PCA3 score improved the AUC of each variable alone in men with PSA less than 50 ng/mL (Fig. 2).

Discussion

In the current study, we investigated the PCA3 urine assay as a new diagnostic marker for predicting prostate cancer in Japanese men. Using the APTIMA PCA3 assay, we found an informative rate of 100%. This rate was similar to that in multiple studies using the APTIMA PCA3 assay and higher than reports using earlier research assays.[7–14] We observed that the incidence of prostate cancer increased as the PCA3 score increased. A total of 69% of men with a PCA3 score of more than 50 had prostate cancer, whereas 5% of men with a PCA3 score of less than 20 had prostate cancer. No men with a PCA3 score less than 10 had prostate cancer. With the PCA3 cut-off of 10, 23 men would avoid the unnecessary biopsy. Thus, PCA3 score can be used to stratify the risk for a positive biopsy in men undergoing biopsy. Several studies have shown that the PCA3 assay has a high specificity for prostate cancer.[8–13] Using the APTIMA PCA3 assay, Groskopf et al. reported that the sensitivity was 69% and the specificity of PCA3 assay was 79%, with a PCA3 score cut-off of 50 in North American men.[10] Haese et al. reported that the sensitivity was 47% and the specificity was 72%, with a PCA3 score cut-off of 35 in European men.[12] Although the study population was small, we observed in Japanese men that the specificity was 85.1% and 74.6%, with a PCA3 score cut-off of 50 and 35, respectively. To our knowledge, this is the first study on assessing the diagnostic accuracy of PCA3 assay in Japanese men, and this result shows that specificity of PCA3 assay in Japan is comparable to that in the multiple reports in North America, as well as Europe, although the sensitivity varies in the different populations.

Several studies have shown that PCA3 score is superior to serum PSA and free PSA in predicting prostate cancer in men undergoing prostate biopsy.[11–13] Using ROC analysis, Marks et al. reported that there was a significant difference in AUC between PCA3 score and PSA (0.678 and 0.524, $P < 0.01$) in patients undergoing repeated biopsy.[13] Furthermore, Haese et al. found in a European study that there was a significant difference in AUC between PCA3 score and free-total PSA ratio (0.658 and 0.578, $P < 0.05$) in men undergoing repeated biopsy.[12] In the present study, there was a significant difference in AUC between PCA3 score (0.8507) and serum PSA (0.7243) in men with PSA less than 50 ng/mL. Furthermore, a remarkable difference (0.8230 and 0.5888, $P < 0.001$) was observed in men with serum PSA between 4 and 10 ng/mL. PCA3 score was supe-

Table 2 Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for positive biopsy result with different prostate cancer gene 3 score cut-off values in patients with prostate-specific antigen less than 50 ng/mL (n = 102)

PCA3 cut-off values	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DA (%)
10	35/35 (100)	23/67 (34.3)	35/79 (44.3)	23/23 (100)	58/102 (56.8)
20	33/35 (94.3)	39/67 (58.2)	33/61 (54.1)	39/41 (95.1)	72/102 (70.6)
35	26/35 (74.3)	50/67 (74.6)	26/43 (60.5)	50/59 (84.7)	76/102 (74.5)
50	23/35 (65.7)	57/67 (85.1)	23/33 (69.7)	57/69 (82.6)	80/102 (78.4)
100	10/35 (28.6)	64/67 (95.5)	10/13 (76.9)	64/89 (71.9)	74/102 (72.5)

DA, diagnostic accuracy; NPV, negative predictive value; PCA3, prostate cancer gene 3; PPV, positive predictive value.

Table 3 Univariate and multivariate logistic regression analysis to predict positive biopsy result in 102 patients with prostate-specific antigen less than 50 ng/mL

Variables	Univariate P-value	Optimal cut-off	Multivariate P-value	Odds ratio	95% CI Lower	95% CI Higher
Age	0.028*	68	0.58	1.39	0.42	4.59
PSA	0.0001*	7.3	0.52	1.61	0.36	6.89
PV	0.018*	36	0.18	2.53	0.65	10.46
PSAD	<0.0001*	0.26	0.027	4.98	1.20	22.67
Repeated biopsy	0.12					
PCA3 score	<0.0001*	26.6	<0.0001	12.38	3.71	51.38

*Variables with P-values <0.05 were entered to multivariate analysis. PCA3, prostate cancer gene 3; PSA, prostate-specific antigen; PSAD, PSA density; PV, prostate volume.

Table 4 Univariate and multivariate logistic regression analysis to predict positive biopsy result in 70 patients with prostate-specific antigen between 4 and 10 ng/mL

Variables	Univariate P-value	Optimal cut-off	Multivariate P-value	Odds ratio	95% CI Lower	95% CI Higher
Age	0.50					
PSA	0.30					
PV	0.0010*	36	0.098	6.60	0.72	151.3
PSAD	0.0077*	0.19	0.32	2.27	0.45	13.13
Repeated biopsy	0.39					
PCA3 score	0.0019*	28.5	0.0002	12.0	3.71	51.38

*Variables with P-values <0.05 were entered to multivariate analysis. PCA3, prostate cancer gene 3; PSA, prostate-specific antigen; PSAD, PSA density; PV, prostate volume.

rior to serum PSA in predicting biopsy outcome in Japanese men, especially with gray zone PSA. AUC of PCA3 score was also superior to PSAD in comparison of AUC, though the difference did not reach significance.

