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ORIGINAL ARTICLE

The significance of micro-lymphatic invasion and pathological Gleason score in prostate cancer patients with pathologically organ-confined disease and negative surgical margins after robot-assisted radical prostatectomy

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Abstract

Background The development process of recurrence in prostate cancer patients with pathologically organ-confined (pT2) disease and negative surgical margins is unclear. The aim of the present study was to determine factors associated with the development of biochemical recurrence following robot-assisted radical prostatectomy among those prostate cancer patients.

Methods We retrospectively reviewed the data of patients who underwent robot-assisted radical prostatectomy without neoadjuvant endocrine therapy. We evaluated prognostic factors in 1096 prostate cancer patients with pT2 disease and negative surgical margins. Univariate and multivariate Cox proportional hazards regression analyses were used to identify independent predictors for biochemical recurrence.

Results Of the 1096 patients, 55 experienced biochemical recurrence during the follow-up period. The 5-year biochemical recurrence-free survival rate for patients with pT2 and negative surgical margins was 91.8%. On univariate analysis, clinical stage, biopsy Gleason score, percent of positive core, pathological Gleason score, and the presence of micro-lymphatic invasion were significantly associated with biochemical recurrence. On a multivariate analysis, the presence of micro-lymphatic invasion and a pathological Gleason score $\geq 4+3$ were significant prognostic factors for biochemical recurrence. Based on these factors, we developed a risk stratification model. The biochemical recurrence-free survival rate differed significantly among the risk groups.

Conclusions The prognosis of prostate cancer patients with pT2 disease and negative surgical margins is favorable. However, patients with the presence of micro-lymphatic invasion and a pathological Gleason score $\geq 4+3$ tend to experience biochemical recurrence more often after surgery. Therefore, careful follow-up might be necessary for those patients.

Keywords Robot-assisted radical prostatectomy · Biochemical recurrence · Pathologically organ-confined disease · Negative surgical margins · Gleason score · Micro-lymphatic invasion

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Introduction

Radical prostatectomy is an effective treatment that has been shown to have cancer-specific survival benefits for localized prostate cancer compared to watchful waiting [1, 2]. As the goal of all surgical oncology procedures is the complete removal of cancer, preoperative prostate-specific antigen (PSA) level, higher Gleason score, higher pathological T stage, and positive surgical margins are considered unfavorable factors associated with the failure of surgery to cure prostate cancer [3, 4].

Extracapsular spread and positive surgical margins are traditional risk factors for biochemical recurrence (BCR) after radical prostatectomy. Therefore, BCR is expected rare among prostate cancer patients with pathologically organ-confined (pT2) disease and negative surgical margins (NSM), because complete tumor resection should be performed in those cases. However, BCR is sometimes encountered after surgery, even for patients with strictly organ-confined disease. Some previous studies have investigated prostate cancer patients with pT2 disease and NSM during the open surgery era [5, 6]. Currently, robot-assisted radical prostatectomy (RARP) instead of open or laparoscopic radical prostatectomy has become a very popular treatment choice for clinically localized prostate cancer. RARP has the potential to decrease the positive surgical margin rate because of its advantage of enhanced vision and fine resection.

It is necessary to clarify the risks of development of BCR in prostate cancer patients with pT2 disease and NSM in the RARP era, because the development process of BCR in those patients should be different from that of patients with poor prognostic factors such as positive surgical margins. However, no study to date has evaluated BCR and its risk factors in prostate cancer patients with pT2 disease and NSM treated with RARP. Therefore, the aim of the present study was to determine prognostic factors associated with BCR following RARP in prostate cancer patients with pT2 disease and NSM and to develop a prediction model of BCR in those patients.

Patients and methods

This retrospective study was approved by our institution's ethics committee.

