ELSEVIER

RUNNING SUTURE FOR VESICOURETHRAL ANASTOMOSIS IN MINILAPAROTOMY RADICAL RETROPUBIC PROSTATECTOMY

TSUNEHARU MIKI, KOJI OKIHARA, OSAMU UKIMURA, SOH USIJIMA, KIMIHIKO YONEDA, YOICHI MIZUTANI, AKIHIRO KAWAUCHI, MINORU KOGA, AND MASAMI TAKEYAMA

ABSTRACT

Introduction. We used a running suture method for vesicourethral anastomosis in patients undergoing minilaparotomy radical retropubic prostatectomy.

Technical Considerations. The vesicourethral anastomosis using a single knot at the 6:30-o'clock position is created with two steps of semicircular running suture. A total of 21 consecutive patients underwent this running suture method using the Endostitch in the hands of a single surgeon (T.M.) between March and November 2004. The running suture procedure was completed in 15 minutes on average. After surgery, no urinary leakage at the anastomotic site was found. Satisfactory continence was achieved in the short term in 100% (0 to 1 pad per day) of cases. However, dilation at the anastomosis using a metal dilator was required in 2 patients immediately after surgery.

Conclusions. The running suture method is considered a feasible alternative in minilaparotomy radical retropubic prostatectomy. UROLOGY **67:** 410–412, 2006. © 2006 Elsevier Inc.

Minilaparotomy radical prostatectomy is reportedly a favorable procedure compared with standard radical prostatectomy in terms of reducing incisional pain and hastening recovery.[1] From March 2004, we have used a 6-cm lower abdominal midline incision for minilaparotomy radical prostatectomy. A minimal incision sometimes makes the anastomosis suture difficult, because the needle driver cannot be handled effectively with the small operative exposure. To manage the anastomosis suture successfully, we have applied a running suture method using the Endostitch and two braided absorbable sutures.

SURGICAL TECHNIQUE

After minilaparotomic removal of the prostate has been accomplished, the bladder neck is everted to ensure a mucosa-to-mucosa anastomosis. Before starting the running suture, two 2-0 braided absorbable sutures (Polysorb 2-0, 120) are tied together at their tail ends (Fig. 1A, upper). The vesicourethral anastomosis is created with two steps of semicircular running suture (a clockwise suture from the 6:30-o'clock to 12-o'clock positions and an anticlockwise suture from the 5:30-o'clock to 12-o'clock positions).

The first needle is passed from outside to inside the urethra at the 6:30-o'clock position (Fig. 1A, lower) using a needle holder (Endostitch) and then from inside the bladder to the outside (Fig. 1B, upper). After passing at the 6:30-o'clock position between the urethra and bladder, a knot is made at the 6:30-o'clock position on the urethra (Fig. 1B, lower).

The needle is repeatedly passed from the urethra (outside to inside) to the bladder neck (inside to outside) in a clockwise and semicircumferential manner to the 12-o'clock position (Fig. 1C, upper).

The surgeon then changes to a second needle and manipulates the same Endostitch device. The needle is passed in the same way but rotating counterclockwise (Fig. 1C, lower). Before reaching the 1-o'clock position, a 20F Foley catheter is placed into the bladder. The balloon on the catheter is filled with 20 mL water. When the running stitch is achieved, gentle traction is exerted on the Foley catheter to bring the bladder in contact with the urethra. Then the operator pulls up each thread gently to attach the urethra

From the Department of Urology, Kyoto Prefectural University of Medicine, Kyoto, Japan; and Department of Urology, Kenporen Osaka Central Hospital, Osaka, Japan

Reprint requests: Koji Okihara, M.D., Ph.D., Department of Urology, Kyoto Prefectural University of Medicine, Kawaramachi Hirokoji, Kyoto 602-8566, Japan. E-mail: okiharakoji@hotmail.com

Submitted: February 11, 2005, accepted (with revisions): August 1, 2005

0090-4295/06/$32.00
doi:10.1016/j.urology.2005.08.003

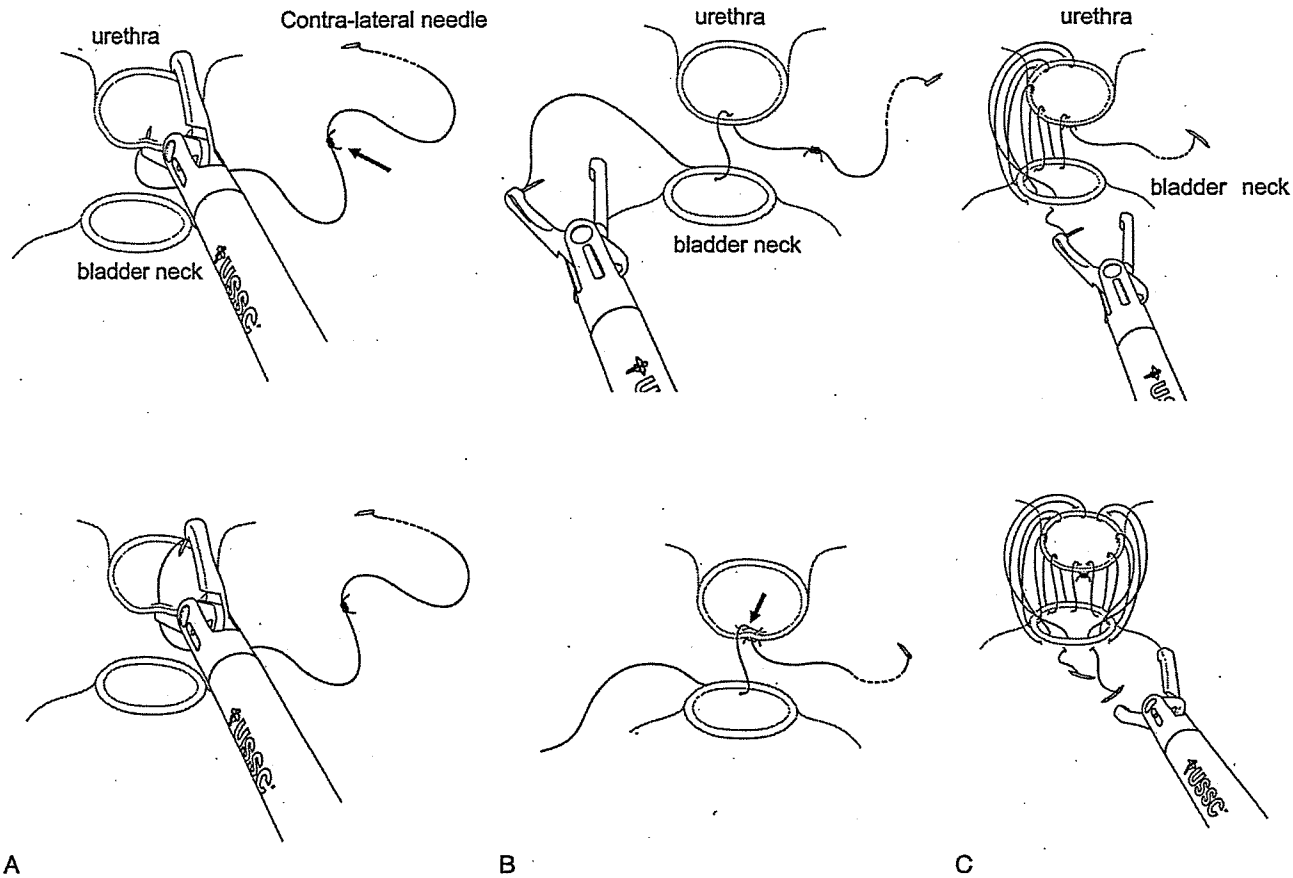


FIGURE 1. *(A) Two 2-0 absorbable monofilaments (Polysorb 2-0, 120) were sutured at the edge of each filament (arrow). We routinely do not leave any stitches in the urethra before excision of prostate (upper). First needle passed from outside to inside of urethra at 6-o'clock position (lower). (B) Needle passed from inside bladder to outside at 6-o'clock position (upper). After passing at 6-o'clock position between urethra and bladder, knot tied at 6-o'clock position on urethra (lower). (C) Needle repeatedly passed from urethra (outside to inside) to bladder neck (inside to outside) in clockwise and semicircumferential manner to 12-o'clock position (upper). Needle passed in same way with anticlockwise rotation (lower).*

and bladder neck mucosa to mucosa. Next, a single suture is made at the 12-o'clock position using knotting forceps. Finally, the bladder is filled with 150 mL saline to test the integrity of the anastomosis.

RESULTS

In the 21 cases in this series, it took between 12 and 20 minutes (average 15) to complete the running suture. Before catheter removal 5 days after surgery, contrast cystography revealed no obvious leakage in any of the patients. The urethral catheter was removed 6 to 7 days after surgery. Without exception, no urinary leakage occurred at the anastomotic site. In the outpatient referral unit, the operating surgeon interviewed the patients regarding the number of pads used per day. During follow-up of at least 3 months, satisfactory continence, defined as 0 (n = 19) to 1 pad (n = 2), was achieved in all patients in this study. Only the initial 2 patients complained of mild urinary dysuria immediately after removal of the catheter. Anastomosis stricture was diagnosed by retrograde urethrography in these 2 patients. Dilation at the anastomosis using a metal dilator was performed successfully in both patients without endoscopic dilation.

COMMENT

Before using the running suture vesicourethral anastomosis, we performed standard minilaparotomy radical retropubic prostatectomy with a 7-cm midline incision using a retractor with specially designed blades. A technique using a special retractor to achieve good exposure was also reported by LaFontaine *et al.*[1] They applied five 2-0 absorbable interrupted sutures for the anastomosis. In their report, four initial sutures were placed in each quadrant of the urethra and one suture was placed at the 6-o'clock position before complete division of the urethra. Leaving the stitches in the urethra made the anastomosis easy, specifically in the minilaparotomic procedure. However, in this method, stitches sometimes become entangled in the urethra immediately before the anastomosis.

TABLE I. *Current reports of running suture for urethrovesical anastomosis in radical prostatectomy*

Reference	RP	Patients (n)	Method for RS	Time Required for RS*(min)	Continence Rate (%)	Urinary Leakage (%)
Hoznek *et al.*,[2] 2000	LRP	30	First RS from 3-o'clock to 9-o'clock position Second RS from 2-o'clock position	23–39 (31)	84 (6 mo)	10
Abbou *et al.*,[4] 2000	LRP	33	Two-step semicircumferential RS	NA	84 (1 mo)	0
van Velthoven *et al.*,[3] 2003	LRP	122	Single knot at 12-o'clock position	14–48 (35)	NA	0
Lee *et al.*,[5] 2004	Ro-RP	100	Single knot at 12-o'clock position (same as Ref. 3)	NA	NA	NA
Menon *et al.*,[6] 2004	Ro-RP	120	First RS from 4-o'clock to 12-o'clock position, two-step RS	5–34 (13)	96 (3 mo)	20

Key: RP = radical prostatectomy; RS = running suture; LRP = laparoscopic RP; Ro-RP = robotic-assisted RP; NA = not available.
** Mean in parentheses.*

To avoid having to disentangle stitches, we routinely leave no stitches in the urethra before excision of the prostate.

LaFontaine *et al.*[1] also reported that in minilaparotomy radical prostatectomy the urethral catheter (18F Foley) was left in place for at least 2 weeks. Similar to their procedure, we started minilaparotomy radical prostatectomy using an interrupted suture for vesicourethral anastomosis in 2001. In our initial series, we left the urethral catheter in place for 10 to 14 days, following their experience. Thereafter, we decided to reduce the time the catheter was indwelling to 6 to 7 days.

