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Original contribution

Quantification of perineural invasion focus after radical prostatectomy could improve predictive power of recurrence☆

Shulin Wu MD, PhD [a,b,1], Ling Xie MD, PhD [c,1], Sharron X. Lin PhD [a], Gregory J. Wirth MD [d], Min Lu MD, PhD [e], Yifen Zhang MD, PhD [c], Michael L. Blute MD [a], Douglas M. Dahl MD [a,], Chin-Lee Wu MD, PhD [a,b,*]**

[a] *Department of Urology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02114, USA*
[b] *Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02114, USA*
[c] *Department of Pathology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, 210023, China*
[d] *Division of Urology, Department of Surgery, Geneva University Hospital, Geneva, 1205, Switzerland*
[e] *Department of Pathology, Peking University Third Hospital, Peking University Health Science Center, Beijing, 100191, China*

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Summary Perineural invasion (PNI) after radical prostatectomy (RP) is a common feature of prostate cancer (PCa) and has been associated with unfavorable tumor characteristics. However, its prognostic relevance is controversial. In this study, we evaluated the impact of both PNI status (PNI+ versus PNI −) and quantified number of PNI focus on the long-term prognosis of biochemical recurrence (BCR) after RP. After reevaluating PNI of a total of 721 patients with localized PCa who underwent RP at our institution between 2000 and 2002, we examined associations between PNI status or PNI focus number and clinicopathological factors including tumor stage, Gleason score, margin status, tumor location, preoperative prostate specific antigen, age, prostate weight as well as BCR outcome. PNI was present in 530 of 721 cases (73.5%) of the RP specimens and was associated with more aggressive disease. BCR occurred in 19.4% of all patients within a median follow-up period of 8.5 years. PNI+ status was associated with poor BCR prognosis in univariate analysis but lost in multivariate analysis. Based on the number of PNI focus, PNI was further divided into 2 distinct group: PNI+ a (≤3) and PNI+ b

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* Corresponding author. Massachusetts General Hospital, Department of Urology and Pathology, Warren Building 225, 55 Fruit Street, Boston, MA 02114, USA.

** Corresponding author. Massachusetts General Hospital, Department of Urology, Gray Building 1102, 55 Fruit Street, Boston, MA 02114, USA.
E-mail addresses: DDAHL@mgh.harvard.edu (D.M. Dahl), cwu2@partners.org (C.-L. Wu).

[1] These authors contributed equally to this work.

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(>3). In a multivariate Cox regression model, PNI+ b (>3) was identified as an independent BCR prognostic factor. Quantification of PNI focus number beside the dichotomized status recording will not only provide more detailed information but also be a novel prognostic indicator for risk stratification. Further external validation will be needed for an optimal cut-off value of the PNI focus number. Our findings will help further research on the relevance of PNI in the pretreatment setting and support ongoing efforts to understand its role of cancer progression.

1. Introduction

Prostate cancer (PCa) is the most commonly diagnosed noncutaneous malignancy in men and the second leading male cancer-specific death cause, with an estimated 174,650 new diagnoses and 31,620 deaths in the USA in 2019 [1]. Radical prostatectomy (RP) remains the primary treatment for localized PCa with excellent oncologic control. However, approximately 20−40% of patients with clinically localized PCa will present biochemical recurrence (BCR) after RP [2−4]. Currently, preoperative prostate-specific antigen (PSA), pathologic T stage (pT), Gleason score (GS), and positive surgical margin (PSM) are widely accepted as high-risk factors of postoperative BCR [5]; however, the predictive power remains unsatisfied for the precise timing of delivering adjuvant treatment without overtreatment.