From August 2006 to December 2018, 2322 patients with clinically localized prostate cancer underwent RARP at our institution. Extended lymph node dissection was performed in patients with D'Amico high-risk prostate cancer, while limited or no lymph node dissection was performed in those with intermediate or low risk. Of the 2322 patients, 1757 patients had pT2 prostate cancer. As 242 patients were treated with neoadjuvant endocrine therapy, 386 patients had positive surgical margins in the pathological specimens, 3 patients underwent adjuvant external beam radiation therapy, 30 patients did not have full data available, we retrospectively reviewed data from the remaining 1096 prostate cancer patients with pT2 disease and NSM in the present study (Fig. 1). Prostatectomy specimens were fixed in 10% formalin and completely inked to enable an accurate assessment of the surgical margin status. Then, the apex and base of each surgical specimen were amputated in the sagittal plane, and the remaining prostate was sectioned transversely at 3- to 5-mm intervals [7]. We evaluated those specimens according to the World Health Organization (WHO) classification [8] and the General Rules for Clinical and Pathological Studies on Prostate Cancer in Japan (4th edition) [7]. A positive surgical margin was defined as tumor extension into the inked surface of the resected specimen. Micro-lymphatic invasion and microvascular invasion were evaluated by immunohistochemical staining (D2-40 and CD31), as previously reported [9, 10].

BCR was defined as two consecutive values of serum PSA level ≥ 0.2 ng/mL. When PSA level never reduced to less

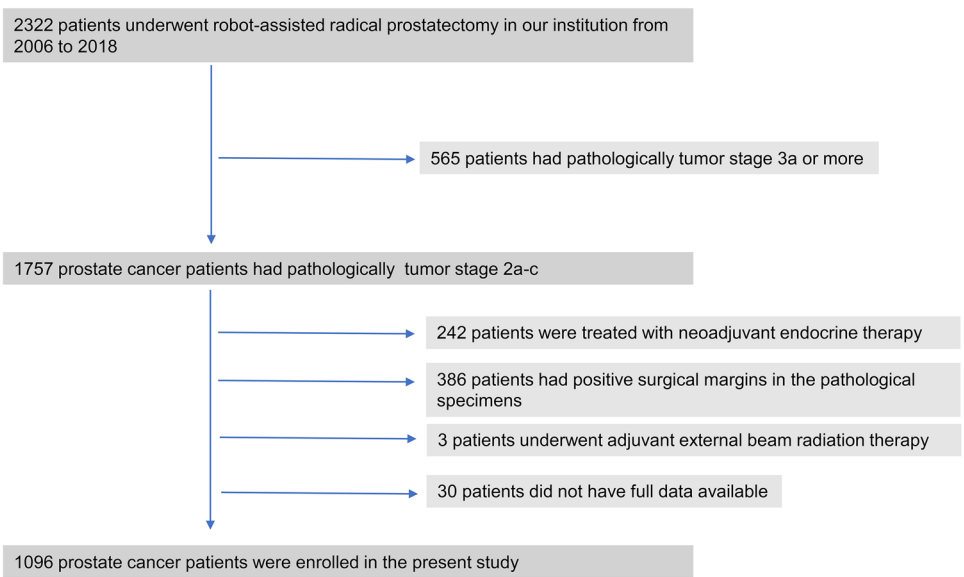

Fig. 1 Study design and inclusion criteria

than 0.2 ng/mL after RARP, the date of BCR was defined as the date of surgery. Statistical analysis of BCR-free survival (BCR-FS) was performed using the Kaplan–Meier method. Univariate and multivariate Cox proportional hazards regression analyses were used to identify prognostic indicators for BCR-FS. To obtain a multivariate model with maximum precision for the significant variables, a stepwise selection procedure was used. To establish risk stratification model, we dichotomize each variable. The cut-off value for each variable was set as previously reported; that is, the value that was best for discriminating between good and poor outcomes (the value that had the most significant p value according to the log–rank test), which was determined by testing all the possible cut-off points [11]. The relative risk (RR) of BCR was estimated using the variable with statistical significance in the multivariate Cox regression analysis, and patients were stratified into groups according to the RR of BCR, as reported previously [11]. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using Stata software (version 14.0; StataCorp, College Station, TX, USA).