To reduce the midline incision by 1 cm inevitably causes difficulty in handling the needle driver if no stitches are to be left in the urethra. To solve this problem, we have applied a laparoscopic instrument for open surgery. The advantage of using the Endostitch is that inserting a hand at the anastomotic site is not required. Moreover, the time needed is significantly shorter with a running suture than with an interrupted suture. The evidence can be found in the Results section. Some clinical investigators have reported a running suture method for laparoscopic radical prostatectomy and robotic-assisted laparoscopic radical prostatectomy[2–6] (Table I). Our technique for the running suture is almost the same as that reported by van Velthoven *et al.*[3] They applied a single-knot method (two semicircles blocked by a knot at the 6-o'clock position and tightened by a single knot at the 12-o'clock position). The difference between their study and ours is the location of the primary knot at the 6-o'clock position (on the urethral side in our series). Compared with other series using laparoscopic surgery, the time required for the running suture in our series is almost similar. In addition, the continence rate in our study was 90% (19 of 21) 3 months after surgery. These results are also similar to those in other reports.

Two of our patients experienced anastomotic stenosis. In laparoscopic radical prostatectomy, Hoznek *et al.*[2] demonstrated that stenosis does not occur at the urethrovesical anastomosis created with running sutures, because the Foley catheter prevents any narrowing of the anastomosis circumference. The reason for the anastomotic stenosis in our series may have been because the suture line was tighter than it is in laparoscopic surgery. We suggest that paying attention to the degree of tightness at the suture line may help avoid anastomotic stenosis in minilaparotomy radical prostatectomy. To assess the incidence of anastomotic stenosis in the running suture method accurately, it will be necessary to have experience with a larger number of cases.

REFERENCES

1. LaFontaine P, Chan D, Partin AW, *et al:* Minilaparotomy radical retropubic prostatectomy: updated technique and results. Semin Urol Oncol 18: 19–27, 2000.
2. Hoznek A, Salomon L, Rabil R, *et al:* Vesicourethral anastomosis during laparoscopic radical prostatectomy: the running suture method. J Endourol 14: 749–753, 2000.
3. van Velthoven RF, Ahlering TE, Peltier A, *et al:* Technique for laparoscopic running urethrovesical anastomosis: the single knot method. Urology 61: 699–702, 2003.
4. Abbou CC, Salomon L, Hoznek A, *et al:* Laparoscopic radical prostatectomy: preliminary results. Urology 55: 630–634, 2000.
5. Lee DI, Eichel L, Skarecky DW, *et al:* Robotic laparoscopic radical prostatectomy with a single assistant. Urology 63: 1172–1175, 2004.
6. Menon M, Hemal AK, Tewari A, *et al:* The technique of apical dissection of the prostate and urethrovesical anastomosis in robotic radical prostatectomy. BJU Int 93: 715–719, 2004.

ELSEVIER

COMPLEXED PSA IMPROVES PROSTATE CANCER DETECTION: RESULTS FROM A MULTICENTER JAPANESE CLINICAL TRIAL

KOJI OKIHARA, OSAMU UKIMURA, TERUKAZU NAKAMURA, SOH USHIJIMA, YOICHI MIZUTANI, AKIHIRO KAWAUCHI, YOSHIO NAYA, MUNEKADO KOJIMA, AND TSUNEHARU MIKI

ABSTRACT

Objectives. To compare the distribution of total and complexed prostate-specific antigen (cPSA) in men with and without prostate cancer with another studied population and to ascertain whether cPSA could enhance the detection of prostate cancer in Japanese men.

Methods. A total of 760 men whose serum total PSA (tPSA) values ranged from 1.0 to 100 ng/mL were enrolled. Serum samples for tPSA and cPSA (ADVIA Centaur) were obtained in all cases. The area under the curve was calculated for comparison of the tPSA and cPSA values. We calculated the number of cancers missed and false-positive results at various cutoff values of cPSA compared with the conventional tPSA threshold of 4.0 ng/mL.

Results. Prostate cancer was detected in 268 (35.3%) of 760 patients. cPSA was greater than 8.3 ng/mL (equivalent to 10.0 ng/mL tPSA) in 46.6% of the men with cancer. The area under the curve for cPSA (0.741) was significantly better than that for tPSA (0.721, $P < 0.001$). At a sensitivity of 85% to 95%, significant differences were found in the corresponding specificity between tPSA and cPSA. cPSA at a 3.0-ng/mL threshold detected an identical number of cancers as a tPSA cutoff of 4.0 ng/mL; however, it decreased the false-positive results by 28 cases.

Conclusions. To our knowledge, this is the first report of the distribution of cPSA in Japanese men using a urologic referral population. cPSA can be an alternative to tPSA as the first screening test. A substantial number of men in Japan with prostate cancer are currently diagnosed with a tPSA value greater than 10.0 ng/mL. UROLOGY **67:** 328–332, 2006. © 2006 Elsevier Inc.

In 1998, a novel assay for complexed prostate-specific antigen (cPSA), which avoided the use of antibodies to α_1-antichymotrypsin (ACT),[1] was developed. The Bayer cPSA assay can measure PSA-ACT and other immunoreactive fractions of PSA. Since the first reports, several retrospective and prospective studies focusing on the clinical usefulness of cPSA with European and American populations have been reported. Most of these studies have concluded that cPSA could enhance prostate cancer detection in men with a serum total PSA (tPSA) level between 4 and 10 ng/mL,[2,3] as well as at levels of 4.0 ng/mL or less.[4–7] Recently, the usefulness of cPSA as a screening tool was assessed in African-American men.[8] Considering the potential ethnic differences in cPSA, we conducted the first study of the new cPSA assay (Bayer ADVIA Centaur cPSA) in Japanese men.[9] The Bayer cPSA assay could replace tPSA as a first screening test and could enhance cancer detection compared with tPSA. The aims of this study were to confirm the established cutoff value of cPSA and to compare the distributions of cPSA in benign and malignant disease in Japanese men with those from an American and European multicenter clinical trial.[10]

MATERIAL AND METHODS

Between January 1999 and August 2004, we archived serum samples collected from outpatients referred to the Kyoto Prefectural University of Medicine (Kyoto), Matsushita Memorial

From the Department of Urology, Kyoto Prefectural University of Medicine, Kyoto, Japan; Department of Urology, Matsushita Memorial Hospital, Osaka, Japan; and Department of Urology, Nagoya Urology Hospital, Nagoya, Japan

Reprint requests: Koji Okihara, M.D., Department of Urology, Kyoto Prefectural University of Medicine, Kawaramachi Hirokoji, Kyoto 602-8566, Japan. E-mail: okiharakoji@hotmail.com

Submitted: February 21, 2005, accepted (with revisions): August 12, 2005

0090-4295/06/$32.00
doi:10.1016/j.urology.2005.08.028

Hospital (Osaka), and Nagoya Urologic Hospital (Nagoya). The samples were collected before any prostatic manipulation and were assayed retrospectively. Participants with a personal history of prostate cancer or symptoms of acute prostatitis and/or urinary tract infection were excluded from this study. Between January 1999 and December 2002, 214 men underwent eight-core transperineal ultrasound-guided prostate needle biopsy (conventional sextant and far lateral portion of the peripheral zone: one core each from the right and left sides).[9] Thereafter, an additional 585 consecutive biopsies were performed in the same manner. The institutional distribution of biopsies was 300 from Kyoto, 295 from Nagoya, and 204 from Osaka. The indication for prostate biopsy was either a tPSA level (Hybritech Tandem-R assay) greater than 4.0 ng/mL or abnormal digital rectal examination (DRE) findings, regardless of the tPSA value.

A blood sample was drawn to measure tPSA (Hybritech Tandem-R assay) and cPSA (Bayer ADVIA Centaur cPSA). As previously described,[9] the Bayer ADVIA Centaur cPSA assay is a simultaneous sandwich immunoassay that uses magnetic particles as the solid phase. In the Centaur assay, a light reagent with polyclonal goat anti-PSA antibody with acridinium eater is applied.

All serum samples were drawn before DRE. Within 24 hours of collection, all samples were centrifuged and stored at −70°C. The minimal specimen volume was 0.7 mL.

The chi-square test was used for each statistical comparison between two specificities at the same sensitivity. For continuous variables, a *t* test or analysis of variance was used to compare groups. Receiver operating characteristic curves were generated by plotting the sensitivity versus (1 − specificity). All statistical calculations were performed using the Statistical Analysis Systems (SAS Institute, Cary, NC) or Statistical Package for Social Sciences, version 10.0 (SPSS, Chicago, Ill) software package. $P < 0.05$ was taken as the level of statistical significance.

RESULTS

Of the 799 men with a complete sample collection, 307 (38%) had positive biopsy results for prostate cancer, and 9 (1.1%) had prostatic intraepithelial neoplasia. In the 799 men, the tPSA value ranged from 1.0 to 6820 ng/mL (median 7.4). The tPSA value was greater than 100.0 ng/mL in 39 men (4.9%). Similar to the recent studies,[8,10] the 39 men were omitted from analysis as outliers for descriptive and comparison purposes. Consequently, we enrolled 760 consecutive men undergoing a first-time prostate biopsy; of these, 268 (35.2%) had histologically confirmed prostate cancer. In the 131 patients with a tPSA value of 4.00 ng/mL or less, prostate biopsies were performed because of abnormal DRE results; of these men, cancer was detected in 18 (13.8%). Of the 268 men with cancer, 77 (29%) were diagnosed with clinical Stage T3 disease. Of the 77 men with Stage T3 disease, 60 (78%) had a tPSA level of 10.01 ng/mL or more. The biopsy Gleason score was less than 7 in 64 (24%) and 7 in 172 (64%) of 268 men.

Of the 760 men, 300 (39.5%) had suspicious DRE findings suggestive of prostate cancer by the supervising urologists at each institution. Of the 760 men, 568 (74.7%) answered the International

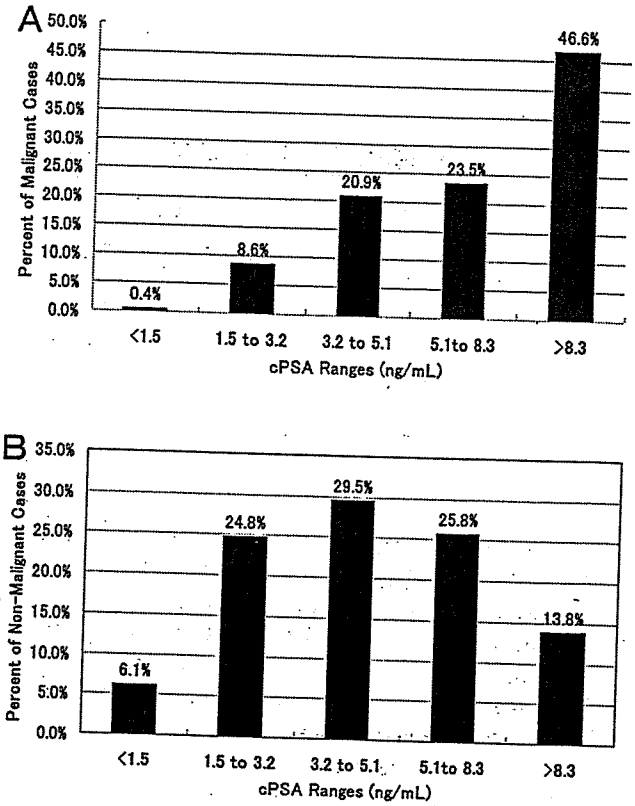


FIGURE 1. *Distribution of cPSA in men (A) with and (B) without biopsy proven prostate cancer.*

Prostate Symptom Score questionnaire before prostate biopsy. The total International Prostate Symptom Score was 0 to 7 (mild symptoms), 8 to 19 (moderate), and 19 to 35 (severe symptoms) for 238 (42.0%), 228 (40.2%), and 102 men (17.8%), respectively. The average patient age for men with cancer was 71.0 ± 7.5 years (median 71), and they were significantly older than the men without cancer (67.2 ± 7.7 years, median 68, $P < 0.001$). Comparing the patient populations from each institution, no significant differences were found in mean age (Kyoto, 67.5 ± 8.0 years; Osaka, 66.9 ± 7.8; and Nagoya, 65.1 ± 9.4) or the incidence of cancer (Kyoto, 38.3% [107 of 279]; Osaka, 35.1% [70 of 199]; and Nagoya, 32.2% [91 of 282]).