The PCA3 urine assay directly detects the PCA3 mRNA copy overexpressed in prostate cancer cells in urine after attentive DRE. The PCA3 score is independent of serum PSA and PV, whereas PSA is correlated with PV.[11,12,14] In the present study, we found a similar result. Nakanishi et al. showed that the PCA3 score was significantly correlated with total tumor volume in prostatectomy specimen (r = 0.269, P = 0.008). In univariable logistic regression
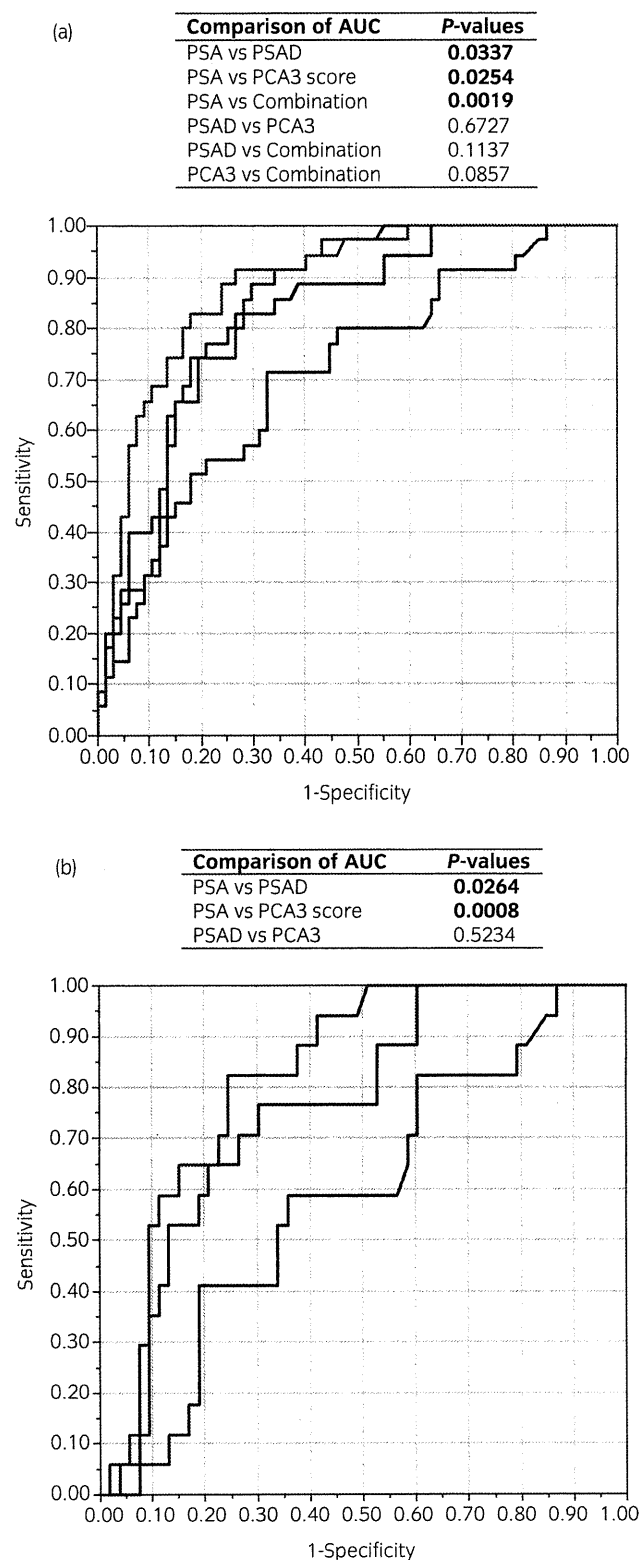
(a)

Comparison of AUC	P-values
PSA vs PSAD	**0.0337**
PSA vs PCA3 score	**0.0254**
PSA vs Combination	**0.0019**
PSAD vs PCA3	0.6727
PSAD vs Combination	0.1137
PCA3 vs Combination	0.0857

(b)

Comparison of AUC	P-values
PSA vs PSAD	**0.0264**
PSA vs PCA3 score	**0.0008**
PSAD vs PCA3	0.5234

