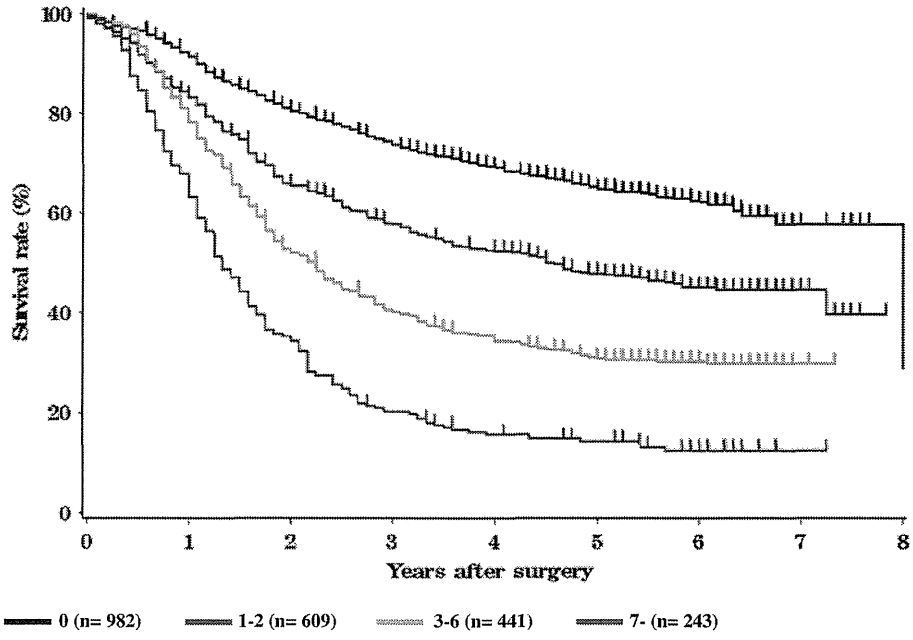

Fig. 17 Survival of patients treated by esophagectomy in relation to number of metastatic node

	Years after surgery							
	1	2	3	4	5	6	7	8
0	91.4%	81.0%	74.4%	69.4%	64.8%	62.2%	57.8%	57.8%
1-2	83.3%	65.7%	57.9%	52.6%	47.9%	45.2%	44.8%	39.8%
3-6	78.3%	52.6%	40.7%	35.5%	30.9%	30.3%	29.8%	29.8%
7-	63.1%	35.4%	20.2%	15.7%	14.3%	12.5%	12.5%	12.5%

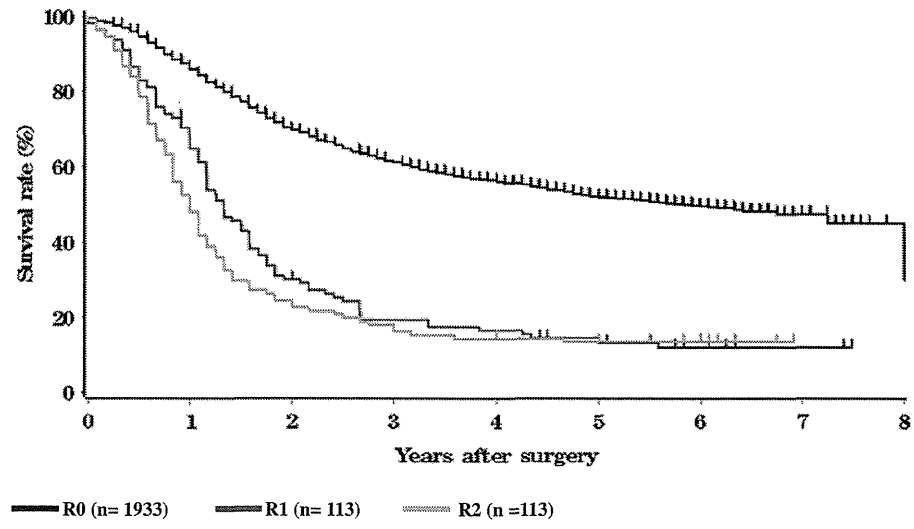

Fig. 18 Survival of patients treated by esophagectomy in relation to residual tumor: R

	Years after surgery							
	1	2	3	4	5	6	7	8
R0	86.1%	70.3%	61.6%	56.5%	52.0%	49.6%	47.5%	45.0%
R1	64.9%	30.2%	19.5%	16.5%	13.5%	12.1%	12.1%	12.1%
R2	48.0%	24.5%	18.1%	14.5%	13.5%	13.5%	13.5%	-

Clinical utility of the prostate cancer gene 3 (PCA3) urine assay in Japanese men undergoing prostate biopsy

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What's known on the subject? and What does the study add?

- It is known that a prostate cancer gene 3 (PCA3) urine assay is superior to serum PSA level or PSA-related indices for predicting a positive biopsy result in European and US men.
- This is the first report on PCA3 in a large cohort of Japanese men. The diagnostic value of the PCA3 score in Japanese men was similar to those reported in European and US men. The study concludes that a combination of PSA density and PCA3 score may be useful for selecting patients who could avoid an unnecessary biopsy.

Objective

- To examine the diagnostic performance of the prostate cancer gene 3 (PCA3) score for prostate cancer in Japanese men undergoing prostate biopsy.

Patients and Methods

- This Japanese, multicentre study included 647 Asian men who underwent extended prostate biopsy with elevated prostate-specific antigen (PSA) and/or abnormal digital rectal examination (DRE).
- Urine samples were collected after DRE.
- The PCA3 score was determined using a PROGENSA PCA3 assay and correlated with biopsy outcome. Its diagnostic accuracy was compared with that of serum PSA level, prostate volume (PV), PSA density (PSAD), and free/total PSA ratio (f/t PSA).

Results

- A total of 633 urine samples were successfully analysed (the informative rate was 98%). Median PSA was 7.6 ng/mL.
- Biopsy revealed cancer in 264 men (41.7%). The PCA3 score for men with prostate cancer was significantly higher than that for men with negative biopsies (median PCA3 score: 49 vs. 18; $P < 0.001$). The rate of positive biopsy was 16.0% in men with a PCA3 score of <20 and 60.6% in those with a PCA3 score of ≥50.
- Using a PCA3 score threshold of 35, sensitivity and specificity were 66.5 and 71.6%, respectively.
- The area under the curve of the PCA3 score was significantly higher than that of the f/t PSA in men with PSA 4–10 ng/mL (0.742 vs 0.647; $P < 0.05$).
- In men with PSAD < 0.15 and PCA3 < 20, only three (4.2%) out of 72 men had prostate cancer.

Conclusions

- The PCA3 score was significantly superior to f/t PSA in predicting a positive biopsy result for prostate cancer in Japanese men with PSA 4–10 ng/mL.
- The combination of PSAD and PCA3 score may be useful for selecting patients who could avoid an unnecessary biopsy.

Keywords

Japanese men, PCA3 urine assay, prostate cancer

Introduction

Serum PSA level has been widely used to detect prostate cancer [1]. It is organ-specific, but not cancer-specific. Several conditions, including BPH and prostatitis, may be associated with an elevated PSA level. An elevated PSA level is likely to be associated with prostate cancer, but the low specificity of PSA limits its use as a screening test and

results in a large number of unnecessary biopsies [2]. Several modifications of PSA-related indices such as PSA isoforms and volume-referenced PSA have been investigated to improve the specificity of PSA in detecting prostate cancer [3–5]. Free/total PSA ratio (f/t PSA) is widely used in clinical practice to differentiate prostate cancer from BPH in men with grey zone PSA levels, but this does not have sufficient specificity to reduce unnecessary biopsies. Thus, more accurate and reliable assessments are needed to select candidates for prostate biopsy.

Bussemakers et al. [6]reported that prostate cancer gene 3 (PCA3) produces an untranslated, prostate-specific messenger RNA (mRNA) that is highly overexpressed in prostate cancer tissue compared with its level in normal or benign tissue. Several studies have shown that PCA3 urine assay is superior to serum PSA level or various PSA isoforms for predicting prostate cancer in European and US men, and it could be used as a diagnostic tool to select biopsy candidates [7–10]. We have previously demonstrated the high specificity of PCA3 urine assay in detecting prostate cancer in a limited number of Japanese men at a single institution [11]. In the present multicentre study, we investigated the diagnostic performance of this assay in a large cohort of Japanese men.

Methods

The study protocol was approved by the institutional review boards and all men provided written informed consent before enrolment in the present study. A total of 647 men with elevated serum PSA levels and/or an abnormal DRE were enrolled. They underwent systematic extended prostate biopsy (≥8 cores) at one of four Japanese institutions (Kyoto Prefectural University of Medicine, Japanese Red Cross Medical Centre, University of Tsukuba and Kinki University) from 2009 to May 2011. The ethnic origin of all patients was Asian. Among the 647 cases, 158 had a previous negative biopsy. The exclusion criteria were as follows: previous history of prostate cancer, taking medication known to affect serum PSA levels, UTIs, and history of invasive treatment for BPH. The first voided urine sample was collected after a DRE, and the urine specimen was examined using a PROGENSA PCA3 assay according to a previously described method [11]. The PCA3 score was determined using PCA3 mRNA copy divided by PSA mRNA copy. The f/t PSA was measured in men with PSA 4–10 ng/mL. Prostate volume (PV) was measured using ultrasonography and PSA density (PSAD) was calculated by dividing PSA by PV. Clinical and pathological outcomes such as clinical stage, Gleason score and percentage of positive cores (% positive cores) in men diagnosed with prostate cancer were correlated with PCA3 score. The % positive cores was calculated as the number of positive cores divided by the number of cores taken, and patients were divided into two groups according to % positive cores (≤33% or >33%). Indolent cancer was defined, according to the Epstein criteria, as clinical stage T1c, PSAD < 0.15, Gleason score ≤ 6, and <3 positive cores on a six-core biopsy, which was replaced by % positive cores ≤ 33 with biopsy sampling of more than six cores [12]. The Mann–Whitney test was used to compare continuous variables among the groups. The chi-squared test was used to assess nominal variables. Bivariate analysis (Pearson's correlation coefficient '*r*') was used to test the linearity of relationships among the variables. Areas under the receiver–operator curves (AUCs) were compared. Multiple stepwise logistic regression analysis was used to determine the significant predictors of positive biopsy among variables such as repeated biopsy or not, PSA, PV, PSAD and PCA3 score. These statistical analyses were performed using commercially available software (SPSS version 12.0, Chicago, IL, USA). A *P* value of <0.05 was considered to indicate statistical significance.