The median tPSA for the cancer group was significantly more than that for the benign group (10.0 ng/mL versus 6.1 ng/mL, $P < 0.001$). Similarly, the cPSA levels were greater in the cancer group (median 7.74 ng/mL, range 0.71 to 65.2) than in the noncancer group (median 5.69 ng/mL, range 0.87 to 51.2, $P < 0.001$). In addition, the 25th and 75th quartiles of cPSA in the cancer group and noncancer group were 4.61 and 16.67 ng/mL and 2.88 and 6.24 ng/mL, respectively.

To determine the equivalency values for cPSA and tPSA, we used the ranges reported by Partin *et al.*[10] The equivalent tPSA value for a cPSA value of 0 to 1.5, 1.5 to 3.2, 3.2 to 5.1, 5.1 to 6.8, 6.8 to 8.3, and greater than 8.3 ng/mL was 0 to 2.0, 2.0 to 4.0,

TABLE I. *Comparison between tPSA and cPSA at various cutoff values*

	Cutoff Value (ng/mL)					
	Hybritech tPSA	cPSA				
	>4.0	>3.4	>3.0	>2.9	>2.8	>2.7
Cancer (n)	250	237	250	251	252	255
Cancer missed (n)	18	31	18	17	16	13
False-positive (n)	380	326	352	367	378	382
No. vs. Hybritech tPSA						
Cancer missed		+13	±0	−1	−2	−5
False-positive results		−54	−28	−13	−2	+2

KEY: PSA = prostate-specific antigen; tPSA = total PSA; cPSA = complexed PSA.

TABLE II. *Comparison of cPSA and tPSA specificities at 80%, 85%, 90%, and 95% sensitivities in all cases*

	80% Sensitivity		85% Sensitivity		90% Sensitivity		95% Sensitivity	
	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)
cPSA	4.27	49.6	3.75	41.3	3.36	33.7	2.76	22.8
tPSA	5.55	43.5	4.95	36.8	4.35	27.6	3.85	18.5
P value	0.0239		0.0042		0.0439		0.0068	

Abbreviations as in Table I.

4.0 to 6.0, 6.0 to 8.0, 8.0 to 10.0, and greater than 10.0, respectively. In men with cancer, the cPSA value was greater than 8.3 ng/mL in 125 (46.6%) and was the largest subgroup. In contrast, the greatest proportion of men without cancer (29.5%, n = 145; Fig. 1) had a cPSA value of 3.2 to 5.1 ng/mL.

We compared the number of patients with a missed cancer or false-positive result between the tPSA and cPSA values (Table I). The number of cancers missed and number of false-positive results using a tPSA cutoff value of 4.0 ng/mL was 18 and 380, respectively. A cPSA threshold of 3.0 ng/mL provided equivalent sensitivity (93.2%) and a better positive predictive value (39.6% versus 41.5%) to a 4.0-ng/mL cutoff for tPSA in the Japanese population. For the comparison in all cases, the area under the curve for cPSA (0.741) was significantly better than that for tPSA (0.721, $P < 0.001$).

The performance characteristics with respect to sensitivity and specificity for all cases are summarized in Table II. At a sensitivity of 80% to 95%, significant differences were found in the corresponding specificities between tPSA and cPSA. At a sensitivity of 90%, the specificity of cPSA improved 6.1% compared with that of tPSA. In addition, we compared the performance characteristics with respect to sensitivity and specificity in men with a tPSA value between 4.01 and 10.00 ng/mL (Table III). Similar to the results in all cases, significant differences were found in the corresponding specificities between tPSA and cPSA at a sensitivity of 80% to 95%.

COMMENT

To assess the utility of new serum markers, it is necessary to analyze their performance in different ethnic populations. The population of men in this study was 100% Asian. In our study, the median age for those with cancer was 71 years and was 68 years for those without cancer. In the study by Partin *et al.*,[10] the study population was 87.9% white. Consequently, those results mainly reflected the performance in one ethnic group. Comparing the two patient populations, the median age for men with and without cancer was 6 and 7 years older, respectively, in our study. In addition, substantial differences in median age have been reported in studies of different national or ethnic populations.[8,11] Just as the incidence of prostate cancer has been increasing in younger men in the United States,[12] the same trend has been observed with the use of PSA screening in Japan.[13] Despite the trend of the young age migration, the median age in Japanese men with prostate cancer was still older than that in the United States.

The distribution of cPSA and tPSA in the population studied also influences the age distribution, as well as the diagnostic performance of the serum marker. To our knowledge, this is the first report of

TABLE III. *Comparison of cPSA and tPSA specificities at 80%, 85%, 90%, and 95% sensitivities in men with total PSA value between 4.01 and 10.00 ng/mL*

	80% Sensitivity		85% Sensitivity		90% Sensitivity		95% Sensitivity	
	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)
cPSA	3.82	31.8	3.50	22.0	3.36	17.9	3.01	11.8
tPSA	5.05	24.7	4.75	17.6	4.55	12.2	4.25	5.7
P value	0.0030		0.0471		0.0025		<0.0001	

Abbreviations as in Table I.

the distribution of cPSA in Japanese men with and without prostate cancer using a urologic referral population. In American and European countries, PSA levels have been shifting downward and what was once considered the traditional gray zone of tPSA (4 to 10 ng/mL) may now be 2 to 6 ng/mL.[10] In contrast to the study by Partin *et al.*,[10] most cPSA levels in men with cancer were more than 8.3 ng/mL (tPSA more than 10.0 ng/mL) in our study. First, the difference in cPSA distribution between the two studies might be explained by the prostate biopsy indication. In our study, biopsy was indicated for a tPSA cutoff of 4.0 ng/mL if the DRE findings were normal. In addition, the proportion of clinical stage, pathologic outcome, and men with urologic symptoms can be associated with the distribution of tPSA and cPSA in a population. Taneja *et al.*[14] reported the distribution of clinical stage and biopsy Gleason score in 410 men with prostate cancer, with the aim of using cPSA as a diagnostic and staging tool. The proportion of clinical Stage T3 disease and biopsy Gleason score less than 7 in their study was 4.0% and 57.6%, respectively, compared with corresponding values of 29% and 24% in our study. We speculate that the striking difference in clinical stage and biopsy outcomes resulted in the distribution of the cPSA range. Furthermore, the proportion of the total International Prostate Symptom Score of 8 or more was 58% in our study. Filella *et al.*[11] demonstrated the usefulness of cPSA and tPSA in the diagnosis of prostate cancer in patients with urologic symptoms. In their study, prostate symptoms were present in 94 (47%) of 200 men referred to the urologic practice, and the average tPSA level for those with cancer was 11.60 ± 10.12 ng/mL (range 1.86 to 49). In our study, it was 18.3 ± 19.5 ng/mL. Despite the substantial differences in the average tPSA value in those with cancer between the two studies, that the PSA value in those with cancer was based on a population with urinary symptoms results in a relatively greater range compared with a screening population. Also, selection bias and study population variances could potentially influence the optimal assay threshold values.

Previously, we established a preliminary optimal cutoff cPSA value (2.8 ng/mL) compared with a tPSA threshold of 4.0 ng/mL.[9] In this study, a 3.0 ng/mL cPSA threshold should be considered the potential cutoff value for comparative analysis of cancers missed and false-positive cases using the conventional 4.0-ng/mL tPSA threshold. In general, the calculated optimal cutoff value will change depending on the population being studied. On the basis of our previous study[9] and this study, however, we concluded that cPSA can be an alternative to tPSA as the first-line prostate cancer detection test in the selected (hospital referral) Japanese population. This position has been supported by the comparison of cPSA and tPSA specificities at a sensitivity of 80% to 95% (Tables II and III). Our next goal is to assess the optimal cutoff value of cPSA in the screening population.

ACKNOWLEDGMENT. To Richard Babaian, M.D. (M.D. Anderson Cancer Center, Houston, Texas) for valuable comments during the preparation of this manuscript.

REFERENCES

1. Allard WJ, Zhou Z, and Yeung KK: Novel immunoassay for the measurement of complexed prostate-specific antigen in serum. Clin Chem 44: 1216–1223, 1998.

2. Djavan B, Remzi M, Zlotta AR, *et al:* Complexed prostate-specific antigen, complexed prostate-specific antigen density of total and transition zone, complexed/total prostate-specific antigen ratio, free-to-total prostate-specific antigen ratio, density of total and transition zone prostate-specific antigen: results of the prospective multicenter European trial. Urology 60(suppl): 4–9, 2002.

3. Brawer MK, Cheli CD, Neaman IE, *et al:* Complexed prostate specific antigen provides significant enhancement of specificity compared with total prostate specific antigen for detecting prostate cancer. J Urol 163: 1476–1480, 2000.

4. Okihara K, Fritsche HA, Johnston DA, *et al:* Can complexed prostate-specific antigen and prostatic volume enhance the prostate cancer detection in men with total PSA values between 2.5 and 4.0 ng/ml? J Urol 165: 1930–1936, 2001.

5. Naya Y, Stamey TA, Cheli CD, *et al:* Can volume measurement of the prostate enhance the performance of complexed prostate-specific antigen? Urology 60(suppl): 36–41, 2002.

6. Horninger W, Cheli CD, Babaian RJ, *et al:* Complexed prostate-specific antigen for early detection of prostate cancer in men with serum prostate-specific antigen levels of 2 to 4 nanograms per milliliter. Urology 60(suppl): 31–35, 2002.

7. Parsons KJ, Brawer MK, Cheli CD, *et al:* Complexed prostate specific antigen (PSA) reduces unnecessary prostate

biopsies in the 2.6 to 4.0 ng/ml range of total PSA. BJU Int 94: 47–50, 2004.

8. Martin B, Cheli CD, Ward LM, *et al:* Complexed PSA performance for prostate cancer detection in an African-American population. Urology 62: 835–839, 2003.

9. Okihara K, Ukimura O, Nakamura T, *et al:* Can complexed prostate specific antigen enhance prostate cancer detection? Eur Urol 46: 57–64, 2004.

10. Partin AW, Brawer MK, Bartsch G, *et al:* Complexed prostate specific antigen improves specificity for prostate cancer detection: results of a prospective multicenter clinical trial. J Urol 170: 1787–1791, 2003.

11. Filella X, Truan D, Alcover J, *et al:* Comparison of several combinations of free, complexed, and total PSA in the diagnosis of prostate cancer in patients with urologic symptoms. Urology 63: 1100–1104, 2004.

12. Jacobsen SJ, Katusic SK, Bergstralh EJ, *et al:* Incidence of prostate cancer diagnosis in the eras before and after serum prostate-specific antigen testing. JAMA 274: 1445–1449, 1995.

13. Okihara K, Nakanishi H, Nakamura T, *et al:* Clinical characteristics of prostate cancer in the eras before and after serum prostate-specific antigen testing in Japanese men. Int J Urol 12: 662–667, 2005.

14. Taneja SS, Hsu EI, Cheli CD, *et al:* Complexed prostate-specific antigen as a staging tool: results based on a multicenter prospective evaluation of complexed prostate-specific antigen in cancer diagnosis. Urology 60(suppl 4A): 10–17, 2002.

Basic Science

Prognostic Significance of Thymidylate Synthase Expression in Patients with Prostate Cancer Undergoing Radical Prostatectomy

Yongnan Li, Yoichi Mizutani, Takumi Shiraishi, Koji Okihara, Osamu Ukimura, Akihiro Kawauchi, Norio Nonomura, Masakazu Fukushima, Toshiyuki Sakai, and Tsuneharu Miki

OBJECTIVES Thymidylate synthase (TS), a key enzyme in DNA synthesis, is overexpressed in a variety of cancer cells. 5-Fluorouracil (5-FU), an anticancer agent used clinically against various cancers, including prostate cancer, inhibits DNA synthesis by binding TS. In this study, we investigated the expression of TS in prostate cancer and its prognostic significance. Its association with the expression of dihydropyrimidine dehydrogenase (DPD), a principal enzyme in the degradation of 5-FU and pyrimidine nucleotides, was also examined.