Fig. 2 (a) Receiver–operator curve (ROC) analysis to predict positive biopsy result in patients with prostate-specific antigen (PSA) less than 50 ng/mL ($n = 102$). Probability of positive biopsy result on the combination of PSA density (PSAD) and prostate cancer gene 3 (PCA3) score was calculated by the following formula: $1 / (1 + \mathrm{Exp} [3.12 - 4.28 \times \mathrm{PSAD} - 0.027 \times \mathrm{PCA3}\ \mathrm{score}])$. *Area under ROC curve (AUC) significantly greater than that of PSA. —, PSA (AUC 0.7243); —, PSAD (AUC 0.8164*); —, PCA3 score (AUC 0.8507*); —, PSAD + PCA3 score (AUC 0.8847*). (b) ROC analysis to predict positive biopsy result in patients with PSA between 4 and 10 ng/mL ($n = 70$). *AUC significantly greater than that of PSA. —, PSA (AUC 0.5888); —, PSAD (AUC 0.7603*); —, PCA3 score (AUC 0.8230*).

tate biopsy. Deras *et al.* have developed prediction models based on logistic regression.[11] When the PCA3 score and PV are added to the model of PSA for predicting prostate cancer, the AUC improved from 0.55 to 0.75. In the present study, we also found that the combination model of PSAD and PCA3 score improved the AUC of PSA from 0.7243 to 0.8847. These results show that the PCA3 score might help better select the patients who might benefit from prostate biopsy in men with elevated PSA.

There was no significant difference in the PCA3 score among subgroups based on the Gleason score of biopsy specimens in the present study. Several reports showed that there was no significant difference in PCA3 score between men with Gleason 6 and 7 in biopsy specimens, whereas there was a significant difference in prostatectomy specimens.[11,13,14] Furthermore, Nakanishi *et al.* showed that a PCA3 score threshold of 25 discriminated significant cancer from low volume/low grade cancer (tumor volume less than 0.5 cc and Gleason 6; $P = 0.007$) in men already diagnosed with prostate cancer, suggesting that PCA3 testing might provide information about treatment decisions.[15]

A limitation of the present study is the relatively small sample size investigated in a single institution. The number of men undergoing repeated biopsy was just 20. We did not find a difference in patients undergoing repeated biopsy, as a consequence of small numbers. Additional studies are ongoing to evaluate the diagnostic accuracy of PCA3 urine assay in Japanese men undergoing repeated biopsy at multi-institutions. We excluded three men with PSA more than 50 ng/mL and prostate cancer in comparison of diagnostic performance of PCA3 score and other variables. A PCA3 urine test would ordinarily be irrelevant in men with extremely high PSA.

In conclusion, PCA3 score was associated with the probability of a positive biopsy. Specificity of PCA3 score for positive biopsy was excellent. PCA3 score and PSAD were the independent predictors for prostate cancer. PCA3 score could improve the prediction for prostate cancer and help better select men who might benefit from prostate biopsy.

analysis, we found that the PCA3 score was significantly associated with biopsy result. We also found in multivariable logistic regression analysis that the PCA3 score ($P < 0.0001$) and PSAD ($P < 0.05$) were independent variables for predicting prostate cancer in men undergoing pros-

Acknowledgments

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Combined Immunotherapy with Low-dose IL-2 Plus IFN-α for Metastatic Renal Cell Carcinoma: Survival Benefit for Selected Patients with Lung Metastasis and Serum Sodium Level

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Objective: To clarify the survival benefit of immunotherapy for renal cell carcinoma patients with lung metastasis using low-dose interleukin-2 plus interferon-α, we examined survival outcomes and factors associated with prognosis.

Methods: This was a multicenter prospective study. Nephrectomized renal cell carcinoma patients with lung metastasis were treated with interleukin-2 (0.7×10^6 unit, 5 days a week) and interferon-α (6×10^6 IU, 3 days a week) for the first 8 weeks, and then with both interleukin-2 and interferon-α, 2 or 3 days a week for 16 additional weeks.

Results: Median follow-up period for 42 patients was 28.3 months (range: 4.2–43.8). Two-year overall survival rate was 82% and the probability of 3 year survival rate was 71%. Median progression-free survival was 10.4 months. While no difference was found in survival among patients assessed as complete response, partial response and no change, survival of patients assessed as NC or better was significantly better than those assessed as progressive disease ($P < 0.0001$). Furthermore, multivariate analyses identified pre-treatment serum sodium ($P = 0.004$) as an independent prognostic factor. The sodium level was also statistically associated with tumor response ($p = 0.035$). Patients with normal sodium level survived significantly longer ($P = 0.0005$) than those with low sodium level showing median survival of 12.2 months.

Conclusions: Combination immunotherapy with low-dose interleukin-2 plus interferon-α showed survival benefit for patients with lung metastasis whose tumor responded as no change or better. This combination immunotherapy could be beneficial for patients selected by metastatic organ and their pre-treatment serum sodium level.

Key words: renal cell carcinoma − interleukin-2 − interferon-α − lung metastasis − sodium

INTRODUCTION

The prognosis for patients with advanced renal cell carcinoma (RCC) is poor. It is reported that the median survival for patients with advanced RCC is 10 months and 5-year survival rate is <15% (1). RCC is highly resistant to conventional cytotoxic chemotherapy, while RCC evokes an immune response, which occasionally results in spontaneous remission (2,3). Such observations provide the rationale for developing immunotherapeutic approaches to treatment and have led us to a clinical investigation of immunostimulatory cytokines, such as interleukin-2 (IL-2) and interferon-α (IFN-α). Positive response rates of 10–20% are reported with these cytokines and some patients achieve a complete and long-lasting remission (4–6).