Perineural invasion (PNI), which is defined as cancer tracking along or around a nerve within the perineural space, is a known risk factor for solid malignancies including pancreas, colon, and rectum, head and neck, biliary tract, and stomach [6,7]. PNI is a commonly identified pathologic feature in PCa, showing a mean frequency of 62.2% and up to 80% if carefully analyzed in RP specimens [8,9]. The previous biological and molecular studies on perineural space and interaction between never and PCa cells suggested that the perineural space may be a microenvironment that promotes both cancer spread and growth [6,9,10]. Clinical significance of PNI in RP specimen has been mainly evaluated based on its status (present or absent) [11−22]. Other approaches such as counting PNI focus number with or without assistance of immunostaining [23,24], measuring PNI diameter [25], recognizing nerve subtypes [26], or targeting extraprostatic PNI only [27] were also applied. The PNI has been consistently reported to associate significantly with adverse pathological features and worse BCR prognosis in univariate analysis. In 1999, the College of American Pathologists published a consensus statement suggesting that PNI could be considered as a potential prognostic factor [28]. However, the independent prognostic significance of PNI in RP specimens in multivariate analysis remains controversial when adjusted by other high-risk factors [13,19,20,29,30].

In the present study, by reviewing pathologic hematoxylin and eosin slides of a cohort of more than 700 patients with a long-term follow-up, we aimed to evaluate the association between intraprostatic PNI (both present/absent status and focus number) and clinicopathological characteristics as well as BCR prognosis following RP for localized PCa.

2. Materials and methods

Following Institutional Review Board approval, using a PCa database of the Departments of Urology and Pathology at Massachusetts General Hospital, a total of 902 patients who underwent RP for localized PCa between 2000 and 2002 were reviewed. After applying exclusion criteria including neoadjuvant treatment or direct postoperative adjuvant therapy, positive lymph nodes, postoperative PSA persistence, lost to PSA follow up, or unavailable pathologic slides, 721 cases were included in this study. RP specimens were inked, and pathological assessments were done as our routine protocol [31,32]. Briefly, the freshly harvested prostate gland was weighted, size measured, and inked. Prostatic apex and bladder neck margin were sampled using the cone method with subsequent radial sectioning, the rest of prostate was divided into right and left halves and further divided into anterior and posterior halves in each side, then the specimen was serially blocked at 3 mm intervals in transverse planes perpendicular to the rectal surface and then formalin-fixed and paraffin-embedded. Twenty-two blocks (including 2 blocks from apex margin, 2 blocks from bladder neck margin, 2 blocks from bilateral seminal vesicle and vas deferens, and at least 16 blocks from 4 quadrants) were obtained from the majority of prostate specimens based on the prostate size. The pathology of the presence of PNI and PNI focus number was reviewed by 2 pathologists (S.W. and L.X.). For cases in which the reviewers could not agree, a third pathologist (C.L.W.) was consulted to reach group consensus. Because the goal of our study was to evaluate the prognostic value of PNI focus number, significant efforts were made to ensure that all the PNI foci were identified and counted in all slides. PNI was defined as positive (PNI+), when PCa infiltration was identified in any layer of the nerve sheath or tumor invasion was involved at least one-third of the nerve