METHODS Fifty-two prostatic tissue specimens were obtained from patients who had undergone radical prostatectomy for prostate cancer without neoadjuvant hormonal therapy. We analyzed the cancerous tissue and normal prostatic tissue specimens for TS expression using immunohistochemistry.

RESULTS TS was expressed at greater levels in the prostate cancer specimens than in the normal prostatic tissue specimens. The patients with prostate cancer with negative TS expression had a longer postoperative recurrence-free rate than did those with positive expression during the 5 years of follow-up. TS expression was significantly decreased in patients who received neoadjuvant hormonal therapy. No relationship was found between the expression of TS and DPD. Patients with prostate cancer with either negative TS or DPD expression had a significantly longer postoperative disease-free rate than those with positive expression of both during the 5 years of follow-up.

CONCLUSIONS The results of the present study have shown for the first time that TS expression could be a prognostic marker for patients with prostate cancer undergoing radical prostatectomy. In addition, the combination of TS and DPD expression might also be helpful for the prediction of the prognosis of patients with prostate cancer. UROLOGY xx: xxx, xxxx. © 2007 Elsevier Inc.

The anticancer agent, 5-fluorouracil (5-FU), is used clinically against various cancers, including prostate cancer.[1,2] Single-agent infusion 5-FU has demonstrated some efficacy against hormone-refractory prostate cancer, and response rates up to 27% have been reported.[3] 5-FU itself is inactive and requires intracellular conversion to 5-fluoro-2′-deoxyuridine 5′-monophosphate. 5-Fluoro-2′-deoxyuridine 5′-monophosphate exerts its cytotoxic activity through the formation of a ternary complex with thymidylate synthase (TS) and 5,10-methylene-tetrahydrofolate, resulting in inhibition of TS and blockage of the DNA synthetic process.[4,5] TS is overexpressed in tumor cells, which show high proliferative activity.[6] Several studies examining the importance of TS expression have indicated that TS expression predicts for overall outcome and the response to 5-FU cytotoxic therapy in several major tumor types.[7–9] Furthermore, the immunohistochemical staining results for TS and dihydropyrimidine dehydrogenase (DPD) predict the response to 5-FU.[10,11]

Our previous studies on renal cell carcinoma and bladder cancer showed that TS activity was greater in the cancerous tissue specimens than in the normal tissue samples and that

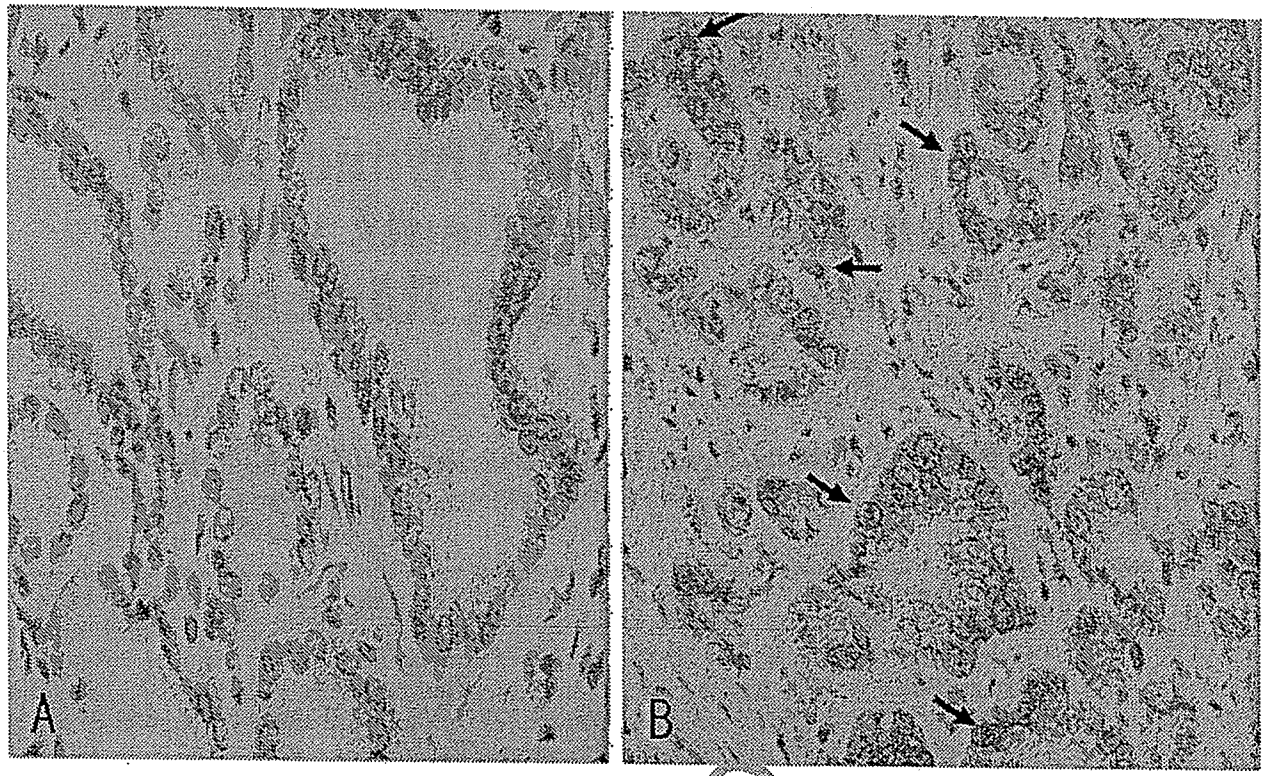
This work was supported in part by the NPO Tsukasa Fellowship and Research Foundation.

From the Department of Urology, Kyoto Prefectural University of Medicine, Kyoto; Department of Urology, Osaka University Faculty of Medicine, Osaka; Cancer Research Laboratory, Taiho Pharmaceutical Company, Limited, Saitama; and Department of Molecular-Targeting Cancer Prevention, Kyoto Prefectural University of Medicine, Kyoto, Japan

Reprint requests: Yoichi Mizutani, M.D., Department of Urology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-Ku, Kyoto 602-8566, Japan. E-mail: ymizutan@koto.kpu-m.ac.jp

Submitted: May 4, 2006, accepted (with revisions): February 8, 2007

0090-4295/xx/$32.00
doi:10.1016/j.urology.2007.02.015



COLOR

Figure 1. Immunohistochemical staining for TS in normal prostatic tissues and prostate cancer. Representative images of tissue microarray samples with absent staining and strong TS expression were examined. TS expression confined to cytoplasm of cells as demonstrated by immunohistochemistry. Original magnification ×200. **(A)** TS staining negative in normal prostatic tissue. **(B)** Positive TS staining (arrows) observed in cytoplasm of prostate cancer tissue.

AQ: 2

the TS activity level correlated with stage progression and increase in bladder cancer grade.[12] In addition, TS activity is a significant prognostic marker in patients with bladder cancer or renal cell carcinoma.[12,13] Reported data on TS activity in prostate cancer are limited, and little is known about the significance of TS in the biology of prostate cancer. The aim of this study was to define whether TS expression is a prognostic marker for patients with prostate cancer.

MATERIAL AND METHODS

Patients

We obtained 52 prostate cancer specimens with adjacent normal prostatic tissues from 1997 to 2005. The patients had not undergone preoperative androgen-deprivation therapy or radiotherapy. The mean patient age was 65 years (range 53 to 75). The 2002 TNM system was used for pathologic staging.[14] The pathologic stage was T2 in 38 patients and T3 in 14. The Gleason grading system was used to determine the Gleason score.[15] The Gleason score of the 52 specimens was grade 4/5 in 1 patient, 4/4 in 1, 4/3 in 9, 3/5 in 2, 3/4 in 18, 3/3 in 8, 3/2 in 8, 2/3 in 3, 2/1 in 1, and 1/2 in 1 patient. In addition, prostate cancer tissue from 48 patients who had undergone neoadjuvant hormonal therapy were examined.

The local human investigations committee approved this study, and all patients provided informed consent.

Immunohistochemistry for TS and DPD

TS and DPD expression was examined by immunohistochemistry, as previously described.[5,16] The sections were incubated with monoclonal antibody TS106 (1:500, dilution, Taiho Pharmaceutical, Saitama, Japan) or incubated with polyclonal rabbit antibody against human DPD[16] (1:2000 dilution, Taiho Pharmaceutical) overnight at 4°C. The secondary antibody was visualized with diaminobenzedine.

Evaluation of TS and DPD Expression

The intensity of the immunoreactivity for TS and DPD was evaluated in normal prostatic tissue and prostate cancer tissue from the same slide in each case. At least 10 high-power fields at 400× magnification were chosen randomly, and more than 1000 carcinoma cells were counted for each section. A pathologist who was unaware of the clinicopathologic data and clinical outcomes of the patients examined cytoplasmic TS and DPD staining results. The intensity of TS and DPD was graded from 0 to 3, and the extent was graded as focal (less than 25% of tumor staining positive) or diffuse (more than 25% of tumor staining positive).[5] A score of 0 or 1 was regarded as negative expression, and a score of 2 or 3 as positive expression. Figure 1 shows representative examples; Fig. 1A shows a TS-negative normal prostate specimen and Fig. 1B TS-positive prostate cancer. F1

Statistical Analysis

For statistical analysis, the Student *t* test and chi-square test were used. Biochemical recurrence was defined as a postopera-

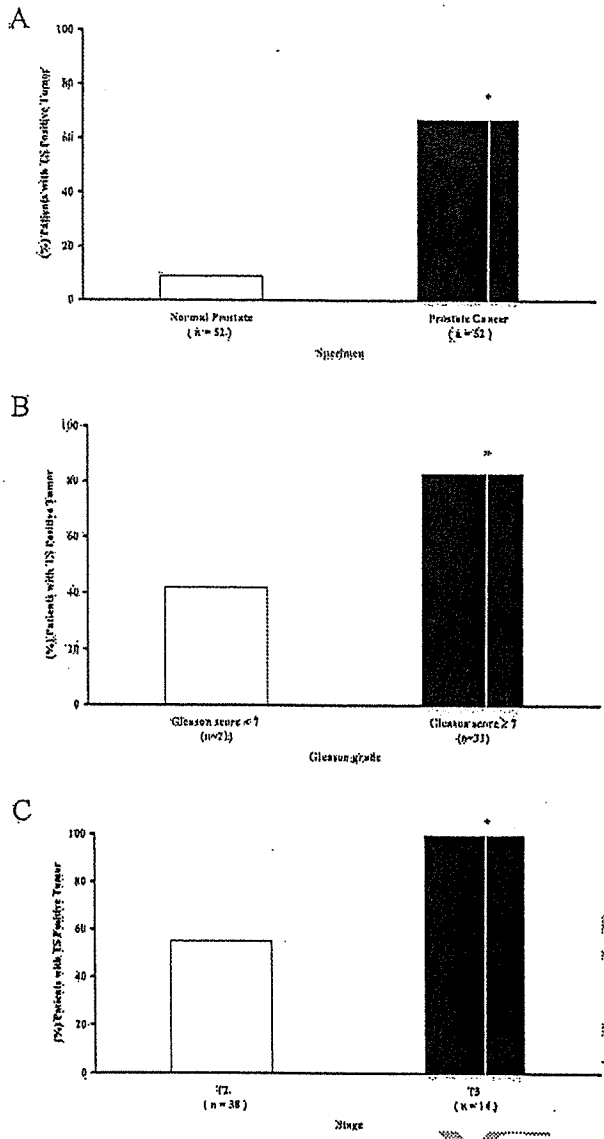


Figure 2. Expression of TS in normal prostatic tissue and prostate cancer. Percentage of TS expression detected by immunohistochemical assay as described in Material and Methods section. **(A)** $^{*}P < 0.0001$ versus normal prostatic tissue; **(B)** $^{*}P = 0.0017$ versus Gleason score less than 7 disease; and **(C)** $^{*}P = 0.0113$ versus Stage T2.