Results

Among 647 urine samples, 633 were successfully analysed (the informative rate was 98%). The median (range) age, PSA level and number of biopsy cores taken were 69 (42–89) years, 7.6 (1.4–1908) ng/mL and 12 (6–59), respectively. Two patients had a six-core biopsy. There was no relationship between the PCA3 score and serum PSA level ($r = 0.049$, nonsignificant). Prostate biopsy was positive for prostate cancer in 264 men (41.7%). The characteristics of the men with positive and negative biopsies are shown in Table 1. The median PCA3 score in men with prostate cancer was significantly higher than that in those without prostate cancer (49 vs 18, $P < 0.001$). The positive rate of biopsy is shown in Table 2. We excluded all men who had a PSA level > 50 ng/mL and men who had initial biopsy with PSA of 20–50 ng/mL from further analyses because of the high yield of positive biopsy results; thus, the remaining 578 men were entered for analysis. The percentage of men with positive biopsy increased with increasing PCA3 score (Fig. 1). In men with a PCA3 score < 20, 16.0% (38/237) had a positive biopsy. When the PCA3 score was ≥50, the percentage of patients with a positive biopsy was 60.6% (106/175). Sensitivity, specificity, positive and negative predictive values of PCA3 scores at different PSA thresholds are shown in Table 3. Using a PCA3 threshold of 35, the sensitivity, specificity and diagnostic accuracy were 66.5, 71.6 and 69.7%, respectively. The AUCs of PSA, PV, PSAD and PCA3 score in 561 available men were 0.583, 0.706, 0.712 and 0.748, respectively. There was a significant difference in AUC between PSA and PCA3 score ($P < 0.001$), but not between PSAD and PCA3 score. Thirty-eight out of 408 men with PSA of 4–10 ng/mL had missing values of either PV or f/t PSA. In 370 available men

Table 1 Characteristics of patients with negative and positive biopsy results.

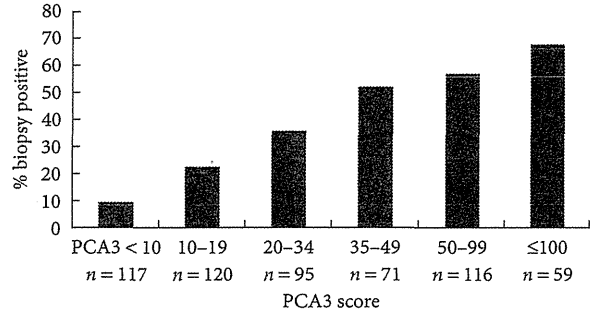
Characteristic	Negative biopsy, $n = 369$	Positive biopsy, $n = 264$	P
	Median (range)	Median (range)	
Age, years	67 (42–89)	71 (49–88)	<0.001
PSA, ng/mL	7.0 (1.4–42.6)	9.0 (2.2–1908)	<0.001
PV*, mL	38 (9.4–130)	29.1 (8.2–109)	<0.001
PSAD	0.18 (0.03–1.38)	0.36 (0.07–80.84)	<0.001
No. of cores	12 (6–59)	12 (6–40)	N.S.
PCA3 score	18 (0–381)	49 (1–288)	<0.001

**22 cases not available. N.S., nonsignificant.*

Table 2 Positive rate of prostate cancer by serum PSA range.

PSA, ng/mL	Total		Initial biopsy		Repeat biopsy	
	n	Prostate cancer (%)	n	Prostate cancer (%)	n	Prostate cancer (%)
≤4	22	5 (22.7)	21	5 (23.8)	1	0 (0)
4–10	408	140 (34.3)	316	120 (38.0)	92	20 (21.7)
10–20	131	64 (48.8)	85	44 (51.8)	46	20 (43.5)
20–50	46	29 (63.0)	29	23 (79.3)	17	6 (37.9)
>50	26	26 (100)	25	25 (100)	1	1 (100)
Total	633	264 (41.7)	476	217 (45.6)	157	47 (29.9)

Fig. 1 Percentage of men with positive biopsy by PCA3 score range ($N = 578$).



with PSA of 4–10 ng/mL, the AUCs of PSA, f/t PSA, PV, PSAD and PCA3 score were 0.557, 0.647, 0.686, 0.692 and 0.742, respectively. There was a significant difference in AUC between f/t PSA and PCA3 score ($P < 0.05$), but not between PSAD and PCA3 score (Fig. 2). On univariate regression analysis, all variables had a significant association with biopsy outcome. Multivariate logistic regression analysis showed that PCA3 score ($P < 0.001$), PSAD ($P < 0.001$), PV ($P < 0.01$) and repeated biopsy ($P < 0.01$) were independent factors predicting biopsy outcome (Table 4). Totals of 21.1% (32/152), 22.9% (27/118) and 51.5% (150/291) of patients had a positive biopsy in patients with PSAD < 0.15, 0.15–0.2 and ≥0.2, respectively. The percentage of men with positive biopsy according to the combination of PSAD and PCA3 score is shown in Fig. 3. The percentage of patients with a positive biopsy increased with higher PCA3 scores in subgroups based on PSAD. Only three (4.1%) out of 72 cases with PSAD < 0.15 and PCA3 score < 20 had prostate cancer. A total of 43% had a positive biopsy in men with PSAD < 0.15 and PCA3 score ≥50. In 264 men diagnosed with prostate cancer, a total of 72, 103 and 89 cases had Gleason scores ≤ 6, 7, and ≥8, respectively. Median (range) PCA3 scores in men with Gleason scores ≤ 6, 7 and ≥8 were 45 (4–280), 51 (4–288), and 45 (1–250), respectively. There was no significant difference in PCA3 score among these three groups. A total of 88, 133, 35 and eight patients had clinical stage T1c, T2, T3, and T4, respectively. Median (range) PCA3 scores in men with clinical stage T1c, T2, T3, and T4 were 44 (6–242), 55 (4–280), 49 (1–288), and 51 (15–123), respectively. There was no significant difference in PCA3 score among the four categorical groups of clinical stage. There was a significant association between PCA3 score and % positive cores ($r = 0.166$, $P < 0.01$). Data on % positive cores was not available in two patients. A total of 164 and 98 cases had % positive cores ≤ 33, and >33, respectively. A total of 12 and 248 cases had indolent cancer and significant cancer, respectively. There was no significant difference in PCA3 score between % positive cores ≤ 33 and >33 (median PCA3 score 47 vs 58), or between indolent cancer and significant cancer (median PCA3 score 39 vs 49).

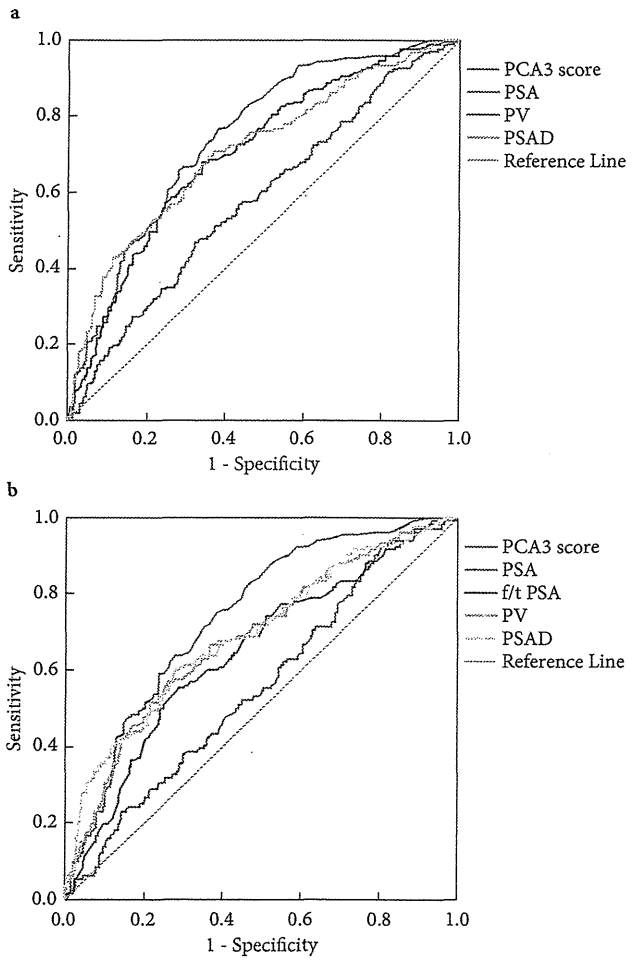
Discussion

In the present study, we investigated the ability of a PCA3 urine assay to predict the prostate biopsy outcome in a

Table 3 Sensitivity, specificity, positive and negative predictive values at different PCA3 score thresholds.

PCA3 score	Sensitivity	Specificity	Positive predictive value	Negative predictive value
10	94.9	29.2	44.3	90.6
20	82.3	54.8	51.9	84.0
35	66.5	71.6	58.1	78.3
50	49.3	81.0	60.6	73.0
100	18.6	94.8	67.8	66.3

Fig. 2 A, Receiver - operator curve (ROC) analysis to predict positive biopsy results ($n = 561$), PSA: 0.583, PV: 0.706, PSAD: 0.712, PCA3 score: 0.748. PSA vs. PCA3 score: $P < 0.001$, PV, PSAD vs. PCA3 score N.S. **B**, ROC analysis to predict positive biopsy result in patients with PSA between 4 and 10 ng/mL ($n = 370$), PSA: 0.557, f/t PSA: 0.647, PV: 0.686, PSAD: 0.692, PCA3 score: 0.742, PSA vs PCA3 score $P < 0.001$, f/t PSA vs. PCA3 score: $P < 0.05$, PV, PSAD vs. PCA3 score N.S.