Among the effective immunotherapy options, administering a high-dose bolus i.v. IL-2, IFN-α and low/intermediate dose of IL-2 plus IFN-α have shown some evidence of anti-tumor activity, but no impact on overall survival (7). A number of uncontrolled studies, however, have shown that low doses of IL-2 plus IFN-α are associated with less toxicity and capable of inducing partial and complete remission with a comparable effect on median survival (8–10). Naito et al. (11) have recently reported a large retrospective study of 1463 Japanese patients that cytokine-based therapy, including IL-2 and IFN-α, improved the prognosis of advanced RCC patients.

Many studies have suggested that the great benefits of the cytokines can be achieved when applied to appropriately selected patients (12,13). Improvements in patient selection will be necessary to ensure that patients who might attain durable remission with IL-2 will not miss this opportunity. The important issue is how these individuals can be selected more accurately. A prognostic model by the Memorial Sloan Kettering Cancer Center (MSKCC) (14) is the most extensively used guide for optimal treatment. In terms of histological characteristics, it has been reported that patients with RCC of clear cell histology respond well to cytokine therapy (15). Although many efforts have been undertaken to clarify clinical or molecular factors associated with response to cytokines, the potential remains largely untapped.

Recently, novel molecular-targeted agents have been developed for the treatment of metastatic RCC (16). These include tyrosine kinase inhibitors, such as sorafenib and sunitinib as well as mammalian target of rapamycin inhibitors. These agents have been designed to target tumor-related angiogenesis and signal transduction. Although we now have an increasing number of effective new agents for patients, extensive experience has shown that they rarely induce durable regressions of metastatic RCC (17,18).

Our previous pilot study has shown that combination treatment with low-dose IL-2 (0.7×10^6unit/person) plus IFN-α is effective for metastatic RCC patients, especially those with metastasis limited to lung (19). In addition, the combination therapy was tolerated well and no additional adverse event was observed in comparison with the monotherapy using either low-dose IL-2 or IFN-α. Thus, in order to confirm the efficacy of the treatment and to explore genetic markers that may be useful in patient selection, we have tried a new prospective and multicenter trial of the combination therapy on patients who had radical nephrectomy, lung metastasis and no previous systemic therapy. The efficacy for tumor responses has already been described in our recent report (20); briefly, the efficacy for patients with metastasis limited to lung has been reproduced with similar response rate of 35.5% and the disease control rate of 80.6%. A separate paper reports that expression levels of HLA-DQA1 and HLA-DQB1 are candidate markers for predicting the tumor response to this combination therapy using oligoDNA microarray analysis after enrichment of the cancer cells with laser microbeam microdissection technology (21).

In this paper, we report survival outcomes of this study and examined factors associated with the prognosis of patients receiving the combination therapy with low-dose IL-2 plus IFN-α. We show that the combination therapy produced superior survival outcomes with a 2-year overall survival rate of 82%. Furthermore, better survival was shown to be significantly associated with tumor responses including NC (no change) and with normal baseline serum sodium level, indicating that the combination immunotherapy will be beneficial to patients selected by their pre-treatment serum sodium in addition to their metastatic organ limited mainly to lung.

PATIENTS AND METHODS

PATIENTS AND TREATMENT

Study design and patient inclusion criteria have been previously described (20). Briefly, this was a prospective, multicenter and open-label trial for Japanese patients with metastatic RCC, who had received radical nephrectomy, measurable lung metastasis, the possibility of providing blood and specimens from primary tumors to determine genetic markers, and who had received no previous systemic treatment. Patients were enrolled from September 2006 to April 2008. The study was approved by the institutional review board at each center.

Administration of IL-2 (Imunace, Shionogi, Osaka, Japan) and IFN-α (Sumiferon, Dainippon Sumitomo, Osaka, Japan) was commenced simultaneously and continued for 8 weeks at following doses: IL-2 administrated by intravenous infusion at 0.7×10^6unit/person per day, 5 days a week and IFN-α subcutaneously or intramuscularly at dose 6×10^6 IU, 3 days a week. From week 9 to week 24, IL-2 and IFN-α were administered 2 or 3 days a week to patients showing evidence of objective response or NC. When this 24-week treatment was completed, progressive disease was detected, or this regimen could not be continued because of severe side effects, subsequent therapy was determined on each case by each center. The patients who were assessed as PD could continue to receive treatment with IL-2 and/or IFN-α

(continuous cytokine therapy) when centers determined it to be beneficial to them, because continuation of cytokine treatment despite progression of disease was reported to add a survival benefit to patients (11) and alternative agents (molecular target drugs) other than cytokines had not been approved in Japan by April 2008. Before their official approval, however, target drugs became available for clinical trials during the present study and were given to some patients who experienced relapse.