AQ: 3

tive serum prostate-specific antigen (PSA) level of 0.1 ng/mL or more.[17] The postoperative recurrence-free rate was determined using the Kaplan-Meier method. The influence of each variable on the recurrence-free rate was analyzed by multivariate analysis of a Cox proportional hazard model. $P < 0.05$ was considered significant.

RESULTS

TS Expression in Prostate Cancer and Normal Prostatic Tissue

TS was expressed in the cytoplasm of both normal prostatic tissue and prostate cancer cells. TS expression was detected in 35 (67%) of 52 prostate cancer samples (Fig. 2A). In contrast, TS expression was detected in 5 (9%) of the 52 normal prostatic tissue specimens. In addition, the intensity of cells that reacted with TS antibodies was significantly greater in the prostate cancer specimens than in the normal prostate samples ($P < 0.0001$, data not shown).

TS Expression in Relation to Pathologic Features and Tumor Stage

The staining percentage of TS expression was greater in patients with Gleason score 7 or greater disease (26 of 31, 83%) than that for patients with Gleason score less than 7 disease (9 of 21, 42%; Fig. 2B). It was also greater in Stage T3 tumor (14 of 14, 100%) than in Stage T2 tumors (21 of 38, 55%; Fig. 2C).

Relationship Between TS Expression and Postoperative Recurrence-Free Rate and Between TS and DPD Expression and Postoperative Recurrence-Free Rate in Patients with Prostate Cancer

Patients with prostate cancer undergoing radical prostatectomy alone were evaluated to determine the postoperative clinical course. From these results, patients with prostate cancer were divided into two groups—those with positive TS expression and those with negative TS expression. At 5 years of follow-up, patients with negative TS expression had a greater recurrence-free rate compared with those with positive TS expression ($P = 0.0183$; Fig. 3A). Using Cox regression analysis for the 52 patients, TS expression seemed to be independent prognostic indicator ($P = 0.021$ on multivariate analysis). The patients were also divided according to the Gleason grade and preoperative serum PSA level at baseline, and the recurrence-free rate of the groups with different TS expression status were analyzed. In Gleason score less than 7 cancer, the TS-negative group had a significantly greater 5-year recurrence-free rate than did the TS-positive group (Fig. 3B). In those with Gleason score 7 or greater cancer, no significant difference was found (Fig. 3C). In those with a PSA of 10 ng/mL or less at baseline, the 5-year recurrence-free rate of TS-negative patients tended to be greater than that of TS-positive patients. However, statistical significance was not reached (Fig. 3D). In those with a PSA level greater than 10 ng/mL, the TS-negative group had a significantly greater 5-year recurrence-free rate compared with the TS-positive group (Fig. 3E). These findings indicate that the TS expression level in prostate cancer could be a prognostic indicator, with negative TS expression a good prognostic sign.

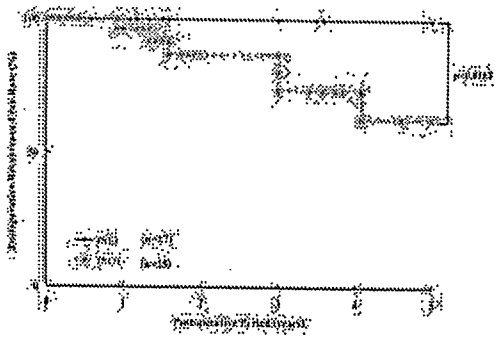
F3

Effect of Neoadjuvant Hormonal Therapy on TS Expression in Prostate Cancer

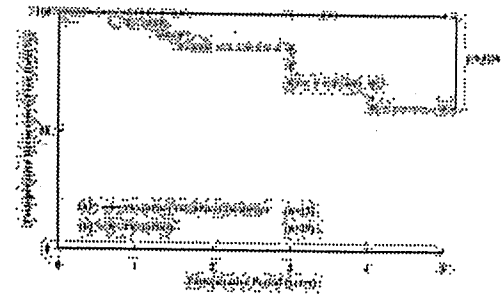
The expression of TS in patients with prostate cancer was greater after radical prostatectomy alone than that after radical prostatectomy plus neoadjuvant hormonal therapy (32 of 52, 61% versus 18 of 48, 37%; Fig. 4A). For patients with Stage T2 prostate cancer, positive TS expression in those who underwent neoadjuvant hormonal

F4

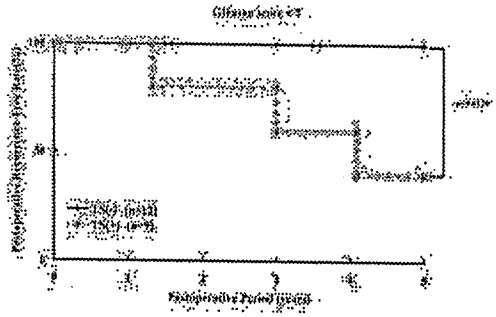
A



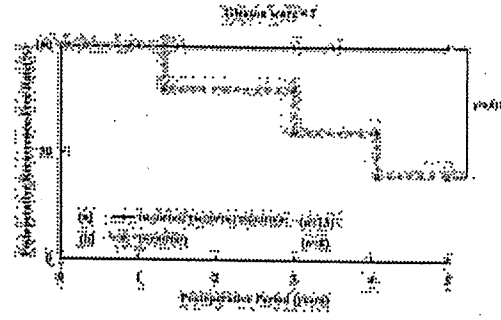
F



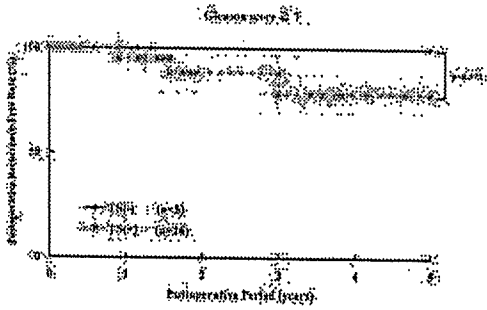
B



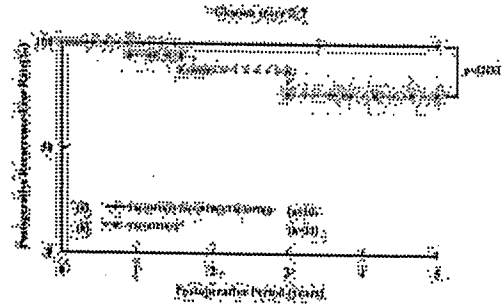
G



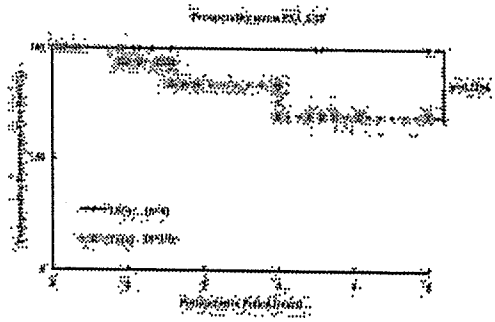
C



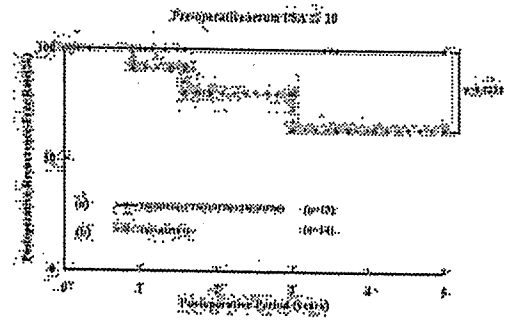
H



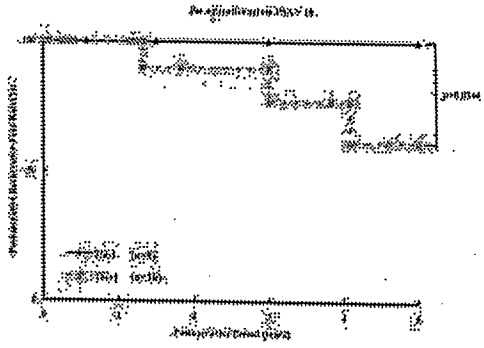
D



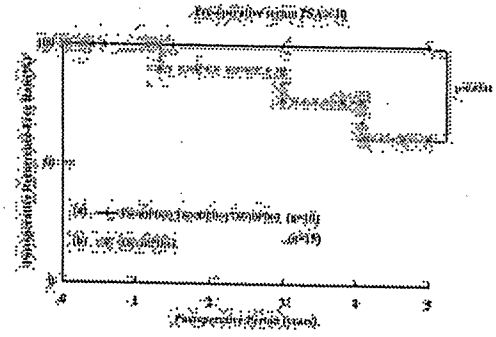
I



E



J



COLOR

therapy (9 of 38, 23%) was less than that in patients who underwent radical prostatectomy alone (21 of 38, 55%; Fig. 4B). However, no significant difference was found in those with Stage T3 prostate cancer (14 of 14, 100% versus 10 of 10, 100%; Fig. 4C). With Gleason score less than 7 prostate cancer, positive TS expression in patients with radical prostatectomy alone (9 of 21, 42%) was significantly greater than that in patients who had undergone neoadjuvant hormonal therapy (1 of 13, 7%; Fig. 4D). A significant difference was also observed in patients with Gleason score 7 or greater prostate cancer between those who underwent radical prostatectomy alone (26 of 31, 83%) and those who also received neoadjuvant hormonal therapy (18 of 35, 51%; Fig. 4E).

These findings suggest that neoadjuvant hormonal therapy might downregulate TS expression in prostate cancer, especially Stage T2 prostate cancer and Gleason score less than 7 disease.

Prognostic Significance of Combined TS and DPD Evaluation

DPD is the initial and rate-limiting enzyme in the three-step pathway of pyrimidine nucleotide catabolism.[18] In contrast, TS is an important enzyme in pyrimidine nucleotides synthesis. Kornmann *et al.*[19] reported that the combination of TS and DPD expression correlated highly with survival. We examined the association between TS and DPD expression in patients with prostate cancer. No significant association was observed between the levels of TS and DPD expression using the chi-square test (data not shown).

We then examined the prognostic significance of a combination of TS and DPD expression using Kaplan-Meier analysis. On the basis of TS and DPD expression, the 52 patients were stratified categorized as those having positive expression of both TS and DPD and all others. We found a significant difference between these two groups ($P = 0.0108$; Fig. 3F). Patients with positive expression of both TS and DPD had a greater rate of postoperative recurrence. The patients also were divided using the Gleason grade and preoperative serum PSA level at baseline, and the recurrence-free rate of the groups with different TS and DPD expression were analyzed. In those with Gleason score less than 7 cancer, the TS-negative group had a significantly greater 5-year recurrence-free rate than did the TS-positive group (Fig. 3G). In patients with Gleason score 7 or greater cancer, no significant difference was found (Fig. 3H). In patients with a PSA level of 10 ng/mL or less, the 5-year recurrence-free rate of the TS-negative group tended to be greater than that of the TS-positive group. However, statistical significance was not reached (Fig. 3I). In those with a PSA level greater than 10 ng/mL, the TS-negative group had a significantly greater 5-year recurrence-free rate than that of the TS-positive group (Fig. 3J).