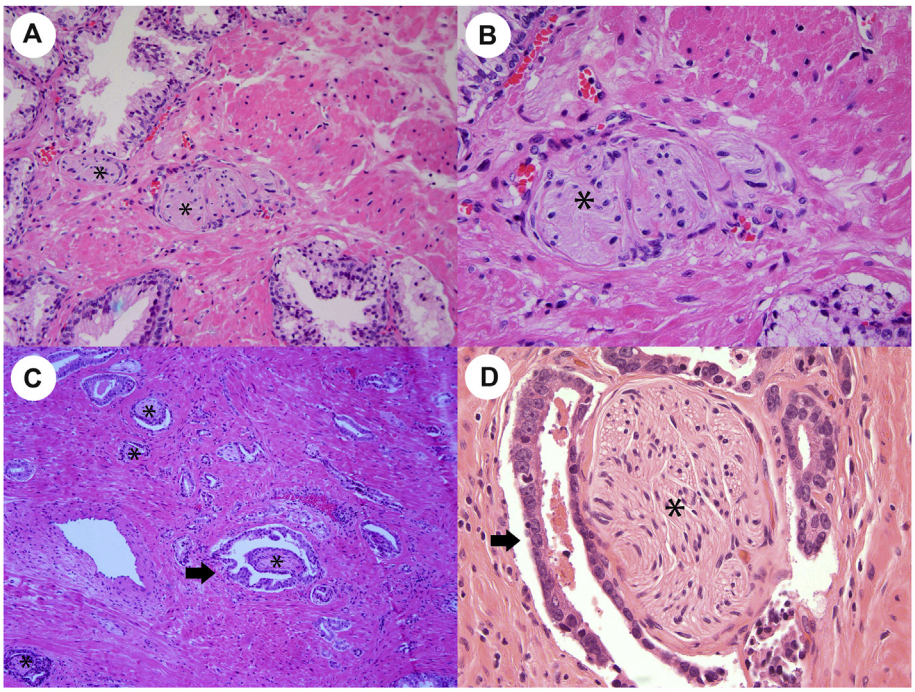

Fig. 1 Representative photomicrographs of radical prostatectomy specimens show nerves (*) with and without perineural invasion (arrows). Nerve bundles without PNI are shown in panel A (x20) and panel B (x40). Extensive PNI can be seen at low and high magnification in panel C (x10) and panel D (x40). PNI, perineural invasion.

circumference, and with this definition, false positive rate with a PNI-like feature could be decreased; however, any PNI lesion would not be overlooked [6]. When counting the PNI focus number, PCa invasion surrounding one solitary nerve was considered as single focus of PNI (Fig. 1). The GS was updated according to the 2014 International Society of Urological Pathology criteria [33]. For tumor dominancy, we defined anterior-dominant, posterior-dominant, and nondominant tumor (anterior or posterior) as well as left (L)-dominant, right (R)-dominant, and nondominant based on the pathology report description regarding quadrant number with PCa involvement and tumor volume in each quadrant.

Postoperative BCR was defined as a postnadir detectable serum PSA level of $\geq$0.2 ng/ml, followed by a confirmatory value. Metastatic disease was defined by the diagnosis of PCa recurrence in a lymph node or at a distant site by clinical impression and radiographic evidence. Information on death was taken from death certificates, patient charts, and physician correspondence.

Descriptive statistics of categorical variables focused on frequencies and proportions. Medians and interquartile ranges (IQRs) were reported for continuous variables. Statistical analysis was performed using the Kruskal–Wallis H test for continuous variables and Pearson's Chi-squared test or Fisher's exact test for categorical variables. Kaplan–Meier survival analysis was performed to estimate probability of remaining free from BCR, comparison of survival distributions was performed with the log-rank test. Multivariate Cox proportional hazards models fitted with variables showing significance on univariate analysis were created to compute hazard ratios (HRs) for predictors of BCR. The Harrell's concordance index (c-index) were used to assess the discrimination ability of the different PNI models. All tests

Table 1 Quantification of PNI focus and univariate Cox regression model for cut-off value decision.

No. of PNI focus	No. (%)	No. (%) of PNI+	HR	95% CI	*P*	PNI+ subgroups	No. (%) of subgroups
PNI− (0)	191(26.5)	−	ref	ref	ref		
PNI+	**530 (73.5)**	**530 (100)**					**530(100)**
1	82 (11.4)	82 (15.5)	2.45	1.06−5.65	**0.036**		
2	61 (8.5)	61 (11.5)	2.46	0.99−6.11	0.053	PNI+a(≤3)	190 (35.9)
3	47 (6.5)	47 (8.9)	2.49	0.97−6.41	0.060		
4	33 (4.6)	33 (6.2)	4.69	1.94−11.3	**0.001**		
5	34 (4.7)	34 (6.4)	4.79	1.98−11.7	**<0.001**	PNI+b(>3)	340 (64.1)
6-84	273 (37.8)	273 (51.5)	6.12	3.26−11.5	**<0.001**		

Abbreviations: PNI, perineural invasion; CI, confidence interval; No.: Number; HR, hazard ratios. Bold in p value showed statistical significance.

Table 2 Clinicopathological characteristics of 721 RP patients between 2000 and 2002 for localized prostate cancer stratified by PNI status and PNI+ subgroups.