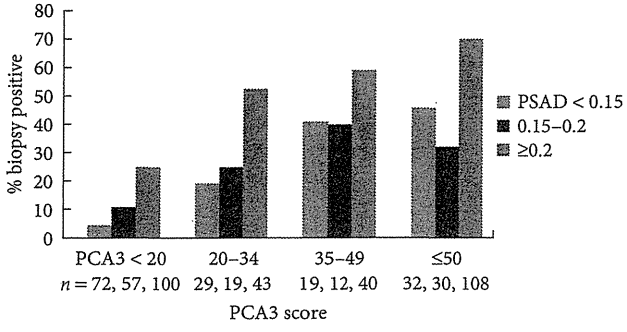
large cohort of patients from four major institutions in Japan. We found the highly informative specimen rate of 98% using a PROGENSA PCA3 assay, which verified the results of multiple studies [7–11]. We observed an increasing incidence of prostate cancer in men with a higher PCA3 score. The diagnostic performance of PCA3 urine assay for prostate cancer was excellent, with an AUC of 0.748 in Japan, compared with other reports in North America and Europe ranging from 0.66 to 0.69 [8–10,13]. Adam et al. [14] reported that the AUC of PCA3 was 0.7054 in a South African setting consisting of 68% black men. At a PCA3 threshold of 35, which is considered to be a better balanced value between sensitivity and specificity, the specificity of the PCA3 score was 71.6% in Japanese men, compared with 72–76% in North American and European men and 50% in South African men. These results showed that the diagnostic performance of PCA3 assay in Japan was similar to that reported in different regions and ethnic groups.

Free/total PSA ratio is widely used to stratify the risk of prostate cancer in men with PSA 4–10 ng/mL, and a lower f/t PSA is more likely to be found in association with prostate cancer [4]. In the present study, we found that the diagnostic performance of PCA3 score surpassed that of f/t PSA in men with PSA 4–10 ng/mL. The AUC of the PCA3 score was highest among the variables analysed and it was significantly higher than that of f/t PSA (0.742 vs 0.647; $P < 0.05$). In the placebo arm of the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial, 1140 men who had a negative baseline biopsy received a PCA3 score before repeat biopsy at 2 years [15]. In its largest repeat biopsy study to date, the AUCs of PCA3 score, PSA, and f/t PSA were 0.693, 0.612 and 0.637, respectively. A significant difference was found between PCA3 score and PSA, but the difference between PCA3 score and f/t PSA did not reach statistical significance ($P = 0.065$). In 463 European men with repeat biopsy, the AUC of PCA3 score was higher than that of f/t PSA (0.658 vs 0.578); however, the difference in AUCs between PCA3 score and f/t PSA did not reach statistical significance either [10]. We confirmed that PCA3 score was significantly superior to f/t PSA and PSA in predicting biopsy outcome in Japanese men with grey zone PSA levels.

Table 4 Univariable and multivariable logistic regression analysis for positive biopsy.

Variable	Univariable			Multivariable		
	OR	95% CI	P	OR	95% CI	P
Repeat biopsy	0.626	0.422–0.930	<0.05	0.521	0.319–0.849	<0.01
PSA	1.058	1.022–1.096	<0.01			N.S.
PV	0.955	0.942–0.968	<0.001	0.978	0.963–0.992	<0.01
PSAD	60.885	18.671–198.536	<0.001	18.883	4.805–74.208	<0.001.
PCA3 score	1.017	1.013–1.022	<0.001	1.015	1.010–1.020	<0.001

Fig. 3 Percentage of men with positive biopsy by combination of PCA3 score range and PSAD range.



Multivariable logistic regression showed that PCA3 score ($P < 0.001$), PSAD ($P < 0.001$), PV ($P < 0.01$) and repeated biopsy ($P < 0.01$) were independent predictors of positive biopsy in the present study. Several studies showed that PCA3 score was significantly cooperating with PSA and PV for predicting prostate cancer [9,10,13]. PSA correlates with PV in men without prostate cancer; thus, PSAD (PSA divided by PV) was used for more accurate assessment to improve the specificity in diagnosis of prostate cancer; however, the commonly used PSAD threshold of 0.15 could not detect > 40% of cancers, resulting in limited usefulness in a routine clinical setting [5]. In the present study, 21.1% of men with PSAD < 0.15 had a positive biopsy. Thus, when a PSAD threshold value of 0.15 was applied in our population, a considerable number of prostate cancer cases were missed. When the PCA3 score was combined with PSAD, we observed that the rate of positive biopsy increased, even in the subgroup of men with PSAD < 0.15 (Fig. 3). When the PCA3 score was <20 in men with PSAD < 0.15, only three (4.1%) out of 72 men had prostate cancer. By contrast, 43% of men with PCA3 score ≥50 and PSAD < 0.15 had a positive biopsy. The combination of PSAD and PCA3 score stratifies the risk of prostate cancer and predicts a low risk of prostate cancer, suggesting that these men could avoid unnecessary biopsy. It is notable that these three patients with cancer (PSAD < 0.15 and PCA3 score <20) had significant cancer with % positive cores of ≤33 and Gleason score > 6 as biopsy pathological features.

Several studies have shown the significant relationship between PCA3 score and tumour volume in prostatectomy specimens and the ability of PCA3 score to discriminate low-volume/low-grade cancer with the aim of selecting patients who are candidates for active surveillance [16,17]. Ploussard et al. [17]reported that a high PCA3 score of >25 was an important predictor of large tumour volume with an odds ratio of 5.4 ($P = 0.1$) and significant cancer with an odds ratio of 12.7 ($P = 0.003$). In the present study, we found a significant relationship between PCA3 score and % positive cores; however, there was no difference in PCA3 score between % positive ≤ 33 and >33, or among subgroups by Gleason score and clinical stage. Further investigation of the correlation between PCA3 score and pathological outcomes on prostatectomy specimens will be needed in Japan.

In the present cohort, men with a wide range of PSA levels were enrolled. A PCA3 urine assay would be irrelevant in men with a high PSA level (the positive rate of prostate cancer was 100% in men with PSA > 50 ng/mL, and 79.3% in men with PSA 20–50 ng/mL at initial biopsy); thus, these were excluded from the analysis for prediction of biopsy outcome. Furthermore, not all men received prostate volume measurement because this was a multi-institutional study. These features might have influenced the results.

In conclusion, this Japanese multicentre study shows that the PCA3 urine assay could improve the prediction of prostate cancer and may help in selecting men who might benefit from prostate biopsy. The percentage of patients with positive biopsy increased with higher PCA3 scores. PCA3 score was superior to f/t PSA for predicting prostate cancer in patients with PSA 4–10 ng/mL. A combination of PSAD and PCA3 score may be useful for selecting patients who could avoid an unnecessary biopsy.

Acknowledgements

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Conflict of Interest

None declared.

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Abbreviations: PCA3, prostate cancer gene 3; f/t PSA, free/total PSA ratio; mRNA, messenger RNA; PV, prostate volume; PSAD, PSA density; AUC, area under the receiver–operator curve.

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RADIATION ONCOLOGY

RESEARCH Open Access

Vaginal tolerance of CT based image-guided high-dose rate interstitial brachytherapy for gynecological malignancies

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Abstract

Background: Purpose of this study was to identify predictors of vaginal ulcer after CT based three-dimensional image-guided high-dose-rate interstitial brachytherapy (HDR-ISBT) for gynecologic malignancies.

Methods: Records were reviewed for 44 female (14 with primary disease and 30 with recurrence) with gynecological malignancies treated with HDR-ISBT with or without external beam radiation therapy. The HDR-ISBT applicator insertion was performed with image guidance by trans-rectal ultrasound and CT.

Results: The median clinical target volume was 35.5 ml (2.4-142.1 ml) and the median delivered dose in equivalent dose in 2 Gy fractions (EQD_2) for target volume D_{90} was 67.7 Gy (48.8-94.2 Gy, doses of external-beam radiation therapy and brachytherapy were combined). For re-irradiation patients, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 91.1 Gy, 100.9 Gy, 260.3 Gy, 212.3 Gy, 170.1 Gy, 117.1 Gy, 105.2 Gy, and 94.7 Gy, respectively. For those without prior radiation therapy, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 56.3 Gy, 54.3 Gy, 147.4 Gy, 126.2 Gy, 108.0 Gy, 103.5 Gy, 94.7 Gy, and 80.7 Gy, respectively. Among five patients with vaginal ulcer, three had prior pelvic radiation therapy in their initial treatment and three consequently suffered from fistula formation. On univariate analysis, re-irradiation and vaginal wall D_{2cc} in EQD_2 was the clinical predictors of vaginal ulcer ($p = 0.035$ and $p = 0.025$, respectively). The ROC analysis revealed that vaginal wall D_{2cc} is the best predictor of vaginal ulcer. The 2-year incidence rates of vaginal ulcer in the patients with vaginal wall D_{2cc} in EQD_2 equal to or less than 145 Gy and over 145 Gy were 3.7% and 23.5%, respectively, with a statistically significant difference ($p = 0.026$).

Conclusions: Re-irradiation and vaginal D_{2cc} is a significant predictor of vaginal ulcer after HDR-ISBT for gynecologic malignancies. Three-dimensional image-guided treatment planning should be performed to ensure adequate target coverage while minimizing vaginal D_{2cc} in order to avoid vagina ulcer.

Keywords: Gynecologic brachytherapy, High-dose-rate brachytherapy, Interstitial brachytherapy, Vaginal ulcer

Introduction

High-dose rate intracavitary brachytherapy (HDR-ICBT) is an established method in the management of gynecological malignancies, especially in cervical cancer. However, in patients with a narrow vagina, short uterine cavity, distal vaginal extension, and bulky tumors in which the optimal dose distribution cannot be obtained by intracavitary brachytherapy (ICBT), interstitial brachytherapy (ISBT) is employed. Also in patients with bulky postoperative central pelvic recurrence, ISBT has proven to be effective [1-5]. With the advent of image-guided brachytherapy it has become possible to assess the dose volume histogram (DVH) in brachytherapy. Several studies have validated the D_{2cc} as a predictor of rectal and bladder toxicities for ICBT [6] or for ISBT [7]. D_{2cc} of the rectum and bladder have been introduced into daily clinical practice of gynecological image-guided brachytherapy. However in ICRU 38 vagina was not recognized as organ at risk

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during brachytherapy tough it is adjacent to target volume and radioactive sources [8].