COMMENT

The role of TS expression in prostate cancer has not been previously studied. Multivariate analysis revealed that the TS expression profile was an independent prognostic indicator of prostate cancer. These results suggest that TS expression in patients with prostate cancer might provide additional prognostic information beyond the orthodox clinical and pathologic prognostic markers. Ichikawa *et al.*[20] reported that patients with colorectal tumor with both low DPD and low TS survived longer than did patients with tumor having the other patterns of TS and DPD expression. Consistent with our findings, the combined evaluation of TS and DPD expression might predict the prognosis more accurately than using one marker in patients with prostate cancer. These data also reflect the report by Jakob *et al.*[11] that immunohistochemistry such as reverse transcriptase polymerase chain reaction is a suitable method to determine the correlation between TS and DPD expression and histopathologic tumor regression. However, the precise mechanisms for this relationship remain unclear at present. Additional studies are necessary to examine the mechanisms.

The benefit of hormonal therapy against advanced prostate cancer has been widely accepted. In addition, the antitumor effect is very high. Because TS is the key

Figure 3. Relationship between TS expression and postoperative recurrence-free rate in patients with prostate cancer and relationship between TS and DPD expression and postoperative recurrence-free rate in patients with prostate cancer. Postoperative recurrence-free rate of 52 patients with prostate cancer undergoing radical prostatectomy alone was determined using Kaplan-Meier method. **(A)** Patients categorized by TS expression. Recurrence-free rate was significantly greater for patients with negative TS expression than for those with positive expression ($P = 0.0183$). **(B)** Patients categorized by TS expression of Gleason score less than 7 disease. Recurrence-free rate was significantly greater for patients with negative TS expression than for those with positive expression ($P = 0.0256$). **(C)** Patients categorized by TS expression of Gleason score 7 or worse. No significant difference observed between two groups of patients ($P = 0.2722$). **(D)** Patients categorized by TS expression of preoperative serum PSA level of 10 ng/mL or less. No significant difference observed between two groups of patients ($P = 0.2296$). **(E)** Patients categorized by TS expression of preoperative serum PSA level greater than 10 ng/mL. Recurrence-free rate significantly greater for patients with negative TS expression than for those with positive expression ($P = 0.0344$). Patients (n = 52) categorized as having positive expression of both TS and DPD (b) and all others (a). **(F)** Significant difference in recurrence rate between two groups ($P = 0.0108$). **(G)** Patients with Gleason score less than 7 disease. **(H)** Patients with Gleason score of 7 or more. **(I)** Patients with tumor with preoperative serum PSA level of 10 ng/mL or less. **(J)** Patients with tumor and preoperative serum PSA level greater than 10 ng/mL.

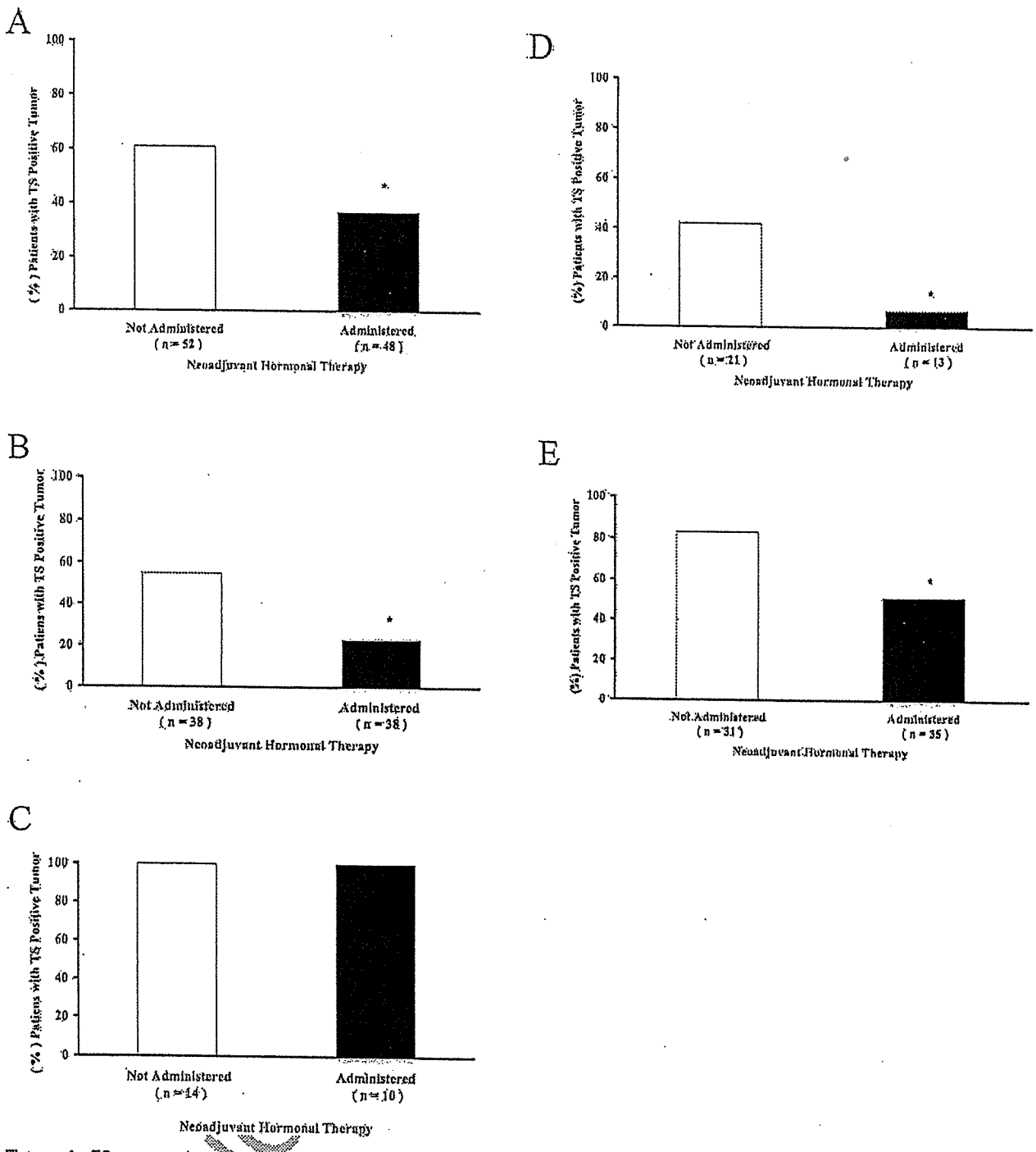


Figure 4. TS expression in prostate cancer with/without neoadjuvant hormonal therapy according to stage and Gleason grade of prostate cancer. TS expression in prostate cancer specimens evaluated by immunohistochemical assay as described in Material and Methods section. **(A)** $^{*}P = 0.0049$ versus not administered. **(B)** Patients with Stage T2 disease ($^{*}P = 0.0078$ versus not administered). **(C)** Patients with Stage T3 tumor. **(D)** Patients with Gleason score less than 7 tumor ($^{*}P = 0.0259$ versus not administered). **(E)** Patients with Gleason score 7 or greater tumor ($^{*}P = 0.0036$ versus not administered).

enzyme in the process of DNA synthesis, we believe it is reasonable to assume that this was the reason the TS expression was greater in the in the prostate cancer specimens of the patients treated without neoadjuvant hormonal therapy than in those treated with surgery and neoadjuvant hormonal therapy. Furthermore, hormonal therapy might directly downregulate TS expression. A study by Ogasawara *et al.*[21] showed that in nude mouse MCF-7 breast cancer xenografts, tumor TS levels were reduced by treatment with a pure antiestrogen. Hung *et al.*[22] also reported that the Ki-67 proliferation index was significantly greater in TS-positive tumors than in TS-negative tumors, suggesting that TS-negative tumors might have a low rate of cell proliferation.

TS is a key enzyme for pyrimidine synthesis. DPD is an important pyrimidine salvage enzyme. No correlation was found between DPD and TS expression in colorectal cancer.[20,23] We reported that no correlation was found between the TS and DPD activity levels in bladder carcinoma[12,24] or renal cell carcinoma.[13] Our data in prostate cancer are consistent with these results.

Immunohistochemical staining for TS and DPD predict the response to 5-FU.[10,11] Previous studies of several cancers have demonstrated that the TS expression level predicted the response to 5-FU-based chemotherapy.[4,5] Greater TS expression was accompanied by a greater response rate to 5-FU-containing chemotherapy. Most of the administrated 5-FU is degraded through the catabolic pathway with DPD.[18] DPD activity is highly associated with 5-FU pharmacokinetics.[25] The efficacy of 5-FU is related to the plasma level of this agent, which is inversely associated with the DPD activity level.[25] Our previous report demonstrated that primary cultured renal cell carcinoma cells with both high TS activity and low DPD activity were more sensitive to 5-FU than those with either low TS activity or high DPD activity.[26] These findings suggest that the TS and DPD expression levels in prostate cancer could be important predictive indicators for 5-FU efficacy. However, other factors might be more important, including the rate of degradation, carrier protein level, and so forth, although the principle TS and DPD expression levels might predict the response to 5-FU.

CONCLUSIONS

The results of our study have demonstrated that TS expression was significantly greater in the cancerous prostate and that positive TS expression was associated with a worse prognosis. These findings suggest that the assessment of TS expression might be useful in the management of prostate cancer. Because TS expression could be used as a prognostic parameter in patients with prostate cancer, an accurate prediction of prognosis might help to select patients for more intensive surgical, hormonal, or chemotherapeutic approaches, including 5-FU. Additional prospective studies are warranted to define the role of TS in selecting patients for adjuvant therapy for prostate cancer.

Acknowledgment: To Hal Gold for assistance in the preparation of this report.

References

1. Kuzel T, Tallman M, Shevrin D, *et al:* A phase II study of continuous infusion 5-fluorouracil in advanced hormone refractory prostate cancer: an Illinois Cancer Center study. Cancer 72: 1965–1968, 1993.
2. Birtle AJ, NewBy JC, and Harland SJ: Epirubicin carboplatin and 5-fluorouracil (ECarboF) chemotherapy in metastatic hormone refractory prostate cancer. Br J Cancer 91: 1472–1476, 2004.
3. Atkins J, Muss HB, Case D, *et al:* High-dose 24 hour infusion of 5-fluorouracil in metastatic prostate cancer: a phase II trial of the Piedmont Oncology Association. J Clin Oncol 14: 526–529, 1996.
4. Foekens JA, Romain S, Look MP, *et al:* Thymidine kinase and thymidylate synthase in advanced breast cancer: response to tamoxifen and chemotherapy. Cancer Res 61: 1421–1425, 2001.
5. Johnston PG, Fisher ER, Rockette HE, *et al:* The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. J Clin Oncol 12: 2640–2647, 1994.
6. Correale P, Del Vecchio MT, Di Genova G, *et al:* 5-Fluorouracil-based chemotherapy enhances the antitumor activity of a thymidylate synthase-directed polyepitopic peptide vaccine. J Natl Cancer Inst 97: 1437–1445, 2005.
7. Lenz HJ, Danenberg KD, Leichman CG, *et al:* p53 and thymidylate synthase expression in untreated stage II colon cancer: associations with recurrence, survival and site. Clin Cancer Res 4: 1227–1234, 1998.
8. Leichman CG, Lenz H, Leichman L, *et al:* Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infused fluorouracil and weekly leucovorin. J Clin Oncol 15: 3223–3229, 1997.
9. Johnston PG, Lenz HJ, Danenberg KD, *et al:* Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. Cancer Res 55: 1407–1412, 1995.
10. Huang CL, Yokomise H, Kobayashi S, *et al:* Intratumoral expression of thymidylate synthase and dihydropyrimidine dehydrogenase in non-small cell lung cancer patients treated with 5-FU-based chemotherapy. Int J Oncol 17: 47–54, 2000.
11. Jakob C, Liersch T, Meyer W, *et al:* Immunochemical analysis of thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase in rectal cancer: correlation with histopathologic tumor regression after 5-fluorouracil-based long-term neoadjuvant chemotherapy. Am J Surg Pathol 29: 1304–1309, 2005.
12. Mizutani Y, Wada H, Ogawa O, *et al:* Prognostic significance of thymidylate synthase activity in bladder carcinoma. Cancer 92: 510–518, 2001.
13. Mizutani Y, Wada H, Yoshida O, *et al:* Significance of thymidylate synthase activity in renal cell carcinoma. Clin Cancer Res 9: 1453–1460, 2003.
14. Gospodarowicz MK, Miller D, Groome PA, *et al:* The process for continuous improvement of the TNM classification. Cancer 100: 1–5, 2004.
15. Gleason DF, and Mellinger GT: Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111: 58–64, 1974.
16. Nozawa H, Tsukui H, Nishida K, *et al:* Dihydropyrimidine dehydrogenase expression in preoperative biopsy and surgically resected specimens of gastric carcinoma. Cancer Chemother Pharmacol 49: 267–273, 2002.
17. Babaian RJ, Troncoso P, Bhadkamkar VA, *et al:* Analysis of clinicopathologic factors predicting outcome after radical prostatectomy. Cancer 91: 1414–1422, 2001.
18. Diasio RB, and Lu Z: Dhydropyrimidine dehydrogenase activity and fluorouracil chemotherapy. J Clin Oncol 12: 2239–2242, 1994.
19. Kornmann M, Schwabe W, Sander S, *et al:* Thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expression levels: predictors for survival in colorectal cancer patients receiving adjuvant 5-fluorouracil. Clin Cancer Res 9: 4116–4124, 2003.
20. Ichikawa W, Uetake H, Shirota Y, *et al:* Combination of dihydropyrimidine dehydrogenase and thymidylate synthase gene expressions in primary tumors as predictive parameters for the efficacy of fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. Clin Cancer Res 9: 786–791, 2003.
21. Ogasawara Y, Doihara H, Shiroma K, *et al:* Effects of experimental chemoendocrine therapy with a combination of a pure antiestrogen and 5-fluorouracil on human breast cancer cells implanted in nude mice. Surg Today 29: 149–156, 1999.