Results

The mean and median follow-up periods after surgery were 35.9 and 29.7 months, respectively. Patient demographics are shown in Table 1. Of the 1096 patients, 55 (5.0%) experienced BCR during the follow-up period. The 3-, 5-, and 7-year BCR-FS rates for prostate cancer patients with pT2 disease and NSM were 95.1%, 91.8%, and 88.5, respectively. The mean time to BCR from surgery was 23.6 ± 21.8 months (median, 17.2 months; range, 0–77.7 months). Of those 55 patients who experienced BCR, 40 patients underwent salvage treatment and 15 patients were under observation without salvage treatment during study period. Thirty patients underwent salvage external beam radiation therapy (EBRT), 5 patients underwent androgen deprivation therapy (ADT) + EBRT, and 5 patients underwent ADT monotherapy. Preoperative variables (age, clinical T stage, serum PSA level, biopsy Gleason score, percent of positive cores) and postoperative variables (pathological T stage, pathological Gleason score, micro-lymphatic invasion, microvascular invasion, perineural invasion) were included in the Cox univariate analysis (Table 2). In univariate analysis, age, PSA level, a pathological T stage, a presence of vascular invasion, and a presence of perineural invasion were not significantly associated with BCR. In contrast, patients with a clinical T stage ≥ 2b, a biopsy Gleason score ≥ 4 + 3, a percent positive core of ≥ 25%, a pathological Gleason score ≥ 4 + 3, and a presence of micro-lymphatic invasion showed significantly lower BCR-FS rates than their respective counterparts (Fig. 2a–e).

Table1 Patient characteristics

Age at surgery (year)	
Median (IQR)	65 (61, 69)
Clinical T stage, *n* (%)	
T1c	835 (76.2%)
T2a	153 (14.0%)
T2b	46 (4.2%)
T2c	59 (5.4%)
T3a	3 (0.3%)
PSA (ng/mL)	
Median (IQR)	6.60 (5.10, -9.10)
Biopsy Gleason sum, *n* (%)	
≤ 6	258 (23.5%)
3 + 4	402 (36.7%)
4 + 3	220 (20.1%)
8	163 (14.9%)
≥ 9	53 (4.8%)
Number of positive cores	
Median (IQR)	2 (1,4)
Percent of positive biopsy cores	
Median (IQR)	20.0 (10.0–33.3)
D'Amico risk classification	
Low risk	208 (19.0%)
Intermediate risk	612 (55.8%)
High risk	276 (25.2%)
Pathological T stage, *n* (%)	
pT2a	189 (17.2%)
pT2b	137 (12.5%)
pT2c	770 (70.3%)
Pathological Gleason sum, *n* (%)	
≤ 6	87 (7.9%)
3 + 4	522 (47.6%)
4 + 3	321 (29.3%)
8	80 (7.3%)
≥ 9	86 (7.8%)
Lymph node involvement, *n* (%)	
Positive	4 (0.4%)
Negative/not resected	1092 (99.6%)
Lymphatic invasion, *n* (%)	
Positive	91 (8.3%)
Negative	1005 (91.7%)
Vascular invasion, *n* (%)	
Positive	141(12.9%)
Negative	955 (87.1%)
Perineural invasion, *n* (%)	
Positive	794 (72.4%)
Negative	302 (27.6%)

IQR interquartile range, *PSA* prostate specific antigen

Table 2 Results of uni- and multivariate analyses using stepwise analysis after dichotomizing to establish risk classification

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age at surgery, < 60 vs. ≥ 60	1.091	0.563–2.113	0.796			
Preoperative PSA level, < 8 vs. ≥ 8	1.490	0.872–2.546	0.145			
Clinical T stage, ≤ T2a vs. ≥ T2b	2.145	1.081–4.257	0.029			
Biopsy Gleason score, ≤ 3 + 4 vs. ≥ 4 + 3	4.058	2.288–7.199	< 0.001			
Percent of positive core, < 25% vs. ≥ 25%	1.744	1.023–2.972	0.041			
D'Amico risk classification, ≤ intermediate vs. high	1.707	1.127–2.587	0.012			
Pathological T stage, T2a vs. ≥ T2b	0.581	0.321–1.053	0.073			
Pathological Gleason score, ≤ 3 + 4 vs. ≥ 4 + 3	5.236	2.757–9.945	< 0.001	4.654	2.429–8.916	< 0.001
Micro-lymphatic invasion, presence vs. absence	3.749	2.011–6.989	< 0.001	2.631	1.398–4.949	0.003
Microvascular invasion, presence vs. absence	0.823	0.352–1.921	0.652			
Perineural invasion, presence vs. absence	1.927	0.992–3.742	0.053			

On the multivariate analysis with stepwise procedure, the presence of micro-lymphatic invasion (hazard ratio [HR], 2.631; 95% confidence interval [CI], 1.398–4.949; $p = 0.003$) and a pathological Gleason score ≥ 4 + 3 (HR, 4.654; 95% CI, 2.429–8.916; $p < 0.001$) were significant prognostic factors for BCR after surgery. The 5-year BCR-FS rates were 96.8% and 84.9% for pathological Gleason score ≤ 3 + 4 and ≥ 4 + 3, respectively. In addition, the 5-year BCR-FS rates were 92.8% and 81.1% for the absence and presence of micro-lymphatic invasion, respectively (Fig. 2d–e).