Variable	PNI status				PNI+ subgroups				
	Total	PNI− (0)	PNI+ (≥1)	*P*	PNI+a (≤3)	PNI+b (>3)	*P* (0 *vs* a)	*P* (a *vs* b)	*P* (all)
Patients, n (%)	721 (100)	191(26.5)	530(73.5)		190(26.3)	340(47.2)			
Median (IQR)									
Age (year)	60 (55−64)	60 (55−64)	60 (55−64)	0.653	59 (53−65)	60 (56−64)	0.695	0.248	0.437
PSA (ng/mL)	5.3 (4.0−7.4)	5.1 (3.8−6.7)	5.4 (4.0−7.7)	0.067	4.8 (3.8−6.6)	5.8 (4.2−8.4)	0.755	**0.001**	**< 0.001**
Prostate size (gm)	41 (34−54)	50 (39−64)	40 (33−50)	**< 0.001**	41 (33−51)	40 (33−50)	**< 0.001**	0.335	**< 0.001**
GS, n (%)				**< 0.001**			**< 0.001**	**< 0.001**	**< 0.001**
<=6	311 (43.1)	150 (78.5)	161 (30.4)		99 (52.1)	62 (18.2)			
3+4	290 (40.2)	33 (17.3)	257 (48.5)		70 (36.8)	187 (55.0)			
4+3	66 (9.2)	8 (4.2)	58 (10.9)		11 (5.8)	47 (13.8)			
≥8	54 (7.5)	0 (0)	54 (10.2)		10 (5.3)	44 (13.0)			
pT stage, n (%)				**< 0.001**			0.062	**< 0.001**	**< 0.001**
pT2	587 (81.4)	189 (98.9)	398 (75.1)		182 (95.8)	216 (63.5)			
≥T3	134 (18.6)	2 (1.1)	132 (24.9)		8 (4.2)	124 (36.5)			
PSM				**< 0.001**			**< 0.001**	**0.034**	**< 0.001**
Negative	563 (78.1)	180 (94.2)	383 (72.3)		148 (77.9)	235 (69.1)			
Positive	158 (21.9)	11 (5.8)	147 (27.7)		42 (22.1)	105 (30.1)			
Tumor dominancy 1				**< 0.001**			0.114	**< 0.001**	**< 0.001**
Anterior-dominant	103 (14.3)	43 (22.5)	60 (11.3)	0.060[a]	39 (20.5)	21 (6.2)		**< 0.001**[a]	
Posterior-dominant	228 (31.6)	71 (37.2)	157 (29.6)	**0.001**[b]	55 (29.0)	102 (30.0)		0.340[b]	
Nondominant (AP)	390 (54.1)	77 (40.3)	313 (59.1)		96 (50.5)	217 (63.8)			
Tumor dominancy 2				0.074			0.103	**0.013**	**0.009**
Left-dominant	155 (21.5)	48 (25.1)	107 (20.2)	0.885[c]	31 (16.3)	76 (22.4)		**0.006**[c]	
Right-dominant	172 (23.9)	52 (27.2)	120 (22.6)		56 (29.5)	64 (18.8)			
Nondominant (LR)	394 (54.6)	91 (47.6)	303 (57.2)		103 (54.2)	200 (58.8)			
No. BCR (%)	140 (19.4)	11 (5.8)	129 (24.3)	**< 0.001**	26 (13.7)	103 (30.3)	**0.010**	**< 0.001**	**< 0.001**
No. Metastasis (%)	25 (3.5)	0 (0)	25 (4.7)	**0.001**	4 (2.1)	21 (6.2)	0.061	**0.034**	**< 0.001**
No. All death (%)	77 (10.7)	19 (10.0)	58 (10.9)	0.123	15 (7.9)	43 (12.7)	0.590	0.110	0.237

Abbreviations: RP, radical prostatectomy; PNI, perineural invasion; GS, Gleason score; PSM, positive surgical margin; BCR, biochemical recurrence; PSA, prostate-specific antigen; IQRs, interquartile ranges. Bold in p value showed statistical significance.

[a] Comparison between posterior-dominant versus anterior-dominant.

[b] Comparison between Non-dominant versus posterior-dominant.

[c] Comparison between right-dominant versus left-dominant.