22. Hung C, Liu D, Masuya D, *et al:* Clinical application of biological markers for treatment of respectable non-small-cell lung cancers. Br J Cancer 92: 1231–1239, 2005.
23. Salonga D, Danenberg KD, Johnson M, *et al:* Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. Clin Cancer Res 6: 1322–1327, 2000.
24. Mizutani Y, Wada H, Yoshida O, *et al:* Prognostic significance of a combination of thymidylate synthase and dihydropyrimidine dehydrogenase in grade 1 and 2 superficial bladder cancer. Oncol Rep 9: 289–292, 2002.
25. Fleming RA, Milano G, Thyss A, *et al:* Correlation between dihydropyrimidine dehydrogenase activity in peripheral mononuclear cells and systemic clearance of fluorouracil in cancer patients. Cancer Res 52: 2899–2902, 1992.
26. Mizutani Y, Wada H, Yoshida O, *et al:* Significance of dihydropyrimidine dehydrogenase activity in renal cell carcinoma. Eur J Cancer 39: 541–547, 2003.

International Journal of Urology (2007) 14, 54–59 doi: 10.1111/j.1442-2042.2006.01655.x

Long-term results of first-line sequential high-dose carboplatin, etoposide and ifosfamide chemotherapy with peripheral blood stem cell support for patients with advanced testicular germ cell tumor

Tsuneharu Miki,[1] Yoichi Mizutani,[1] Hideyuki Akaza,[2] Seiichiro Ozono,[3] Taiji Tsukamoto,[4] Toshiro Terachi,[5] Katsusuke Naito,[6] Norio Nonomura,[7] Isao Hara,[8] Osamu Yoshida[9] and The Japan Blood Cell Transplantation Study Group for Testicular Germ Cell Tumor*

[1]Department of Urology, Kyoto Prefectural University of Medicine, Graduate School of Medical Sciences, Kyoto, [2]Department of Urology, University of Tsukuba, School of Medicine, Ibaragi, [3]Department of Urology, Hamamatsu University School of Medicine, Shizuoka, [4]Department of Urology, Sapporo Medical University, Hokkaido, [5]Department of Urology, School of Medicine, Tokai University, Kanagawa, [6]Department of Urology, Yamaguchi University, School of Medicine, Yamaguchi, [7]Department of Urology, Osaka University, Graduate School of Medicine, Faculty of Medicine, Osaka, [8]Department of Urology, Kobe University, Graduate School of Medicine, School of Medicine, Hyogo, and [9]Nara Medical University, Nara, Japan

Objective: Standard chemotherapy shows relatively low long-term survival in patients with poor-risk testicular germ cell tumor (GCT). First-line high-dose chemotherapy (HD-CT) may improve the result. High-dose carboplatin, etoposide, ifosfamide chemotherapy followed by autologous peripheral blood stem cell transplantation (PBSCT) was investigated as first-line chemotherapy in patients with advanced testicular GCT.

Methods: Fifty-five previously untreated testicular GCT patients with Indiana 'advanced disease' criteria received three cycles of bleomycin, etoposide and cisplatin (BEP) followed by one cycle of HD-CT plus PBSCT, if elevated serum tumor markers were observed after three cycles of the BEP regimen.

Results: Thirty patients were treated with BEP alone, because the tumor marker(s) declined to normal range. Twenty-five patients received BEP and HD-CT. One patient died of rhabdomyolysis due to HD-CT. Three and six (13% and 25%) out of 24 patients treated with BEP and HD-CT achieved marker-negative and marker-positive partial responses, respectively. The other patients achieved no change. Fifteen (63%) are alive and 14 (58%) are free of disease at a median follow-up time of 54 months. Severe toxicity included treatment-related death (4%).

Conclusions: HD-CT with peripheral stem cell support can be successfully applied in a multicenter setting. HD-CT demonstrated modest anticancer activity for Japanese patients with advanced testicular GCT and was well tolerated. This regimen might be examined for further investigation in randomized trials in first-line chemotherapy for patients with poor-risk testicular GCT.

Key words: chemotherapy, germ cell tumor, high-dose, peripheral blood stem cell transplantation (PBSCT), testis.

Introduction

Cisplatin-based combination chemotherapy has improved the prognosis of patients with metastatic germ cell tumor (GCT), and the long-term cure rate is approximately 80%.[1,2] However, patients with the 'advanced disease' criteria according to the Indiana University classification or the 'poor prognosis' criteria of the International Germ Cell Cancer Collaborative Group classification show survival rates of only 50–60% following standard-dose cisplatin-based chemotherapy.[3–5] Several attempts have been undertaken to improve the outcome of this patient group, including the use of double-dose cisplatin regimens or alternating dose-dense chemotherapy sequences.[6–8] However, doubling the dose of cisplatin did not lead to an improved outcome as compared with a standard cisplatin-dose regimen. Recently, high-dose chemotherapy (HD-CT) followed by autologous peripheral stem cell support or autologous bone marrow support has also been studied in these patients.[9–11] The rationale for the use of HD-CT in patients with GCT is based on preclinical and clinical data suggesting a dose–response relationship for certain drugs used in the treatment of GCT, particularly for etoposide and ifosfamide.[12,13] Dose finding studies in the high-dose setting, usually using a combination of carboplatin, etoposide and cyclophosphamide, ifosfamide, or thiotepa, have been conducted in heavily pretreated patients with relapsed or refractory disease.[14,15]

Although single center phase II trials have reported 2-year survival rates of 70–80% using first-line HD-CT approaches in poor prognosis patients, results of large phase II trials or phase III trials are lacking.[9–11] In addition, it is unclear at present whether the reported survival rates of 70–80% are maintained with longer follow up. The present study investigated the long-term results of first-line HD-CT with autologous stem cell support in Japanese patients with advanced testicular GCT in a multicenter setting. Patients with relapsed testicular GCT were excluded in this study.

Correspondence: Tsuneharu Miki, MD, Department of Urology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan. Email: tmiki@koto.kpu-m.ac.jp

*Japan Blood Cell Transplantation Study Group for Testicular Germ Cell Tumor: Sapporo Medical University, Yamagata University, Tohoku University, Jichi Medical School, University of Tsukuba, Ibaraki Prefectural Central Hospital, Chiba University, The Jikei University, Nippon Medical School, Tokyo Women's Medical University, Kyorin University, Nagaoka Chuo General Hospital, Niigata Cancer Center, Nagoya Daini Red Cross Hospital, Shiga University of Medical Science, Nara Medical University, Kyoto University, Kyoto Prefectural University of Medicine, Aiseikai Yamashina Hospital, Kansai Medical University, Osaka University, Osaka City University, Seichokai Fuchu Hospital, Japanese Red Cross Wakayama Medical Center, Kobe University, Kobe City General Hospital, Kawasaki Medical School, Hiroshima University, Yamaguchi University, Ehime University, University of the Ryukyu.

Received 30 January 2006; accepted 19 July 2006.

Methods

Patients

Fifty-five patients with advanced testicular GCT were entered onto this institutional review board-approved prospective trial between March 1996 and December 1999. All patients gave informed consent before they were enrolled onto the study.

Eligibility included 'advanced disease' criteria according to the Indiana classification.[3] According to the International Germ Cell Cancer Collaborative Group (IGCCCG) criteria, the numbers of good, intermediate and poor prognosis were 7, 26 and 22, respectively. All patients had histologically confirmed testicular GCT and no prior chemotherapy. Patients were also required to have a pretreatment leukocyte count greater than 3000/μL, pretreatment platelet count greater than 100 000/μL, glomerular filtration rate of 60 mL/min or higher, and serum creatinine level less than or equal to 1.5 times the upper level of the institutional norm.

Before chemotherapy, each patient was evaluated with a history and physical examination, chest radiography, computed tomography (CT) of the abdomen/pelvis, and screening chemistries, which included serum tumor markers (alpha-fetoprotein [AFP], human chorionic gonadotropin-β [HCG-β] and lactate dehydrogenase [LDH]). CT of chest or brain and bone scintigraphy were performed as indicated. All patients had chest CT, if chest X-ray suggested metastases.

Mobilization and harvest of peripheral blood stem cells

A dose of 5 μg/kg of recombinant human granulocyte colony-stimulating factor (G-CSF) was given to each patients s.c. once daily from the day of the nadir of neutrophil count after bleomycin, etoposide and cisplatin (BEP) combination chemotherapy. After white blood cells (WBC) recovered to 5000/μL, leukapheresis collections of peripheral blood stem cells were carried out for 2–3 consecutive days using a CS 3000 blood cell separator (Baxter Limited, Deerfields, IL, USA). A total volume of 10–15 L of blood was processed in each patient. Mononuclear cells containing hematopoietic stem/progenitor cells were cryopreserved in liquid nitrogen. The target harvest was more than 2.0×10^6 CD34-positive nucleated cells/kg patient bodyweight.

Conventional-dose chemotherapy

All patients were treated with three cycles of BEP as induction chemotherapy. The doses of anticancer agents, treatment schedule and treatment-related toxicity have been described previously (bleomycin 30 mg, i.v., days 2, 9, 16; etoposide 100 mg/m^2, i.v., days 1–5; cisplatin 20 mg/m^2, i.v., days 1–5).[16] After three cycles of BEP therapy, patients whose tumor marker(s) were still elevated received one cycle of HD-CT with peripheral blood stem cell transplantation (PBSCT). If the serum tumor markers declined to normal range, the patients did not receive HD-CT.

High-dose chemotherapy

For treatment with HD-CT and autologous PBSCT, performance status 0 or 1 was required. HD-CT consisted of 1250 mg/m^2 of carboplatin, 1500 mg/m^2 of etoposide, and 7.5 g/m^2 of ifosphamide followed by 300 mg/m^2 of Mesna (bolus i.v. every 8 h, days 1–5). HD-CT was administered in five divided doses from day −7 to day −3. PBSCT was given i.v. on day 0. All patients received 5 μg/kg of G-CSF s.c., beginning the day following PBSCT and continuing until neutrophil count recovery. If all abnormally elevated serum tumor marker values returned to normal, surgery was performed when it was necessary to resect residual tumors.