OUTCOME VARIABLES

The efficacy of tumor response has reported in our recent paper (20). Tumor response was assessed by up to 24 weeks plus an additional 4-week follow-up after commencement of the treatment according to the criteria of the Japanese Urological Association (JUA) (22) which is similar to the WHO criteria (23). We used JUA criteria instead of RECIST in order to compare the efficacy with our previous pilot study (19). Response evaluation was reviewed by external independent radiologists following investigators' assessment and further confirmed by central assessment. Progression-free survival (PFS) was defined as the time from the date of registration to disease progression or death, whichever occurred first. Overall survival was defined as the time from registration until death from any cause. Baseline serum sodium was determined in each center and low sodium level was determined based on the criterion of each center.

STATISTICAL ANALYSIS

For time-to-event endpoints, medians and 95% confidence intervals (CI) were estimated using the Kaplan–Meier method and the differences were assessed using log rank test. Uni- and multivariate survival analyses were based on the Cox proportional hazards regression model. Univariate parameters with $P < 0.05$ were used in the multivariate analyses using the backward selection.

RESULTS

PATIENT CHARACTERISTICS

From September 2006 to April 2008, a total of 44 Japanese patients were enrolled in this study and treated with low-dose IL-2 plus IFN-α therapy as a first-line setting. One patient was excluded due to violation of inclusion criteria and one discontinued treatment in the first week by withdrawal of consent. The baseline characteristics of 42 patients, which have been previously described in part (20), are shown in Table 1. All patients had undergone radical nephrectomy and had lung metastasis. Thirty-one patients (73.8%) had metastasis limited to lung. Others (11 patients) had multiple organ metastases, including lymph node, bone, liver, pancreas, adrenal gland and/or cardiac membranes in addition to lung. The number of measurable metastatic

Table 1. Patient characteristics

	n	%
Gender		
Male	32	76.2
Female	10	23.8
Age		
Less than 65	28	66.7
65 or greater	14	33.3
ECOG PS		
0	33	78.6
1	9	21.4
Nephrectomy		
Yes	42	100
No	0	0
Pathological T stage		
pT1	9	21.4
pT2	9	21.4
pT3	23	54.8
pT4	1	2.4
Histology		
Clear cell	38	90.5
Papillary	1	2.4
Mixed	3	7.1
Metastatic organ		
Lung	42	100
Lymph node	7	16.7
Bone	5	11.9
Others	7	16.7
Number of metastatic organ		
Single (lung only)	31	73.8
Multiple	11	26.2
Number of metastatic lesion		
1	3	7.1
2	9	21.4
3–5	16	38.1
6–10	12	28.6
17–26	2	4.8
MSKCC risk group		
Favorable	1	2.4
Intermediate	29	69
Poor	12	28.6

ECOG, Eastern Cooperative Oncology Group; Others, included liver, pancreas and cardiac membrane; MSKCC, Memorial Sloan Kettering Cancer Center.

lesions in each patient varied from 1 to 26 with a median number of 4. Among patients with only lung metastasis, the number of lesions varied from 1 to 16 with a median
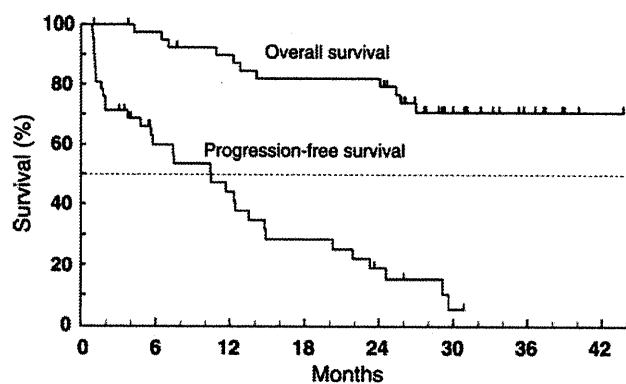
Figure 1. Kaplan–Meier estimates for progression-free survival (PFS) and overall survival (OS) for patients receiving first-line IL-2 plus IFN-α. Median PFS was 10.4 months. OS has not been reached to median during observation period (median: 28.3 months, range: 4.2–43.8).

number of 3. Thirty-eight (90.5%) of 42 patients had pure clear cell carcinoma, 1 papillary and others (3 patients) had mixed cell type with clear cell carcinoma. Based on MSKCC prognostic criteria (14), patients were categorized mostly in the intermediate (69.0%) and poor (28.6%) risk groups with only one patient categorized as favorable group (2.4%). To utilize the primary tumor specimens for marker analysis, the present study had mainly enrolled patients (92.9%: 39/42) who had metastasis at nephrectomy, which is one of the risk factors in the MSKCC criteria.