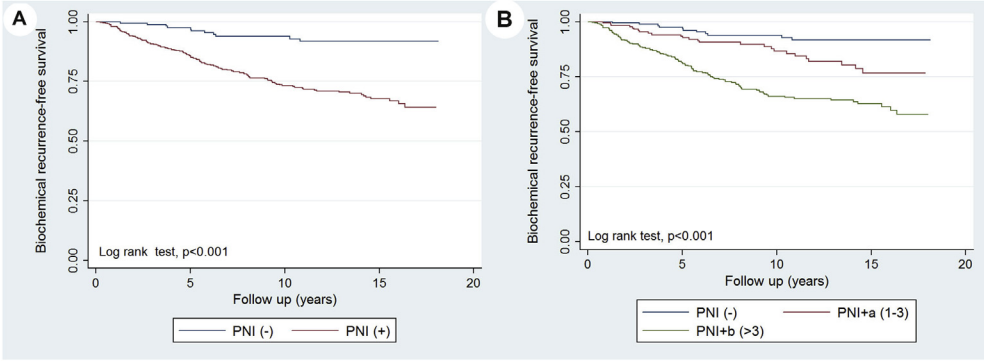

Fig. 2 Kaplan−Meier curve showing biochemical recurrence-free survival stratified by the PNI dichotomized present status (A) and subgroups of quantified PNI focus numbers (B). PNI, perineural invasion.

were 2-sided with statistical significance set at $p < 0.05$. All statistical analyses were performed with Stata14 (College Station, TX).

3. Results

Of the total 721 RP cases, PNI was presented in 530 cases (73.5%), and the total amount of PNI focus ranged from 0 to 84, with a median foci number of 6 (IQR: 2−18) of PNI+ cases (Table 1). To find the best cut-off foci number to further categorize the PNI+ subgroups, we performed univariate Cox proportional hazard analysis, and we found that the BCR prognostic power of PNI+ cases with 1, 2, or 3 foci were similar, and they all showed lower HR (<2.5) with or without statistical significance when compared with PNI− cases. On the contrary, PNI+ cases with 4 or more foci consistently showed statistically higher HR (>4.5) when compared with PNI− cases. Based on these results, we further divided PNI+ cases into a and b two subgroups according to the foci number (PNI+ a group: focus number≤3, PNI+ b group: focus number>3) (Table 1).

The baseline clinicopathological characteristics and the distribution of PNI status and PNI+ subgroups in our cohort are shown in Table 2. PNI+ was significantly prevalent in high-grade tumors (GS ≥ 4+ 3: 21% versus 4.2%) and in advanced stage (pT3: 24.9% versus 1.1%) when compared with PNI− cases. PNI+ was also correlated with PSM positively and with lower prostate weight negatively. For tumor anterior-posterior dominancy analysis, PNI+ showed significantly higher frequency in nondominant tumor group (usually with higher tumor volume) than either posterior-dominant tumor or anterior-dominant tumor (59.1% versus 29.6% versus 11.3%, $P < 0.001$). In addition, the posterior-dominant tumor showed a trend of higher frequency of PNI+ than anterior-

Table 3 Univariate and multivariate analyses for BCR of 721 RP patients.

Variable	Univariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (year)	1.00	0.98−1.03	0.843	–	–	–
PSA (ng/mL)	1.08	1.04−1.11	**<0.001**	1.04	0.99−1.08	0.091
Prostate weight (gram)	0.99	0.98−0.99	**0.015**	0.99	0.98−1.00	0.117
RP GS						
<=6	ref			ref		
3+4	3.36	2.07−5.47	**<0.001**	2.14	1.25−3.67	**0.006**
4+3	6.44	3.63−11.4	**<0.001**	3.68	1.95−6.95	**<0.001**
≥8	11.7	6.75−20.4	**<0.001**	5.18	2.70−9.94	**<0.001**
pT3 *vs* pT2	3.55	2.54−4.98	**<0.001**	1.45	0.97−2.17	0.071
PSM (+) *vs* PSM(−)	3.25	2.32−4.53	**<0.001**	1.79	1.22−2.63	**0.003**
RP PNI (status)				**Model1**		
PNI(+) *vs* PNI(−)	4.57	2.47−8.45	**<0.001**	1.80	0.89−3.61	0.101
RP PNI (quantification)				**Model2**		
PNI (−)	ref			ref		
PNI+ a (≤3)	2.46	1.22−4.98	**0.012**	1.44	0.67−3.09	0.347
PNI+ b (>3)	5.82	3.13−10.8	**<0.001**	2.12	1.02−4.40	**0.044**