The purpose of this study was to retrospectively analyze the incidence of vaginal morbidities after HDR-ISBT for gynecological cancers and to find clinical and dosimetric factors which affect the incidence of the vaginal morbidities.

Methods

The inclusion criteria of this single institutional retrospective study were patients with gynecological malignancies who were treated by HDR-ISBT with or without external beam radiation therapy (EBRT) with a follow-up length exceeding 6 months or more. Patients with distant metastasis outside of pelvis were excluded from current study. HDR-ISBT was applied for both primary and salvage intents. Patients with superficial vaginal disease with thickness less than 5 mm were treated with HDR-ICBT and did not treated by HDR-ISBT; therefore these patients were not included in this analysis. Also HDR-ISBT was not applied for those patients who had distant metastasis or for those patients with far advanced tumors which had not responded to EBRT performed before HDR-ISBT. These patients were treated with EBRT alone. One patient who succumbed to progressive cancer in 5.5 months after ISBT was also excluded in this analysis. The medical records of all patients with gynecological malignancies treated with HDR-ISBT at the National Cancer Center Hospital, Tokyo, Japan, between 2008 and 2011 were retrieved and 44 patients were included in this study.

In the patients without prior pelvic irradiation, pelvic EBRT was delivered before HDR-ISBT. The common EBRT portals were whole pelvic irradiation including gross tumor volume (GTV) with adequate margin as well as the pelvic lymph nodes basin up to the level of the common iliac (L4/5 junction). If the tumor involved the lower third of the vagina, or there were clinically palpable inguinal nodes, inguinal regions were also included in the EBRT portals. The initial 20-40 Gy was delivered to the whole pelvis with a 4-fields box technique and then pelvic irradiation was administered with a central shield being employed to reduce exposure of organs at risk (OAR). The total dose delivered to the pelvic side wall was up to 50 Gy in a conventional fractionation. In patients with a history of prior pelvic radiation therapy or in feeble elderly patients, no EBRT or smaller EBRT fields with a reduced total dose were employed. HDR-ISBT was basically performed after the central shield was inserted. However for those patients treated without EBRT, HDR-ISBT was applied as solitary radiotherapy modality. The detailed procedure of gynecological HDR-ISBT was described elsewhere [9]. In brief, transperineal needle applicator insertion was performed under either general or local anesthesia with the patients in lithotomy position and guided by trans-rectal ultrasound (TRUS) or CT which can be taken with the patients lying in lithotomy position with the applicators in place. For advanced large disease, a Syed-Neblett perineal template (Best Medical International, Inc., Springfield, VA) was used in order to sufficiently cover lateral disease extent. For rather localized small disease, with limited parametrial and/or paracolpial invasion, free-hand needle applicator insertion with or without a vaginal applicator was used with fewer needles inserted compared to the Syed-Neblett perineal template. Treatment planning was performed with brachytherapy planning system (Oncentra® Nucletron, Veenendaal, The Netherlands) using CT images taken by the large bore CT simulator (Aquilion LG®, Toshiba, Tokyo, Japan), which allows imaging of the patients in lithotomy position. Although different applicator was used throughout the patients, the calculation method applied was the same. The clinical target volume (CTV) was defined based on the CT image obtained after needle insertion, as well as physical examination immediately before needle insertion, the intra-operative TRUS image and the most recent MRI were also taken into account. Reference points were set on the surface of CTV and prescribed dose was delivered to those points. HDR-ISRT treatment plan was calculated initially by geometrical optimization or volume optimization and then manual graphical modification was followed to enclose the CTV by the prescription dose while minimizing high dose to OAR. The median HDR-ISBT dose was 24 Gy (range, 18-54 Gy), and median HDR-ISBT dose per fraction was 6 Gy (range, 4-6 Gy). HDR-ISBT was performed twice daily with each fraction 6 hours apart. HDR-ISBT was performed with MicroSelectron HDR (Nucletron, Veenendaal, The Netherlands) using Ir-192.

At the discretion of the attending physician, weekly CDDP 40 mg/m^2 was used in 10 patients concurrently with EBRT. In general, patients with bulky disease, good performance status and adequate organ function were selected for the candidate for the administration of concurrent chemoradiation. Patients were seen in follow up 1 week after HDR-ISBT for a skin check, then every 1-2 months for 2 years, every 3-4 months for 5 years, and every 6-12 months thereafter.

When adding doses of EBRT, HDR-ISBT, and HDR-ICBT, we used the equivalent dose in 2 Gy fractions (EQD_2) according to the LQ model [10,11]. For re-irradiated patients, prior central pelvic EBRT doses were also added to EQD_2 for OARs. For those who had prior HDR-ICBT without DVH parameters of OARs because of lack of three dimensional dose calculations, it was difficult to estimate EQD_2 for OARs. Therefore, prescribed dose for tumor in EQD_2 ($\alpha/\beta = 10$) was converted to EQD_2 for late responding tissue ($\alpha/\beta = 3$) and added

together. Time interval between prior RT and the current RT was not taken into consideration in this analysis.

Rectum and bladder were contoured as a whole organ. Vaginal wall was extracted with a thickness of 4 mm on all CT images according to the Vienna group [12]. As for rectum and bladder, dosimetric parameter of D_{2cc} was used because these values have been validated by several studies [6-8]. On the other hand, there is no validated parameter for vaginal dose; therefore $D_{0.5cc}$, D_{1cc}, D_{4cc}, D_{6cc}, and D_{8cc} were calculated along with D_{2cc} for vaginal wall dose volume parameters.

Late vaginal morbidities were retrospectively evaluated according to LENT-SOMA scales [13]. Because morbidity scores were evaluated retrospectively in this study, we focused on only vaginal ulcer which could be regarded as one of the severest symptoms and could be retrieved accurately from medical records.

Student's unpaired t-test was used to compare the continuous variables and Pearson's chi-square test to compare categorical variables. A p value of < 0.05 was considered as statistically significant. In addition, calculation of the area under the curve (AUC) of receiver operating characteristics (ROC) was used to determine the most predictive dosimetric parameter of vaginal ulcer. The predictive values of parameters were evaluated based on the AUC. The optimal threshold for each parameter was defined as the point yielding the minimal value for $(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$, which is the point on the ROC curve closest to the upper left-hand corner [14]. The obtained cutoff point was used for dividing patients into two groups and the incidences of vaginal ulcer were calculated by Kaplan-Meier method with the difference evaluated by log-lank test. All statistical analyses were performed using SPSS Statistics version 18.0 (SAS Institute, Tokyo, Japan).

This retrospective study was approved by the institutional review board of the National Cancer Center.

Results

There were 44 patients who met the eligibility criteria and 36 patients were alive at the time of the analysis (May 2012). The median follow-up length of living patients was 18.3 months (range, 7.6-39.5 months). The pretreatment characteristics of the 44 patients included in this study are summarized in Table 1. Median age was 56 years (range, 25-89 years). HDR-ISBT was applied as the primary therapy in 14 patients (31.8%) and as the salvage therapy in 30 patients (68.2%). Eight patients (18.2%) had previously received pelvic irradiation, in the form of EBRT and/or ICBT. Twenty four patients were treated with Syed-Neblett perineal template, 17 with free-hand with vaginal applicator and three with free-hand without vaginal applicator. Treatment details are summarized in Table 2. Ten patients underwent concurrent chemotherapy. In most cases HDR-ISBT dose per fraction was 6 Gy. Median total EQD_2 of CTV D_{90} was 67.7 Gy. Median EQD_2 of D_{2cc} for rectum and bladder was 60.8 Gy and 58.1 Gy, respectively. Median EQD_2 of $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc}, and D_{8cc} for vaginal wall were 210.7 Gy, 167.3 Gy, 131.5 Gy, 111.6 Gy, 100.0 Gy, and 83.2 Gy, respectively. Table 3 shows EQD_2 of rectum, bladder and vaginal wall for the patients with or without prior pelvic radiation therapy. For re-irradiation patients, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 91.1 Gy, 100.9 Gy, 260.3 Gy, 212.3 Gy, 170.1 Gy, 117.1 Gy, 105.2 Gy, and 94.7 Gy, respectively. For those without prior radiation therapy, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 56.3 Gy, 54.3 Gy, 147.4 Gy, 126.2 Gy, 108.0 Gy, 103.5 Gy, 94.7 Gy, and 80.7 Gy, respectively (Table 3). In EQD_2 of D_{2cc} for rectum, bladder and vaginal wall the difference was statistically significant ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively).

As for late morbidities of vagina, five patients experienced vaginal ulcer after HDR-ISBT. All of vaginal ulcer occurred within two years after completion of the HDR-ISBT. Patient characteristics and objective/management

Table 1 Patients characteristics (n = 44)

		Patients (n)
Median age (years, range)		56 (25-89)
Primary site	Cervix	24 (54.6%)
	Vagina	12 (27.3%)
	Corpus	5 (11.3%)
	Ovary	2 (4.5%)
	Vulva	1 (2.3%)
Primary therapy		14 (31.8%)
	Cervical cancer	4 (9.1%)
	Vaginal cancer	10 (22.7%)
Salvage therapy		30 (68.2%)
	Post ope regidual tumor	5 (11.4%)
	Post ope recurrent tumor	21 (47.7%)
	Post RT recurrent tumor	4 (9.1%)
Histology	Scc	25 (56.8%)
	Adeno	16 (36.4%)
	Others	3 (6.8%)
Prior pelvic RT*	Yes	8 (18.2%)
	No	36 (81.8%)
Median tumor size (cm, range)		3.6 (1.0-8.0)
Pelvic LN† metastais	Yes	11 (25%)
	No	33 (75%)

*RT radiation therapy.
†LN lymph node.