The patients were stratified into three groups according to the significant risk factors. The RR of BCR was calculated by considering these two significant factors obtained from the multivariate analysis according to the following formula: RR = exp ([0.967 × the presence of micro-lymphatic invasion] + [1.538 × pathological Gleason score]). In this equation, the presence of micro-lymphatic invasion was assigned a value of 1 or 0 for presence or absence of micro-lymphatic invasion, respectively, and pathological Gleason score was assigned a value of 1 or 0 for Gleason score of ≥ 4 + 3 or ≤ 3 + 4, respectively. The patients were stratified into three groups according to the RR of BCR. Low-risk group was defined as patients with RR = 1 (0 risk factor), intermediate-risk group was defined as patients with RR = 2.361–4.654 (1 risk factor), and high-risk group was defined as patients with RR = 12.244 (2 risk factors). The 5-year BCR-FS rate was 96.7% in the low-risk group, 87.3% in the intermediate-risk group, and 74.2% in the high-risk group. The BCR-FS curves for the different risk groups are shown in Fig. 3. The BCR-FS rate differed significantly between the groups.

Discussion

Radical prostatectomy improves overall and cancer-specific survival rates for patients with intermediate-risk and high-risk localized prostate cancer [12, 13]. Radical prostatectomy aims to achieve complete resection of the tumor. In the absence of detected metastases, prostate cancer patients with pT2 disease and NSM should achieve the highest cure rates after surgery with definitive monotherapy. Budaus et al. reported that the 5-year BCR-FS rate for these patients was 95% in their open radical prostatectomy series [14]. In the present study, the 5-year BCR-FS rate for those patients was 91.8%, indicating an excellent prognosis after surgery. However, 55 (5.0%) patients experienced BCR after RARP during the follow-up periods, and some of them needed additional treatment such as ADT or EBRT for the recurrent prostate cancer. Wilczak et al. investigated large number of assessment about micro-lymphatic invasion after radical prostatectomy. In their study, nodal metastasis found in 4.3% of patients with an absence of micro-lymphatic invasion. On the other hand, nodal metastasis were found 41% in patients with a presence of micro-lymphatic invasion. They suggested that the presence of micro-lymphatic invasion was significantly associated with the presence of lymph node metastasis [15]. Since only 0.4% patients had lymph node involvement in the present study of organ confined disease, we could not asses the relationship between micro-lymphatic invasion and lymph node involvement. However, we investigated