Evaluation procedures

Serum tumor markers were determined before each treatment cycle and 4 weeks after the end of therapy. Evaluation of measurable disease by radiographic means was performed after HD-CT cycle and 4–6 weeks after the end of treatment. Subsequent follow-up tests including CT scans, serum tumor marker values and routine blood tests were performed at 3-month intervals during the first 2 years and then every 6 months up to 5 years of follow up.

Response to first-line HD-CT was defined according to World Health Organization (WHO) criteria.[17] Complete response (CR) was defined as the disappearance of all evidence of disease for at least 6 weeks when documented by imaging and all tumor markers. Partial response (PR) was defined as at least 50% reduction in the product of perpendicular diameters of each indicator lesion. PR was divided into two categories, partial response with tumor marker normalization (PR^{m-}) and marker positive partial response (PR^{m+}). Progressive disease (PD) was defined as 25% increase in the product from any lesion or the appearance of any new lesion. No change (NC) was defined as that which did not meet any of the above criteria. NC was also divided to two categories, no change with tumor marker normalization (NC^{m-}) and marker positive no change (NC^{m+}), Response and duration of survival were measured from the date of initiation of HD-CT.

Statistical analysis

Disease-specific survival was determined by the Kaplan–Meier method. For statistical analysis, a χ^2 test was used.

Results

Patient characteristics

Fifty-five patients, ranging in age 16–51 years (median, 27 years), were entered into this trial. Patient characteristics are summarized in Table 1a. Approximately 75% of all patients had lung metastasis and involvement of abdominal lymph nodes. Liver metastasis was present in 20% of patients. Four percent of patients had bone metastasis and 7% had central nervous system metastasis at diagnosis. Their histological types of GCT were four pure seminomas and 51 non-seminomas.

Response and survival

Five of 55 patients treated with three cycles of BEP achieved CR. Twenty-five of 50 patients who achieved PR or NC by induction BEP had normal concentrations of serum tumor markers. These 30 patients received another cycle of BEP therapy

Thus, following three cycles of induction BEP therapy, the remaining 25 patients whose tumor marker(s) (AFP, HCG-β and/or lactate dehydrogenase [LDH]) were still elevated when treated with one cycle of HD-CT with PBSCT. The patient characteristics are summarized in Table 1b. According to the IGCCCG criteria, the numbers of good, intermediate and poor prognoses were 1, 11 and 13, respectively. Because one patient died of rhabdomyolysis due to HD-CT, 24 patients were available to evaluate. Table 2 and Figure 1 summarize the outcome data. No patient achieved CR after one cycle of HD-CT. In all,

Table 1 Patient characteristics

Characteristics	No. of patients
(a)	
Patients age (years)	55
Median	27
Range	16–51
Histology	
Seminoma	4
Non-seminoma	51
Number of metastatic sites	
1	8
2	36
3 or more	11
Sites of metastasis	
Lung	48
Retroperitoneal lymph node	45
Mediastinal lymph node	12
Supraclavicular lymph node	3
Liver	11
Brain	4
Bone	2
Serum tumor markers	
HCG-β (ng/mL)	
Median elevated value	42
Range	0.1–120 000
AFP (ng/mL)	
Median elevated value	205
Range	10–62 274
LDH (IU/L)	
Median elevated value	1 550
Range	213–7 479
(b)	
Patients age (years)	25
Median	27
Range	16–45
Histology	
Seminoma	1
Non-seminoma	24
Number of metastatic sites	
1	5
2	13
3 or more	7
Sites of metastasis	
Lung	24
Retroperitoneal lymph node	19
Mediastinal lymph node	5
Liver	8
Brain	2
Serum tumor markers	
HCG-β (ng/mL)	
Median elevated value	1.35
Range	0.2–9.59
AFP (ng/mL)	
Median elevated value	52
Range	9–2 383
LDH (IU/L)	
Median elevated value	548
Range	419–781
Follow-up (months)	
Median	54
Range	10–80

AFP, alpha-fetoprotein; HCG-β, human chorionic gonadotropin-β; LDH, lactate dehydrogenase.

Table 2 Outcome data

Outcome	No. of patients (%)
Partial response	9 (37.5)
Marker-negative	3 (12.5)
Marker-positive	6 (25.0)
No change	15 (62.5)
Marker-negative	4 (16.7)
Marker-positive	11 (45.8)
Overall response rate	37.5

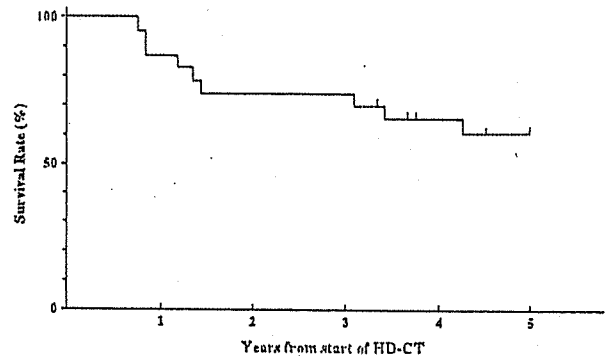


Fig. 1 Disease-specific survival of testicular germ cell tumor (GCT) patients treated with high-dose chemotherapy (HD-CT). Disease-specific survival rate was determined by the Kaplan–Meier method.

nine (38%) of 24 patients achieved PR. Marker-negative PR were achieved in only three patients. Fifteen NC and no PD were observed.

Seven patients showed high serum levels of AFP before HD-CT. The serum levels of AFP in six patients decreased after chemotherapy, but the levels in three patients were higher than normal range. In addition, serum AFP levels in one patient increased after HD-CT. Nineteen patients had high levels of serum HCG-β. The high serum HCG-β levels in five patients became less than the sensitivity of examination after HD-CT. Although the levels of serum HCG-β in 11 patients decreased after HD-CT, the serum levels were higher than normal range. The increased and same serum HCG-β levels in one and two patients were observed after HD-CT. Five patients demonstrated high serum LDH levels. The serum LDH level in three patients decreased to normal range after HD-CT, and the levels in the other two patients decreased, but to more than normal range.

Seventeen of 24 patients underwent operations for residual tumors. The patients included eight PR (marker-negative PR, three; marker-positive PR, five) and nine NC (marker-negative NC, four; marker-positive NC, five). Salvage surgery was performed for 10 patients (five PR and five NC) with positive marker(s). The pathological examinations revealed nine necrosis (marker-negative, three; marker-positive, six), one mature teratoma (marker-negative, one) and seven viable malignant tumors (marker-negative, three; marker-positive, four).

Twenty-one patients received additional therapy after first-line HD-CT and/or surgical resection of residual masses. Three patients received additional HD-CT, and nine patients received salvage chemotherapy with VIP (etoposide, ifosfamide and cisplatin), taxol or camptothecin, and radiation.

Table 3 Relationship between survival and various characteristics

Characteristics	Outcome (patient no.)	
	Alive (n = 15)	Dead (n = 9)
Serum tumor marker after HD-CT:		
Positive	10	7
Negative	5	2
IGCCCG criteria:		
Good	1	0
Intermediate	5	6
Poor	9	3
Histology: choricarcinoma		
(+)	7	3
(−)	8	6
Histology: yolk sac tumor		
(+)	5	4
(−)	10	5

There was no statistical significance by χ^2 test. HD-CT, high-dose chemotherapy; IGCCCG, International Germ Cell Cancer Collaborative Group.

Table 4 Adverse events

Adverse events	Grade				
	0	1	2	3	4
Neutropenia	0	0	0	1	22
Thrombocytopenia	0	0	3	4	16
Anemia	0	0	11	6	6
Fever	19	2	2	0	0
Mucositis	7	6	9	1	0
Nausea/vomiting	1	6	8	8	0
Diarrhea	11	5	6	1	0
Liver toxicity	13	4	4	1	1
Renal toxicity	21	1	0	1	0
Skin	20	2	1	0	0

The median duration of follow up is 54 months with a range of 9–80 months. Fourteen patients are currently alive and free of GCT and one patient remains alive with disease. All six patients with marker-positive status after HD-CT, whose pathological examinations of salvage surgery revealed necrosis, are alive. Nine have died of disease. The 3-year and 5-year survival rates were approximately 75% and 63%, respectively (Fig. 1). There was no correlation between survival and various characteristics (Table 3).

Adverse effects

Table 4 describes the toxicity in this study according to the WHO scale. There was a toxic death caused by rhabdomyolysis due to HD-CT.

Neutropenia was significant in all patients, and all patients except one experienced WHO grade 4 neutropenia. Nine patients had neutropenia with fever. The median duration of neutropenia less than 500/μL was 9 days (8–15 days). All patients received G-CSF, and the median duration of use of G-CSF was 11 days (8–21 days). Similarly, grade 2–4 thrombocytopenia/anemia were reported in all patients. The median duration of thrombocytopenia less than 20 000/μL was 9 days (0–18 days). All patients received platelet transfusions during HD-CT. The median amount of platelet transfusion was 55 units (20–200 units). Twenty patients received red blood cell transfusions during the chemotherapy. The median amount of transfusion was 4 units (0–11 units). Discontinuity of chemotherapy was not necessary for this hematological toxicity.

As expected, the other most common non-hematological side-effects were mucositis and nausea/vomiting. Diarrhea was also common. The most frequent grade 3/4 complications were nausea/vomiting, which were sufficiently controlled with anti-emetic therapy. Severe neurotoxicity was rare.

No specific investigations regarding late toxicity have yet been performed. At present, no patient developed therapy-related secondary leukemia.

Discussion

This study on first-line chemotherapy with HD-CT/PBSCT consisting of carboplatin, etoposide and ifosfamide was carried out in cooperation with 30 centers within the Japan Blood Cell Transplantation Study Group. The objectives of this trial were to determine the outcome, feasibility and toxicity of HD-CT/PBSCT in a multicenter setting. The rationale for HD-CT is based on the assumption that the front-line use of HD-CT may induce cell death in a higher fraction of sensitive and intermediate-sensitive GCT before drug resistance develops. This assumption is based on the observation in several solid tumor types including lymphomas and testicular cancer, that applying chemotherapy with a higher dose-intensity may lead to improved outcome.[18,19] Several phase II studies on the use of first-line HD-CT in testicular GCT have investigated schedules consisting of two to three standard-dose cycles followed by high-dose cycles.[9–11] These phase II studies have reported 2-year survival rates of 70–80% following first-line HD-CT, indicating that a 15–20% survival advantage may be achievable with first-line HD-CT as compared with standard BEP therapy.[9–11] In the phase II study, Motzer *et al.* demonstrated that first-line high-dose chemotherapy is well tolerated, and suggested a survival advantage following this approach compared to a historical control group treated with vinblastine, actinomycin-D, cyclophosphamide, cisplatin and bleomycin.[9] In a subsequent trial by the same investigators, poor prognosis patients with insufficient marker decline following two cycles of standard-dose VIP therapy received two cycles of high-dose carboplatin, etoposide and cyclophosphamide therapy followed by autologous stem cell support. Among 58 patients treated with this approach, 50% remained disease-free as compared to 25% of control patients who only received standard-dose therapy.[10] The present study demonstrated that first-line HD-CT with PBSCT achieved a 37.5% response rate, with NC in another 62.5% of patients, and that the 2-year survival rate was 75% following this chemotherapy. This result is comparable to those phase II studies.

The only randomized study investigating HD-CT as part of the first line chemotherapy for poor-risk GCT applied HD-CT with autologous bone marrow transplantation as consolidation after three cycles at standard doses was by Chevreau *et al.*[20] In this study, patients received four cycles of cisplatin, etoposide, vinblastine and bleomycin (PVeVB) at standard doses or three cycles of PVeVB followed by one cycle of high-dose cisplatin, etoposide and cyclophosphamide. This study failed to demonstrate a survival advantage for the high-dose group. The results of this trial are difficult to interpret, because the standard regimen contained double dose cisplatin, with approximately 30% of