Table 2 Treatment details (n = 44)

	Median range
Central pelvic dose of EBRT* (Gy)	30 (0-50)
No. of needles used in HDR-ISBT†	15 (5-29)
HDR-ISBT† fractions	4 (3-9)
HDR-ISBT† dose per fraction (Gy)	6 (4-6)
CTV†† (ml)	35.1 (2.4-142.1)
CTV†† D_{90} in EQD_2‖ (Gy)	67.7 (48.8-94.2)
Rectum D_{2cc}¶ in EQD_2‖ (Gy)	60.8 (30.5-114.3)
Bladder D_{2cc}¶ in EQD_2‖ (Gy)	58.1 (7.3-120.3)
Vaginal wall $D_{0.5cc}$¶ in EQD_2‖ (Gy)	210.7 (51.5-468.1)
Vaginal wall D_{1cc}¶ in EQD_2‖ (Gy)	167.3 (49.9-352.1)
Vaginal wall D_{2cc}¶ in EQD_2‖ (Gy)	131.5 (43.7-294.4)
Vaginal wall D_{4cc}¶ in EQD_2‖ (Gy)	111.6 (34.0-200.8)
Vaginal wall D_{6cc}¶ in EQD_2‖ (Gy)	100.0 (20.4-173.7)
Vaginal wall D_{8cc}¶ in EQD_2‖ (Gy)	83.2 (10.3-144.4)
Concurrent chemotherapy	
Yes	10 patients
No	34 patients

*EBRT: external beam radiation therapy.
†HDR-ISBT: high-dose-rate interstitial brachytherapy.
††CTV: clinical target volume.
‖EQD2: equivalent dose in 2 Gy fractions.
¶D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6 and 8 cm3 of tissue.

Table 3 DVH parameters for bladder and vaginal wall with or withour prior radiation therapy

	Prior pelvic RT[J] (+) (n = 8)	Prior pelvic RT[J] (-) (n = 36)	*p* value
eMedian rectum D_{2cc}† (EQD_2*, Gy, range)	91.1 (71.0-114.3)	56.3 (30.5-82.7)	< 0.001*
Median bladder D_{2cc}† (EQD2*, Gy, range)	100.9 (69.7-120.3)	54.3 (7.3-82.7)	< 0.001*
Median vaginal wall $D_{0.5cc}$† (EQD2*, Gy, range)	260.3 (59.9-349.3)	147.4 (47.9-267.3)	0.109
Median vaginal wall D_{1cc}† (EQD2*, Gy, range)	212.3 (58.2-277.5)	126.2(33.6-182.7)	0.013
Median vaginal wall D_{2cc}† (EQD2*, Gy, range)	170.1 (56.6-247.5)	108.0 (31.7-150.9)	0.001*
Median vaginal wall D_{4cc}† (EQD2*, Gy, range)	117.1 (34.0-200.8)	103.5 (39.1-139.4)	0.139
Median vaginal wall D_{6cc}† (EQD2*, Gy, range)	105.2 (33.0-173.7)	94.7 (20.4-138.7)	0.097
Median vaginal wall D_{8cc}† (EQD2*, Gy, range)	94.7 (32.4-144.4)	80.7 (10.3-130.4)	0.105

[J]RT: radiation therapy.
*EQD2: equivalent dose in 2 Gy fractions.
†D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6, and 8 cm3 of tissue.

scores of vaginal ulcer according to LENT-SOMA are summarized in Table 4. Two patients had superficial and > 1 cm^2 vaginal ulcer and three had vaginal fistula (two vesicovaginal fistulae and one vesicovaginorectal fistula). Three out of the five patients had prior pelvic irradiation and the interval between prior pelvic irradiation and secondary pelvic irradiation was 15, 27, and 40 months, respectively. All of the three patients with vaginal fistula received hyperbaric oxygen therapy without success. Two underwent surgical intervention (one total cystectomy for massive hematuria and one nephrostomy) for their vesicovaginal fistula, while one was followed up conservatively with a persistent vesicovaginal fistula. The other two patients with grade 2 vaginal ulcer were treated conservatively. The overall 2-year actuarial incidence of vaginal ulcer was 11.4%; 37.5% for re-irradiation patients and 5.6% for those without prior radiation therapy (Figure 1a). Comparison of dose-volume parameters of the vaginal wall is shown in Table 5 for the patient with and without vaginal ulcer. It was shown that the incidence of vaginal ulcer in the patients with prior pelvic irradiation was statistically higher than that of the patients without prior pelvic irradiation ($p = 0.035$). It was also shown that the mean EQD_2 of vaginal wall D_{2cc} of patients with or without vaginal ulcer was statistically different ($p = 0.025$). There was no relationship between administration of concurrent chemotherapy and manifestation of vaginal ulcer ($p = 0.256$), number of needles used in HDR-ISBT ($p = 0.293$) nor bladder D_{2cc} EQD_2 ($p = 0.091$). The ROC analysis revealed that vaginal wall D_{2cc} was the best dosimetric parameter predicting the incidence of vaginal ulcer and the cutoff value of 145 Gy in vaginal wall D_{2cc} provided the lowest p value in log-rank test (Table 6). Figure 1b shows Kaplan-Meyer curve for the incidence of vaginal ulcer stratified by vaginal wall D_{2cc} 145 Gy in EQD_2. The 2-year incidence rates of vaginal ulcer in the patients with vaginal wall D_{2cc} equal to or less than 145 Gy in EQD_2 and over 145 Gy were 3.7% and 23.5%, respectively, with a statistically significant difference ($p = 0.026$).

Discussion

Although the Manchester method of ICBT for the cervical cancer was developed to avoid the occurrence of radiation induced vaginal ulcer and necrosis, vaginal ulcer is now very rarely encountered because vaginal wall is relatively radioresistant and typical ICBT delivers radiation dose less than the tolerance of the relatively radioresistant vaginal wall. In a retrospective study of cervical cancer patients using EBRT and the film based low-dose rate (LDR) brachytherapy, Samuel et al. showed that vaginal tolerance dose was above 150 Gy [15]. In recent advancement of image guided brachytherapy (IGBT), rectum and bladder doses were recommended to be

Table 4 Patient characteristics who developed vaginal ulcer

Patient no.	Age at HDR-ISBT*	Primary site	Prior pelvic RT	Interval between prior RT and HDR-ISBT* (mo)	HDR-ISBT* with/without EBRT††	Total vaginal wall $D_{0.5cc}$#/D_{1cc}#/D_{2cc}# in EQD_2## (Gy)	LENT SOMA¶ objective score	LENT SOMA¶ management score
1	40	Cervix	WPRT† 45 Gy/25fr + EBRT†† boost 15 Gy/5fr	27	HDR-ISBT* 36 Gy/9fr	272.1/202.6/169.1	4	4
2	51	Cervix	None	None	WPRT† 30 Gy/15fr + CS‖ 20 Gy/10fr + HDR-ISBT* 24 Gy/4fr	215.2/171.8/145.4	4	3
3	64	Corpus	None	None	WPRT† 30 Gy/15fr + HDR-ISBT* 30 Gy/5fr	196.6/141.5/109.1	2	1
4	64	Cervix	WPRT† 40 Gy/20fr + CS 10 Gy/5 + HDR-ICBT 18 Gy/3fr	40	HDR-ISBT* 48 Gy/8fr	465.4/352.1/294.4	2	1
5	67	Cervix	WPRT† 50 Gy/50fr + HDR-ICBT 12 Gy/3fr	15	HDR-ISBT* 42 Gy/7fr	234.0/211.1/193.5	4	3

*HDR-ISBT: high-dose-rate interstitial brachytherapy.
†WPRT: whole pelvis radiation therapy.
††EBRT: external beam radiation therapy.
‖CS: radiation therapy with center shielding.
¶LENT-SOMA: Late Effects of Normal Tissues - Subjective, Objective, Management, Analytic.
#D0.5cc, D1cc, D2cc: most exposed 0.5, 1 and 2 cm3 of tissue.
##EQD2: equivalent dose in 2 Gy fractions.

reported in the treatment of ICBT for cervical cancer but vagina was not mentioned as OAR [9]. In the GEC-ESTRO working group (II) or American Brachytherapy Society guidelines, vagina was taken into consideration for OAR but it was stated that the vaginal dose volume parameters still need to be defined [16,17]. Dimopoulos et al. reported clinical result of primary vaginal cancer treated with IGBT and they experienced two vaginal fistulae and one periurethral necrosis. However they did not specify DVH parameters of vaginal wall with vaginal complication [18]. Lee et al. reported in detail the toxicity analysis of CT based HDR-ISBT for gynecologic malignancies. They reported that D_{2cc} for the rectum was a reliable predictor of late rectal complication; however because of limited number of events it was not able to explore the DHV parameters for vaginal complication [5]. Recently, Vienna group tried to find out DVH parameters that correlate with vaginal late morbidities but vaginal D_{2cc} did not relate with the vaginal morbidities [12]. The calculation method was the same as