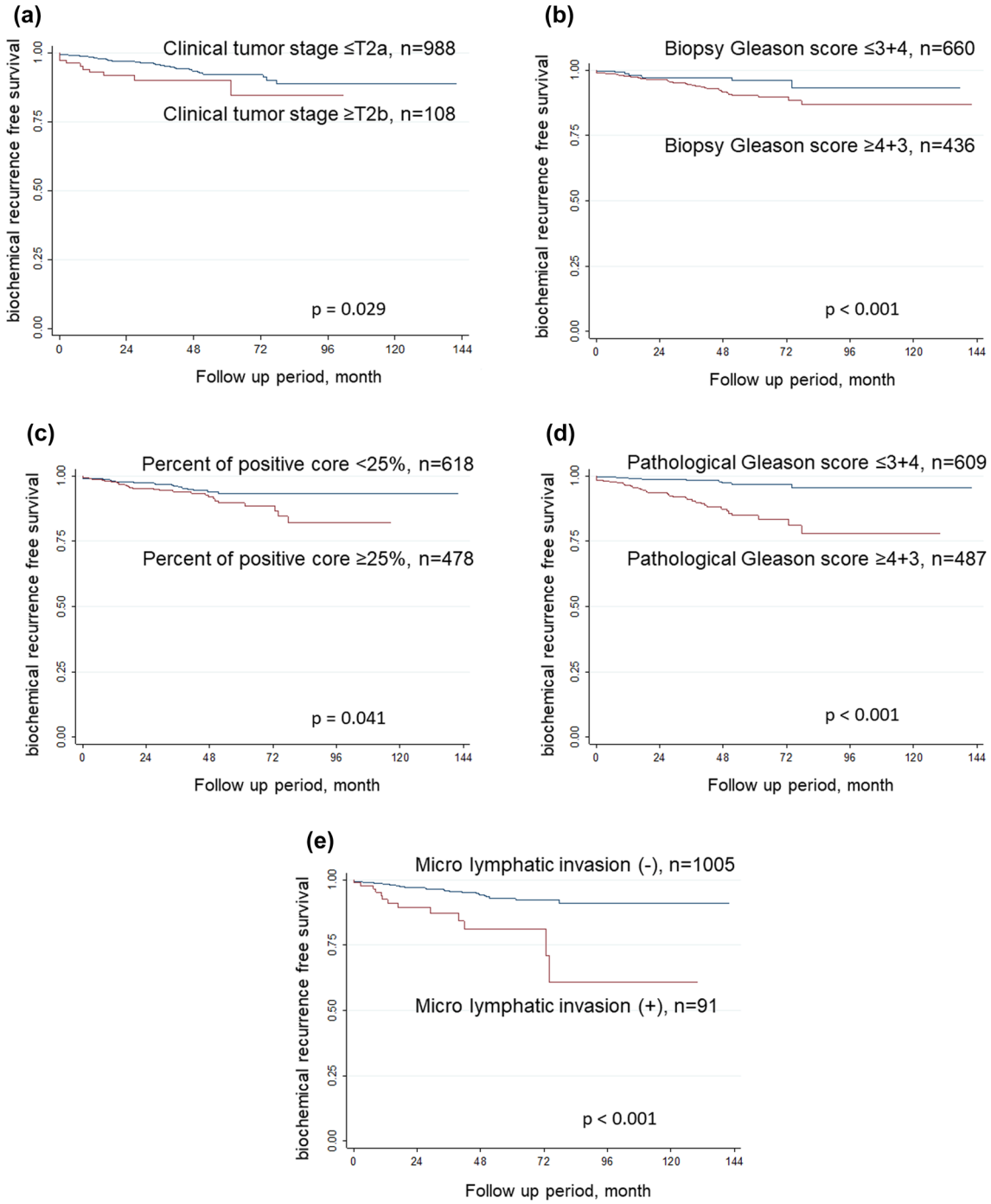

Fig. 2 **a** Kaplan–Meier analysis according to the clinical tumor stage. Significance: $p < 0.05$. The clinical tumor stage ≤ T2a vs. the clinical tumor stage ≥ T2b. **b** Kaplan–Meier analysis according to the biopsy Gleason score. Significance: $p < 0.05$. Biopsy Gleason score ≤ 3 + 4 vs. biopsy Gleason score ≥ 4 + 3. **c** Kaplan–Meier analysis according to percent of positive core. Significance: $p < 0.05$. Percent of positive core ≤ < 25% vs. percent of positive core ≥ 25%. **d** Kaplan–Meier analysis according to the pathological Gleason score. Significance: $p < 0.05$. Pathological Gleason score ≤ 3 + 4 vs. pathological Gleason score ≥ 4 + 3. **e** Kaplan–Meier analysis according to micro-lymphatic invasion. Significance: $p < 0.05$. Presence of micro-lymphatic invasion vs. absence of micro lymphatic invasion

patients with BCR after EBRT to research the effect of salvage EBRT. Eight patients experienced PSA elevation after salvage EBRT during study period. Interestingly of those 8 patients, 6 patients had micro-lymphatic invasion. Therefore, it was suggested that the presence of micro-lymphatic invasion also might be associated with failure of salvage radiation therapy.