OVERALL SURVIVAL AND PFS

Median follow-up period for 42 patients was 28.3 months (range: 4.2–43.8). The overall survival had not reached the median by June 2010. In the first 12 months and the next 12 months after the registration, 3 and 4 deaths had occurred, during these respective periods. The 1- and 2-year overall survival rates were 89.9% (95% CI: 75.4–96.1) and 82.0% (66–91%), respectively. Figure 1 shows the overall survival curve estimated by the Kaplan–Meier method. The probability of 3-year survival rate was estimated to be 70.9% (54–83%). The patients (n = 7) who died in 2 years had either multiple organ metastases (n = 4) or poor risks (n = 5) by MSKCC criteria (14), although 7 of 12 poor risk patients have survived for over 2 years (data not shown).

The median PFS was 10.4 months (5.6–14.8) (Fig. 1). While one of the two patients assessed as complete response (CR) has relapsed after a follow-up period of 13 months but surviving over 32.2 months, another patient remained with no evidence of disease for over 25 months by continued therapy with IL-2 plus IFN-α. One patient with papillary type RCC (type not classified) in the lung, who had responded to the combination therapy (assessed as PR), was progression free for 10 months and survived for over 29 months.

Survival was compared between patient groups with only lung metastasis (n = 31) and with extrapulmonary organs (n = 11). The difference was not statistically significant, but patients with only lung metastasis tended to survive longer
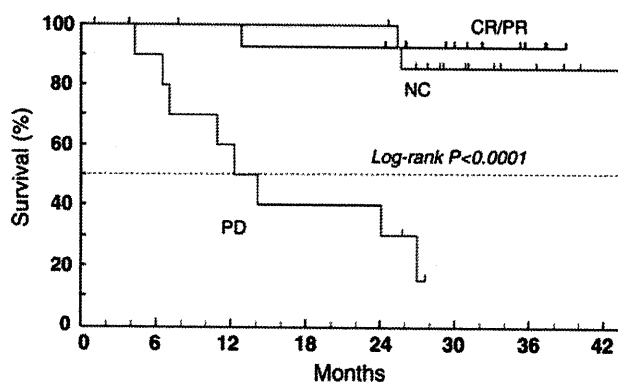

Figure 2. Cause-specific survival and tumor response of patients treated with IL-2 plus IFN-α. The tumor response was assessed by up to 24 weeks plus additional 4-week follow-up after the first dose (20). There was no difference between survival of patients assessed as complete response (CR)/partial response (PR) or no change (NC), while for those assessed as PD it was significantly different ($P < 0.0001$). For the PD subpopulation, the median survival time was 13.2 months, while the survival for CR/PR or NC has not been reached to median during observation period (median: 28.3 months, range: 4.2–43.8).

than those with extrapulmonary metastasis (log-rank $P = 0.0745$, data not shown). The 2-year survival rates of patients with only lung metastasis and with extrapulmonary metastases were 89.7% (71.3–96.5) and 61.4% (26.6–83.5), respectively.

RELATIONSHIP BETWEEN TUMOR RESPONSE AND CAUSE-SPECIFIC SURVIVAL

In our subgroup analysis, a strong correlation was found between diagnosis of tumor response (20) (the response assessed by 24 weeks after the first dose) and cause-specific survival (Fig. 2). In the patient group achieving CR or PR (n = 15), only one death occurred in 24 months with a 2-year survival rate of 92.9% (59.1–99.0) and no death occurred in patients assessed as NC (n = 16) in 24 months. Thus, the 2-year survival rate was 96.6% (77.9–99.5) for patients achieving objective response or NC. A patient diagnosed as PR who had died after 12 months had baseline characteristics, including multiple organ metastases (lung plus mediastinal lymph node), 16 lung metastatic lesions and poor risk factors (<1 year from initial visit to metastasis, >10 mg/dl high corrected calcium and low hemoglobin) by MSKCC prognostic criteria.

In contrast, 6 deaths had occurred in patients diagnosed as PD (n = 11) in 24 months with a 2-year survival rate of 40.0% (12.3–67.0). The median survival time was 13.2 months (7.0–27.0) for the PD subpopulation. All of the 6 patients have been assessed as PD by 8 weeks from the first dose with a median of 4 weeks.

PROGNOSTIC AND PREDICTIVE FACTORS

To identify clinical factors predicting prognosis in patients who received the combined IL-2 plus IFN-α therapy,

Table 2. Univariate and multivariate analyses of baseline parameters for overall survival of patients receiving IL-2 plus IFN-α

Risk factors	Categories	Univariate analyses			Multivariate analyses		
		Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
Sodium	Low vs. N[a]	6.48	1.94–21.6	0.002	16.1	2.45–105	0.004**
Lymphocyte	Low vs. N[a]	7.91	2.04–30.8	0.003	14.7	2.25–96.6	0.005**
Corrected Ca	>10 mg/dl	5.51	1.56–19.4	0.008	13.2	1.83–94.2	0.010**
Albumin	Low vs. N[a]	4.72	1.02–21.8	0.047	1.94	0.28–13.4	0.500
CRP	>0.3 mg/dl	5.35	1.15–24.9	0.032	1.04	0.14–7.57	0.966

[a]N, normal.
**$P < 0.05$ on multivariate analysis.