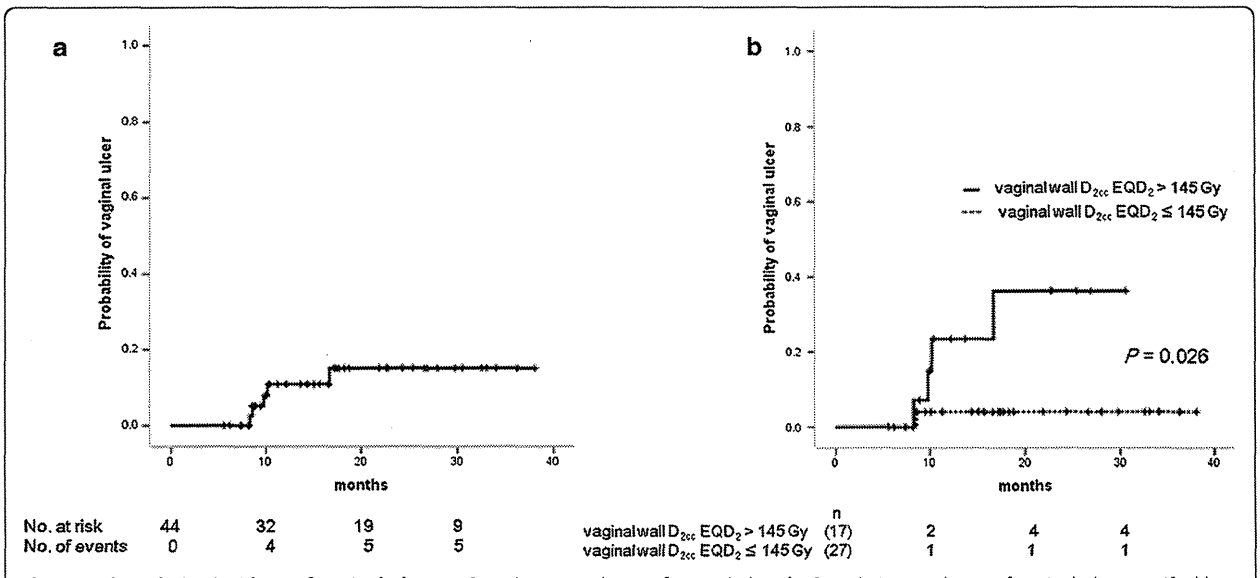

Figure 1 Cumulative incidene of vaginal ulcer. a: Cumulative incidence of vaginal ulcer. **b**: Cumulative incidence of vaginal ulcer stratified by vaginal wall D_{2cc} 145 Gy in EQD_2.

Table 5 Clinical predictors of vaginal ulcer

Characteristic		Vaginal ulcer (+) (n = 5)	Vaginal ulcer (-) (n = 39)	p value
Prior pelvic RT*		3	5	
	Yes	2	34	0.035*
	No			
Concurrent chemotherapy				
	Yes	0	10	0.256
	No	5	29	
Median number of needles used in HDR-ISBT[ʃ] (range)		14 (10-24)	15 (5-29)	0.293
Median CTV[†] (ml, range)		54.7 (17.7-114.0)	34.7 (2.4-142.1)	0.271
Median rectum D_{2cc}[††] (EQD_2[‖], Gy, range)		84.2 (34.0-100.7)	57.9 (30.5-114.3)	0.118
Median bladder D_{2cc}[††] (EQD_2[‖], Gy, range)		69.3 (37.4-113.5)	57.7 (7.3-120.3)	0.091
Median vaginal wall $D_{0.5cc}$[††] (EQD_2[‖], Gy, range)		206.4 (106.6-349.3)	149.4 (47.9-310.1)	0.243
Median vaginal wall D_{1cc}[††] (EQD_2[‖], Gy, range)		169.1 (91.6-277.5)	127.9 (33.6-220.8)	0.096
Median vaginal wall D_{2cc}[††] (EQD_2[‖], Gy, range)		152.5 (71.1-247.5)	109.0 (31.7-201.9)	0.025*
Median vaginal wall D_{4cc}[††] (EQD_2[‖], Gy, range)		115.5 (83.8-200.8)	110.6 (34.0-153.2)	0.152
Median vaginal wall D_{6cc}[††] (EQD_2[‖], Gy, range)		102.5 (60.4-173.7)	99.5 (20.4-146.3)	0.266
Median vaginal wall D_{8cc}[††] (EQD_2[‖], Gy, range)		82.0 (47.6-144.4)	84.3 (10.3-140.3)	0.511

*RT: radiation therapy.
[ʃ]HDR-ISBT.
[†]CTV: clinical target volume.
[‖]EQD2: equivalent dose in 2 Gy fractions.
[††]D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6, and 8 cm3 of tissue.

the current study, which was composed of EBRT and ICBT/ISBT and normalized to 2 Gy per fraction (EQD_2) using the linear-quadratic model with α/β of 3 Gy for the vaginal morbidities [10-12,16]. The difference between Vienna group and the current study was that there were more patients with severe vaginal morbidities in the current study, presumably because there were more patients who received re-irradiation and current study excluded the patients treated with HDR-ICBT. HDR-ISBT delivers higher dose to the vaginal wall than HDR-ICBT because the multiple needle applicators directly contact vaginal wall. According to the current results, after vaginal wall $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc}, and D_{8cc} having been compared, vaginal wall D_{2cc} was found to be the most relevant DVH parameter predicting the incidence of vaginal ulcer. ROC analysis also showed that vaginal wall D_{2cc} of 145 Gy in EQD_2 can be used as clinical cutoff dose predicting vaginal ulcer. This figure is quite similar to the vaginal tolerance dose of 150 Gy derived from a retrospective study of LDR brachytherapy which was previously mentioned [15]. The current report is the first one concerning about vaginal DVH parameter and complication using modern era of three-dimensional image-guided brachytherapy. It was also found in this study that the history of prior pelvic irradiation was another significant predictive factor for vaginal ulcer (Table 5). Lee et al. reported a patient with colovaginal fistula with previous EBRT [5,7]. As shown in Table 3,

Table 6 Dosimetric predictors for the development of vaginal ulcer

Parameter	ROC[†] AUC*	Cutoff[¶]	2-y incidence ofvaginal ulcer (%)	P value[#]
Vaginal wall $D_{0.5cc}$[‖] (EQD_2[††])	0.667	≤195 Gy	0.0	0.058
		>195 Gy	18.5	
Vaginal wall D_{1cc}[‖] (EQD_2[††])	0.682	≤171 Gy	4.2	0.091
		>171 Gy	20.0	
Vaginal wall D_{2cc}[‖] (EQD2[††])	0.733	≤145 Gy	3.7	0.026*
		>145 Gy	23.5	
Vaginal wall D_{4cc}[‖] (EQD_2[††])	0.618	≤83 Gy	0.0	0.119
		>83 Gy	15.6	
Vaginal wall D_{6cc}[‖] (EQD_2[††])	0.569	≤86 Gy	5.6	0.323
		>86 Gy	15.4	
Vaginal wall D_{8cc}[‖] (EQD_2[††])	0.559	≤75 Gy	5.6	0.323
		>75 Gy	15.4	

*AUC: area under the curve.
[†]ROC: receiver operator characteristic.
[††]EQD2: equivalent dose in 2 Gy fractions.
[‖]D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6, and 8 cm3 of tissue.
[¶]Cutoff refers to the most predictive value from the AUC of ROC curve.
[#] Univariate analysis by log-rank test.

both rectum and bladder D_{2cc} was significantly higher in patients with prior pelvic irradiation than those without prior pelvic irradiation. However both rectum and bladder D_{2cc} was not in itself a significant prognostic factor for vaginal ulcer and could not be used as a surrogate indicator (Table 5).

There were several limitations in this study. Contouring of the vagina was not based on MRI but CT, which is inferior to MRI in tissue contrast. However because 41 out of 44 patients were inserted either cylinder or mold into their vagina, contouring of vagina was considered to be precise. The time interval between the prior pelvic RT and HDR-ISBT was not taken into consideration for the calculation of the total dose for OARs. Additionally, this study was a retrospective study with small number of patients with heterogeneous tumor origin, heterogeneous treatment applied, small number of events, and with short follow-up period. Therefore we should be cautious about the results of the current study. However even tumor origin differed greatly in current cohorts of study, it is considered to be feasible because the main concern in current study was focused on only the vaginal toxicity.

It should be stressed that with the introduction of HDR-ISBT in gynecological malignancies and increment of vaginal dose, vaginal tolerance dose must be taken into consideration. Further discussion and validation of vaginal DVH parameters in image-guided brachytherapy in a multicenter prospective study is needed.

Conclusions

The DVH parameters for vagina are essential for treatment planning and optimization in image based HDR-ISBT in gynecological malignancies. Vaginal wall D_{2cc} in EQD_2 should be monitored and be kept under 145 Gy in order to avoid vaginal ulcer. Also in patients with prior pelvic irradiation, vaginal wall dose including the prior radiation dose should be kept lower than 145 Gy.

Consent

Written informed consent was obtained from the patient for the publication of this report and any accompanying images.

Abbreviations

HDR-ISBT: High-dose rate interstitial brachytherapy; EQD_2: Dose in equivalent in 2 Gy fractions; ICBT: Intracavitary brachytherapy; ISBT: Interstitial brachytherapy; DVH: Dose volume histogram; EBRT: External beam radiation therapy; GTV: Gross tumor volume; CTV: Clinical target volume; OAR: Organ at risk; AUC: Area under the curve; ROC: Receiver operating characteristics; IGBT: Image guided brachytherapy; PDR: Pulsed dose rate.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TK, MS, RY, KH, MK, SS, KT, KY, KI, MM, and YI performed the treatment. NM and JI analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Original Article

Analysis of Ki-67 Expression With Neoadjuvant Anastrozole or Tamoxifen in Patients Receiving Goserelin for Premenopausal Breast Cancer

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BACKGROUND: The increasing costs associated with large-scale adjuvant trials mean that the prognostic value of biologic markers is increasingly important. The expression of nuclear antigen Ki-67, a marker of cell proliferation, has been correlated with treatment efficacy and is being investigated for its value as a predictive marker of therapeutic response. In the current study, the authors explored correlations between Ki-67 expression and tumor response, estrogen receptor (ER) status, progesterone receptor (PgR) status, and histopathologic response from the STAGE study (Study of Tamoxifen or Arimidex, combined with Goserelin acetate to compare Efficacy and safety). **METHODS:** In a phase 3, double-blind, randomized trial (National Clinical Trials identifier NCT00605267), premenopausal women with ER-positive, early stage breast cancer received either anastrozole plus goserelin or tamoxifen plus goserelin for 24 weeks before surgery. The Ki-67 index, hormone receptor (ER and PgR) status, and histopathologic responses were determined from histopathologic samples that were obtained from core-needle biopsies at baseline and at surgery. Tumor response was determined by using magnetic resonance imaging or computed tomography. **RESULTS:** In total, 197 patients were randomized to receive either anastrozole plus goserelin (n = 98) or tamoxifen plus goserelin (n = 99). The best overall tumor response was better for the anastrozole group compared with the tamoxifen group both among patients who had a baseline Ki-67 index ≥20% and among those who had a baseline Ki-67 index <20%. There was no apparent correlation between baseline ER status and the Ki-67 index in either group. Positive PgR status was reduced from baseline to week 24 in the anastrozole group. **CONCLUSIONS:** In premenopausal women with ER-positive breast cancer, anastrozole produced a greater best overall tumor response compared with tamoxifen regardless of the baseline Ki-67 index. *Cancer* 2013;119:704-13. © *2012 American Cancer Society.*

KEYWORDS: anastrozole, aromatase inhibitor, biomarker, neoadjuvant, Ki-67, premenopausal breast cancer.