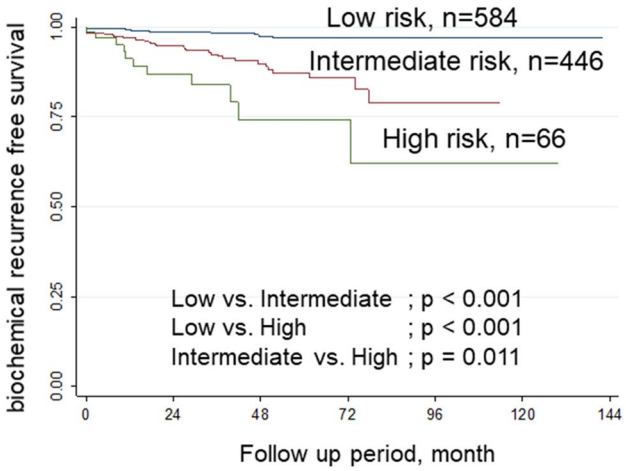

Fig. 3 Kaplan–Meier analysis of the BCRFS rate according to the risk stratification model. The BCR-free survival rate differed significantly between each group

The present study showed that the pathological Gleason score and the presence of micro-lymphatic invasion were significant predictors of BCR during multivariate analysis. These results are likely acceptable, because high Gleason scores traditionally have been worse prognostic characteristics of patients with prostate cancer. Many previous studies showed that the pathological Gleason score is one of the most powerful predictors of BCR after radical prostatectomy for patients with prostate cancer and a cornerstone for counseling and treating patients [16–18]. In the present study of prostate cancer patients with pT2 disease and NSM, patients with pathological Gleason scores $\leq 3+4$ had significantly better BCR-FS rates after RARP than those with pathological Gleason scores $\geq 4+3$. Interestingly, even if patients have pT2 prostate cancer and NSM, those with higher pathological Gleason scores have poorer prognoses. Based on our results, careful observation is required for prostate cancer patients with pT2 disease and NSM if they have a pathological Gleason score $\geq 4+3$ and/or the presence of micro-lymphatic invasion.

It is conceivable that the presence of disseminated diseases via micro-lymphatic invasion also causes BCR, even in prostate cancer patients with pT2 disease and NSM. The results of the present study suggested that patients with micro-lymphatic invasion might already have sufficient metastatic potential, even with pathologically organ-confined prostate cancer. Although we evaluated metastasis in all patients using prostate magnetic resonance imaging, computed tomography, and ^{99m}Tc-methylene diphosphonate bone scan preoperatively, microscopic metastatic lesions could not be detected. The initial entry of tumor cells into the circulation through small vessels could be an important step in tumor dissemination [19]. Previous reports have shown that lymphovascular invasion (LVI) has long been recognized as an essential step toward metastasis in various urologic cancers and that LVI was an independent prognostic factor for BCR after surgery in patients with prostate cancer [20–23]. In addition, other studies showed that a substantial number of patients with metastases had LVI [24, 25]. In an open radical prostatectomy series, Mitsuzuka et al. also demonstrated that LVI is a significant predictor of BCR in patients with pT2 prostate cancer [5]. In the present study, we further classified LVI by micro-lymphatic invasion and microvascular invasion using immunohistochemical staining. No study to date investigated LVI by dividing into micro-lymphatic invasion and microvascular invasion. Many studies have focused on identifying determinants of metastasis until now. Existing circulating tumor cells do not necessarily engraft to other organs. Therefore, the mechanism of engrafting to other organs has been controversial. In the present study, micro-lymphatic invasion was an independent prognostic factor for BCR in the multivariate analysis, although microvascular invasion was not significant. Therefore, we postulated lymphogenous metastasis via micro-lymphatic invasion might be more important mechanism than hematogenous metastasis in prostate cancer patients with pT2 disease and NSM.

Although we believe that this study provides important insights into BCR after RARP, this study has some limitations. This was a retrospective study that involved analysis of data collected from patients who underwent RARP by a single institution which is supposed to be the most experienced in the performance of RARP in our country. Therefore, this retrospective study has a potential selection bias. To our knowledge, this study might be the first report to investigate the prognostic significance of Gleason score for BCR in patients with pT2 and NSM prostate cancer treated with RARP. Further studies are warranted to confirm our results concerning BCR after RARP.

Conclusion

The prognosis of prostate cancer patients with pT2 disease and NSM is favorable. However, patients with the presence of micro-lymphatic invasion and a pathological Gleason score $\geq 4+3$ tend to experience BCR significantly more often after surgery. The risk stratification model which we established was useful. Careful follow-up might be necessary for prostate cancer patients with pT2 disease and NSM if those patients have the presence of micro-lymphatic invasion and/or a pathological Gleason score $\geq 4+3$.

Compliance with ethical standards

Conflict of interest No author has any conflict of interest.

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