Table 3. Correlation between pre-treatment serum sodium and tumor response to IL-2 plus IFN-α

	Sodium level, *n* (%)	
	Normal	Low
n	34	8
Tumor response		
CR/PR	15 (44.1)	0 (0)
NC	13 (38.2)	3 (37.5)
PD	6 (17.6)	5 (62.5)
Clinical benefit		
CR/PR/NC	28 (82.4)	3 (37.5)
p-value*		
CR/PR vs. NC/PD	0.035	
CR/PR/NC vs. PD	0.020	

The tumor response was assessed by up to 24 weeks plus additional 4-week follow-up after the first dose (20). Response evaluation was reviewed by external independent radiologists following investigators' assessment, and further confirmed by central assessment.
p-value: Fisher's precision test.

univariate and multivariate analyses using the Cox proportional hazard regression model were performed on baseline parameters, including pathological, blood and urinary tests. Survival was significantly associated with corrected calcium, CRP, serum albumin, sodium and lymphocyte count on univariate analyses (Table 2). Multivariate analyses showed that baseline serum sodium ($P = 0.004$), lymphocyte count ($P = 0.005$) and corrected calcium ($P = 0.010$) were independent risk factors for shorter survival, although a small number of patients in the present study seemed to exclude some potential factors. Serum sodium level was also found to be associated with tumor response to this therapy (Table 3; responder (CR/PR) vs. non-responder: $P = 0.035$). Furthermore, more strong correlation ($P = 0.020$) was found between patients with clinical benefit (CR/PR/NC) and without benefit (PD). Using the Kaplan–Meier estimate and log-rank test, serum sodium
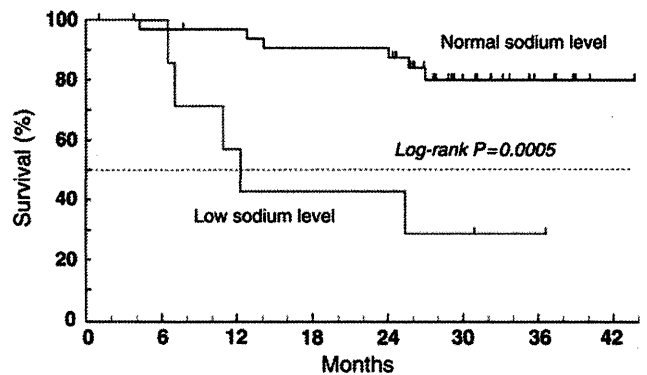

Figure 3. Survival and baseline serum sodium level of patients treated with IL-2 plus IFN-α. Survival was significantly different between patients with normal and low sodium levels ($P = 0.0005$). The median survival time of patients with low sodium level was 12.2 months, while the survival for patients with normal sodium level has not been reached to median during observation period (median: 28.3 months, range: 4.2–43.8).

levels were also shown to be statistically significant predictor of survival time ($P = 0.0005$, Fig. 3). The 2-year survival rates for patients with normal sodium and low level of sodium were 90.7% (73.9–96.9) and 42.9% (9.8–73.4), respectively. The median survival time of patients with low sodium level was 12.2 months.

In MSKCC risk factors (14), corrected calcium was shown to be the only factor associated with survival on multivariate analyses. Prognostic groups by MSKCC criteria were also found to have a correlation with survival. Because only one patient was categorized in a favorable group, survival for intermediate ($n = 29$) plus favorable group was compared with that of the poor group ($n = 12$), and the difference was statistically significant ($P = 0.036$, data not shown). The 2-year survival rates for the favorable/intermediate and poor groups were 92.9% (74.3–98.2) and 58.3% (27.0–80.1), respectively. The median survival of the poor group was 25.4 months.

DISCUSSION

Our previous pilot study has shown that combination therapy with low-dose IL-2 plus IFN-α is effective for metastatic

RCC patients, particularly those with metastasis limited to lung (19). The present trial has confirmed the efficacy of tumor response for patients with lung metastasis (20) and the present study further showed that this regimen provides a good survival benefit. The treatment was well tolerated and no additional adverse events occurred to those observed with monotherapy using either low-dose IL-2 or IFN-α (20). The overall survival did not reach the median in the median follow-up of 28.3 months (range: 4.2–43.8). The median PFS was 10.4 months with 1- and 2-year survival rate of 89.9 and 82.2%, and the probability of 3-year survival rate of 70.9%. While the data from the USA showed that the 1- and 3-year survival rates were 54 and 19%, respectively, in 463 metastatic RCC patients who received IFN-α (14), a large retrospective study on Japanese patients (11), 82% of whom had received cytokine therapy, including IFN-α and/or IL-2, showed 64.2 and 35.2% of 1- and 3-year survival rates, respectively. The 1- and 3-year survival rates of the present study are similar to or even better than those (86 and 46%, respectively) of favorable risk subpopulation in a randomized trial of IFN-α with/without IL-2 and fluorouracil (24).