INTRODUCTION

In addition to ablative surgery, radiotherapy, and cytotoxic chemotherapy, an additional standard treatment option for premenopausal women with estrogen receptor (ER)-positive breast cancer is the ER antagonist tamoxifen, either alone or in combination with ovarian function suppression.[1] Temporary and potentially reversible ovarian suppression can be achieved by treatment with a luteinizing hormone-releasing hormone analog, such as goserelin. Goserelin in combination with tamoxifen has demonstrated improved progression-free survival and disease-free survival compared with goserelin alone in premenopausal women with hormone receptor-positive (ER-positive and/or progesterone receptor [PgR]-positive) breast cancer in the advanced[2] and adjuvant[3] settings.

Nonsteroidal aromatase inhibitors (AIs), including anastrozole and letrozole, and the irreversible steroidal aromatase inactivator exemestane have demonstrated improved efficacy compared with tamoxifen in the advanced[4-7] and adjuvant[8-

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[12] treatment settings. Therefore, AIs in combination with ovarian suppression have been evaluated for the treatment of premenopausal women with ER-positive breast cancer.[13,14]

Neoadjuvant treatment for breast cancer provides an opportunity for downstaging of large tumors to allow patients to undergo breast-conserving surgery rather than mastectomy. Chemotherapy can offer an effective neoadjuvant treatment; however, increasing evidence suggests that ER-positive tumors are less sensitive to chemotherapy.[15] It has been demonstrated that neoadjuvant endocrine therapy has efficacy in the treatment of ER-positive disease among postmenopausal women, resulting in similar objective response rates and rates of breast-conserving surgery for AIs compared with more cytotoxic chemotherapy.[16] Therefore, the role of neoadjuvant endocrine therapy in premenopausal women is also of interest.

With the increasing costs associated with large-scale adjuvant trials, both the prognostic value of biologic markers and the long-term predictive value of short-term trials are increasingly important. The expression of nuclear antigen Ki-67, a marker of cell proliferation, reportedly has been correlated with treatment efficacy and is being investigated for its value as a predictive marker of therapeutic response.[17] In a cross-trial comparison, an increased reduction in Ki-67 expression after neoadjuvant treatment with anastrozole compared with tamoxifen was observed consistently; and increased progression-free survival has been reported for anastrozole versus tamoxifen in the adjuvant Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial.[8,18,19]

The STAGE study (Study of Tamoxifen or Arimidex Combined With Goserelin Acetate to Compare Efficacy and Safety) was the first randomized trial to compare anastrozole plus goserelin versus tamoxifen plus goserelin in the neoadjuvant setting (24 weeks of therapy) in premenopausal women with ER-positive and human epidermal growth factor receptor 2 (HER2)-negative, operable breast cancer. The patients who received anastrozole plus goserelin in that trial had a superior best overall tumor response compared with the patients who received tamoxifen plus goserelin, as measured on magnetic resonance imaging (MRI) or computed tomography (CT) studies (anastrozole plus goserelin, 64.3%; tamoxifen plus goserelin, 37.4%; estimated difference, 26.9%; 95% confidence interval [CI], 13.5-40.4; $P < .001$). The treatment effect was consistently in favor of anastrozole, regardless of the measurement methods (caliper and ultrasound). The histopathologic response rate also was better in the anastrozole group (anastrozole plus goserelin, 41.8%; tamoxifen plus goserelin, 27.3%; estimated difference, 14.6%; 95% CI, 1.4-27.7; $P = .032$). Both treatment regimens were well tolerated, consistent with the known safety profiles of anastrozole, tamoxifen, and goserelin.[20] The geometric mean Ki-67 index at baseline was 21.9% in the anastrozole group and 21.6% in the tamoxifen group. At week 24, the Ki-67 index was reduced in both treatment groups (to 2.9% in the anastrozole group and to 8% in the tamoxifen group). The reduction from baseline to week 24 was significantly greater with anastrozole than with tamoxifen. The estimated ratio of reduction between groups was 0.35 (95% CI, 0.24-0.51; $P < .001$).[20] Here, we report an exploratory analysis of the STAGE study that investigated potential correlations between the Ki-67 index and the best overall tumor response, ER status, PgR status, or histopathologic response.

MATERIALS AND METHODS

Study Design and Patients

In this phase 3, double-blind, randomized, parallel-group, multicenter trial, the participating patients were premenopausal women ≥20 years with ER-positive and HER2-negative breast cancer who had operable and measurable lesions (tumors measuring 2-5 cm, negative lymph node status [N0], and no metastases [M0]). Inclusion and exclusion criteria have been described previously.[20]

Patients were randomized 1:1 to receive either oral anastrozole 1 mg daily with a tamoxifen placebo or oral tamoxifen 20 mg daily with an anastrozole placebo. Both treatment groups received goserelin 3.6 mg as a subcutaneous injection every 28 days. Treatment continued for 24 weeks before surgery or until patients met any criterion for discontinuation.

The primary study endpoint was the best overall tumor response during the 24-week neoadjuvant treatment period. Secondary endpoints included histopathologic response, changes in estrone (E_1) and estradiol (E_2) serum and breast tumor tissue concentrations, changes in Ki-67 expression, and tolerability. For this exploratory analysis, we assessed correlations between Ki-67 expression and tumor response, ER status, PgR status, or histopathologic response.

The protocol was approved by an institutional review board at all study sites, and all enrolled patients provided written informed consent. The study (National Clinical Trials identifier NCT00605267) was conducted in accordance with the Declaration of Helsinki and good clinical practice, the applicable local regulatory requirements, and the AstraZeneca policy on Bioethics.

Assessments

Tumor measurements were performed using caliper measurements, ultrasound, or MRI or CT studies. The

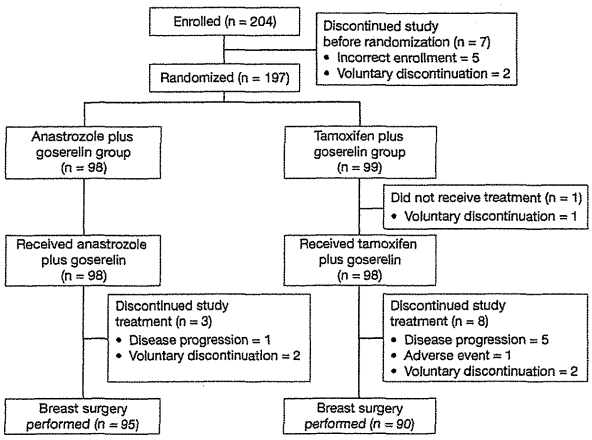

Figure 1. This is a CONSORT (Consolidated Standards of Reporting Trials) diagram of the current study.

primary analysis indicated that the best overall tumor response for anastrozole versus tamoxifen was consistent, regardless of the measurement method used.[20] We present tumor response data from the MRI or CT measurements at day 0 and at 24 weeks. The objective tumor response was assessed according to modified Response Evaluation Criteria in Solid Tumors (RECIST).[21]

The status of Ki-67, ER, and PgR was determined using histopathologic core-needle biopsy specimens that were collected at baseline and at surgery. Tissue sections were fixed in formalin and stored at room temperature before immunohistochemical staining. Ki-67 expression was determined by staining sections with an anti-MIB-1 antibody at a central laboratory (SRL Inc., Tokyo, Japan) for assessment by a central review board. For all slides, photomicrographs were taken from 3 to 5 hotspots at ×20 magnification using light microscopy. Two pathologists independently assessed the photomicrographs, and the Ki-67 index was calculated as the ratio of Ki-67-positive cancer cells from a total of 1000 cancer cells. ER-positive status and PgR-positive status at baseline were defined as ≥10% staining of cancer cell nuclei determined by a pathologist at each individual study site (nuclei were assessed using mouse monoclonal antibody clones 6F11 and 16, respectively). Staining for ER and PgR also was assessed in parallel using Allred scores by the Central Pathologist Review Committee.[22] An Allred score (the proportion score plus the intensity score) of ≥3 defined ER or PgR positivity, a score from ≥3 to <7 indicated medium expression, and a score of ≥7 indicated rich expression.

Histopathologic effects were assessed by comparing histopathologic samples that were obtained at baseline and at surgery. For the assessment of histopathologic response, the following categories were used: grade 0 indicated no response; grade 1a, marked change in <1 of 3 cancer cells; grade 1b, marked changes in ≥1 of 3 but <2 of 3 cancer cells; grade 2, marked changes in ≥2 of 3 cancer cells; and grade 3, necrosis or disappearance of all cancer cells and replacement of all cancer cells by granuloma-like and/or fibrous tissue. The histopathologic response was defined as the proportion of patients whose tumors were classified as grade 1b, 2, or 3.[23,24]

Post hoc subset analyses were used to determine correlations between the baseline Ki-67 index (≥20% vs <20%) and the best overall tumor response. The percentage change in the Ki-67 index for responders (patients whose best overall tumor response was a complete or partial response) versus nonresponders (patients whose best overall tumor response was stable or progressive disease) also was compared. Correlations between the baseline Ki-67 index and the histopathologic response at week 24 also were evaluated, and we used post hoc analyses to investigate correlations between changes in the Ki-67 index from baseline to week 24 and ER or PgR status at baseline. Positive ER and PgR status (Allred score ≥3) also was assessed at baseline and at week 24. Preoperative Endocrine Prognostic Index (PEPI) scores, which were calculated post hoc as the sum of risk points weighted by the size of the hazard ratio for tumor size, pathologic lymph node status, ER status, and Ki-67 expression for both recurrence-free and breast cancer-specific survival, were determined for each patient at surgery according to the methods described by Ellis and colleagues.[25]

Statistical Analysis

The sample size calculation and the main statistical analyses have been described previously.[20] All randomized patients were included in the intent-to-treat analysis set.

In a post hoc exploratory analysis, chi-square tests were performed to compare the best overall tumor response at week 24 between baseline Ki-67 index categories (≥20% vs <20%) within each treatment group and between treatment groups within each baseline Ki-67 index category. A chi-square test also was used to compare the histopathologic response at 24 weeks between the baseline Ki-67 index categories within each treatment group. All tests were made at the nominal 2-sided significance level of .05.

RESULTS

Patients

In total, 197 patients were randomized to receive either anastrozole plus goserelin (n = 98) or tamoxifen plus goserelin (n = 99) (Fig. 1). Patient demographics and

TABLE 1. Patient Demographics and Baseline Tumor Characteristics

Characteristic	No. of Patients (%)	
	Anastrozole Plus Goserelin	Tamoxifen Plus Goserelin
No. of patients	98	99
Age: Median [range]	44 [28-54]	44 [30-53]
Body mass index: Mean±SD, kg/m^2	22.2±3.5	22.1±3.3
Histology type		
Infiltrating ductal carcinoma	87 (88.8)	91 (91.9)
Infiltrating lobular carcinoma	3 (3.1)	3 (3)
Other[a]	8 (8.2)	5 (5.1)
Tumor grade		
1	42 (42.9)	48 (48.5)
2	36 (36.7)	26 (26.3)
3	4 (4.1)	14 (14.1)
Not assessable	1 (1)	0 (0)
Not done	15 (15.3)	11 (11.1)
Hormone receptor status		
ER positive	98 (100)	99 (100)
PgR positive	93 (94.9)	87 (87.9)
HER2 negative	98 (100)	99 (100)

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor; SD, standard deviation.
[a] Other included adenocarcinoma (n = 3).

baseline characteristics generally were well balanced between the treatment groups (Table 1). Paired samples for calculating changes in the Ki-67 index from baseline to week 24 were available for 89 patients in the anastrozole plus goserelin group and for 86 patients in the tamoxifen plus goserelin group.

Correlation of the Baseline Ki-67 Index and Best Overall Tumor Response

With a mean baseline Ki-67 index of 21.9% and 21.6% in the anastrozole and tamoxifen treatment groups, respectively, we used post hoc subset analyses to compare patients according to their baseline Ki-67 index (≥20 vs <20%). For anastrozole versus tamoxifen, best overall tumor response from baseline to week 24 was better with anastrozole plus goserelin versus tamoxifen plus goserelin both in patients who had a baseline Ki-67 index ≥20% (73.2% vs 44.8%; $P = .002$) and in patients who had a baseline Ki-67 index <20% (52.5% vs 29%; $P = .035$) (Fig. 2A).

Within the treatment groups, the best overall tumor response from baseline to 24 weeks, as measured by MRI or CT, was significantly better with anastrozole plus goserelin for patients who had a baseline Ki-67 index ≥20% than for those who had a baseline Ki-67 index <20% (73.2% vs 52.5%; $P = .036$). Among patients in the tamoxifen plus goserelin group, the best overall tumor response was 44.8% for patients who had a baseline Ki-67 index ≥20% and 29% for those who had a baseline Ki-67 index <20% ($P = .118$) (Fig. 2A).

Correlation of the Baseline Ki-67 Index and Histopathologic Response

There was no significant difference in the histopathologic response between patients who had a baseline Ki-67 index ≥20% versus patients who had a baseline Ki-67 index <20% in either treatment group (Fig. 2B).

Correlation of Change in the Ki-67 Index and Responders/Nonresponders

A waterfall plot of changes in the Ki-67 index for individual patients, illustrated according to responders or nonresponders, is provided in Figure 3. There was no apparent relation between a change in Ki-67 expression from baseline to week 24 for responders and nonresponders in either treatment group.

Correlation of the Baseline Ki-67 Index and Estrogen Receptor or Progesterone Receptor Status

In both treatment groups, positive ER status, as determined by the Allred score, was observed in 100% of patients at baseline and at week 24, and >90% of patients in both treatment groups were ER rich (baseline Allred score, ≥7). Therefore, it was not possible to determine any potential relation between the baseline ER Allred score and the percentage change in Ki-67 expression from baseline to week 24 in either treatment group.

In the anastrozole plus goserelin group, 98.9% of patients were positive for PgR expression at baseline, and 34.4% were positive for PgR expression at week 24. The percentage of patients with positive PgR status was not altered from baseline (91.9%) to week 24 (89.5%) in the tamoxifen plus goserelin group (Fig. 4A). In both treatment groups, the mean decrease in the Ki-67 index was greater in patients who had a baseline PgR Allred score ≥7 (anastrozole group, −88.8%; tamoxifen group, −67.6%), compared with patients who had a baseline PgR Allred score <7 (anastrozole group, −74.1%; tamoxifen group, −32.8%) (Fig. 4B).

Preoperative Endocrine Prognostic Index Score

In the anastrozole treatment group, 33.3% of patients had a PEPI score of 0 compared with 11.4% in the tamoxifen group. Fewer patients (21.4%) had a PEPI score ≥4 in the anastrozole group compared with patients in the tamoxifen group (36.7%; $P = .002$) (Table 2).

DISCUSSION

In this exploratory analysis, we investigated changes in Ki-67 expression among patients from the STAGE study, a

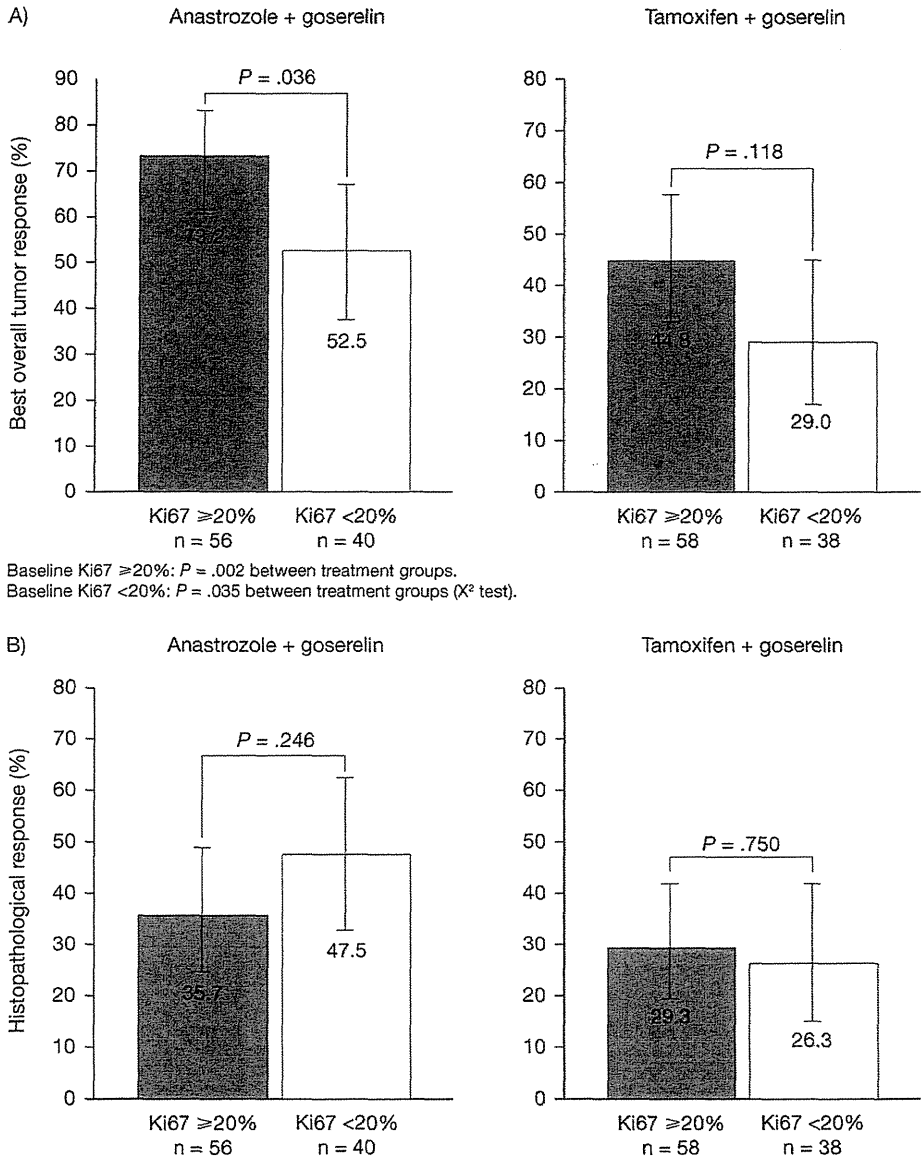

Figure 2. These charts illustrate the baseline Ki-67 index (≥20% vs <20%) according to (A) the best overall tumor response and (B) the histopathologic response at 24 weeks. Magnetic resonance imaging or computed tomography was used to measure responses. The best tumor response was defined a complete or partial response during the 24-week treatment period.

phase 3 randomized trial that compared tumor response for anastrozole plus goserelin versus response tamoxifen plus goserelin during 24 weeks of neoadjuvant treatment in premenopausal women with ER-positive breast cancer. The primary analysis indicated that the reduction in the Ki-67 index for patients who received goserelin was greater with anastrozole coadministration compared with tamoxifen, suggesting a greater inhibitory effect on tumor cell proliferation with this treatment combination.[20] Given the reported clinical prognostic value of Ki-67 expression after short-term neoadjuvant endocrine therapy for breast cancer,[19] this is in concordance with our finding that anastrozole combined with goserelin demonstrates a superior best overall tumor response compared with tamoxifen plus goserelin. Although Ki-67 is perceived as a reliable predictive endpoint, the outcomes of