It is noted that patients enrolled in this study were categorized mostly in intermediate (69.0%) and poor (28.6%) risk groups with only one patient categorized as favorable in the MSKCC prognostic model. To utilize the primary tumor specimens for gene marker analysis, the present study had mainly enrolled patients who had metastasis at nephrectomy, which is one of the risk factors in the MSKCC criteria. Despite the small proportion of favorable patients, on the whole, the survival outcomes were superior.

One reason for the better outcomes in the present study can be attributed to our patient selection by the criteria that included prior radical nephrectomy, ECOG performance status of 0–1 and limited metastasis mainly to lung. Upfront nephrectomy has been shown to enhance survival time for immunotherapy of metastatic RCC patients (25). In fact, nephrectomy improved the median survival period from 10.3 to 14.3 months in patients with only lung metastasis (26). In addition, racial differences may affect the survival of metastatic RCC patients as reported in one study (27).

The baseline serum sodium was found to have a significant positive correlation with tumor response and survival. Most recently, Jeppesen et al. (28) have reported that the level of baseline serum sodium is one of the prognostic and predictive factors in metastatic RCC patients who have been treated with IL-2-based therapy with/without IFN-α. In their work, low serum sodium has been shown to be a prognostic factor for short survival and a predictive factor for a lack of response to the immunotherapy. In the present study, the responders were found only in patients with normal sodium levels. The survival was significantly longer in patients with normal sodium than those with low sodium ($P = 0.0005$). Thus, our observations in the present study were consistent both with prognostic and predictive values of the serum sodium. These results imply that the tumor response and

survival can be further improved by patient selection with baseline serum sodium levels in addition to the pathological criteria, including limited metastasis to lung.

Furthermore, the present study showed that tumor responses were closely associated with survival. The survival of patients assessed as NC was not different from those as CR or PR, while survival for patients assessed as PD was significantly shorter than those assessed as the objective response or NC ($P < 0.0001$). Since similar observations have been shown in our previous pilot study of IL-2 plus IFN-α combination therapy (19), our two independent prospective trials demonstrated that patients showing objective responses or NC can anticipate a survival benefit from this combination therapy. This finding is in agreement with previous reports on IL-2-based immunotherapy (29,30). In the present study, patients who died within 2 years had been diagnosed as PD by 8 weeks from the first dose. Thus, it might be possible to consider that the patients who are assessed as not PD in the first 2 months could continue the combination therapy and could benefit from the treatment.

It is of interest to mention that IFN-α has recently been shown to play a role in the dynamic balance between activated regulatory and effector T cells (31,32). Pace et al. (31) have reported that IFN-α inhibits IL-2-induced regulatory T cell (Treg) proliferation and function through antigen-presenting cell activation. IL-2 plays important roles in tumor immunity by enhancing dendritic cell function, and T cell and NK cell effector activities, while IL-2 also delivers essential signals for the activation of Treg, which suppresses the functions of effector T cells in their homeostasis (33). Therefore, the combination of IL-2 with IFN-α may enhance antitumor activity through suppression of Treg with the aid of IFN-α as suggested by Tatsugami et al. (34).

Administration of targeted agents has become a routine practice for treatment of patients with metastatic RCC. However, none of the novel targeted agents seem to be curative. Furthermore, both randomized and expanded-access trials on sunitinib and sorafenib have shown that PFS and overall survival of both agents have been reported not to be significantly different between treatment-naïve and cytokine-refractory patients (17,18,35–38), indicating that the agents are as effective for patients who are refractory to cytokines. From above, it is thought to be possible to improve the survival benefit for metastatic RCC patients, if the combination therapy with IL-2 plus IFN-α is chosen as the first-line treatment, seeing it has better outcomes, even to the extent that complete remission can be expected. In the case of a patient who is refractory to this treatment, an alternative treatment with targeted agents can commence without delay and provide additional benefits.

A more accurate patient selection would ensure that the benefits they receive from the treatment are maximized. Our separate paper reports that expression levels of HLA-DQA1 and HLA-DQB1, the genes known to form heterodimers in antigen presentation process, are candidate markers for predicting the tumor response to the combination therapy with

IL-2 plus IFN-α (21). Exclusion of patients with tumors lacking either expression of these two genes is likely to improve the response rate to IL-2 plus IFN-α from 36 to 67%, indicating that a pretreatment genetic test would provide useful information in narrowing down the patients in order to improve the efficacy of this treatment and reduce unnecessary medical costs. Thus, by extending the patient selection criteria to metastatic organs, baseline sodium levels and a genetic test, the efficacy of the treatment can improve further.

Although the present study is a non-randomized prospective study, including a relatively small number of patients with a short follow-up period, the results showed that the combination therapy with low-dose IL-2 plus IFN-α provides survival benefits for selected patients who had limited metastases mainly to lung. Furthermore, the present study suggests that if patients are selected by their baseline serum sodium levels, combined immunotherapy would be a great benefit for them.

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