Abbreviations: CI, confidence interval; RP, radical prostatectomy; PNI, perineural invasion; GS, Gleason score; PSM, positive surgical margin; BCR, biochemical recurrence; PSA, prostate-specific antigen; HR, hazard ratio. Bold in p value showed statistical significance.

dominant tumor without reaching a statistical significance ($P = 0.060$). When the tumor laterality dominancy analyses were done, PNI+ only showed a trend of higher frequency in nondominant tumor than either side-dominant tumor (57.2% versus 22.6% (R) versus 20.2% (L), $P = 0.074$), and the PNI+ frequency on right-dominant tumor was similar to left-dominant tumor even litter higher ($P = 0.885$). For the oncological outcomes, patients with PNI+ showed significantly higher BCR rate (24.3% versus 5.8%, $P < 0.001$) and metastasis rate (4.7% versus 0, $P = 0.001$) but similar all-cause death rate (10.9% versus 10.0%, $P = 0.123$) when compared with patients with PNI− status.

When comparing between two PNI+ subgroups, PNI+ b group showed significantly more advanced pathological features and poor BCR and metastasis outcomes than the PNI+ a group in PSA level (5.8 versus 4.8 ng/mL, $P = 0.001$), tumor grade (GS $\geq$ 4+3: 26.8% versus 11.1%, $P < 0.001$), pT3 stage (36.5% versus 4.2%, $P < 0.001$), PSM frequency (30.1% versus 22.1%, $P = 0.034$), BCR rate (30.3% versus 13.7%, $P < 0.001$), and metastasis rate (6.2% versus 2.1%, $P = 0.034$).

BCR occurred in 19.4% of patients in a median follow-up of 8.5 years. For the BCR prognosis analysis, Kaplan−Meier survival curve showed widening gap between patients with PNI+ versus patients with PNI− ($P < 0.001$, Fig. 2A), and the gap became wider between PNI+ b versus PNI− ($P < 0.001$, Fig. 2B). On univariate analysis, PSA, GS, pT stage, PSM, prostate weight, PNI status, and PNI+ subgroups were significantly ($P < 0.05$) associated with BCR prognosis.

On multivariate analysis, we further tested the independent prognosis value of PNI in models with either PNI status (model 1) or PNI+ subgroups (model 2) adjusted by variables which showed statistical significance on univariate analysis. We found, by decreasing HRs, GS, PSM status, and PNI+ subgroups were significantly independent prognostic factors (Table 3). PNI status lost its statistical significance when adjusted by GS and PSM. We found that including PNI+ subgroups to the Cox model improved its predictive accuracy slightly (Harrell's C = 0.77 versus 0.76).

4. Discussion

PNI status has been considered to be a potential prognostic factor based on its biological significance on PCa progression [10,34−37]. The presence of PNI on biopsy specimen was reported as a predictive factor for worse oncological outcome after RP [9]. However, the paradox of RP PNI as an independent prognostic factor remained because of its heterogenous presentations and the various number of foci on the RP specimens [19,20,30].

In the present study, when PNI status was recorded by the traditional dichotomization method, PNI+ was significantly associated with pathological features of cancer aggressiveness and with poor BCR prognosis in univariate analysis. The presence of PNI lost its statistical significance as an independent prognostic indicator in multivariate analysis reemphasizing the current evidence that the high prevalence of PNI may broadly co-exist with other known factors of tumor invasiveness.

To quantify the PNI focus, we simply counted all the PNI foci in all the slides of the RP specimens, although we were unable to distinct if the PNI foci were from the same perineural space when they were from neighbor slides. We think PNI foci from the same perineural space would not bring significant bias, and this method could be clinically practical. Based on the ability to predict BCR outcome in univariate analysis, we found 3 foci could be used as cut-off value to further divide PNI+ patients into PNI+ a (focus number $\leq$3) and PNI+ b (focus number>3). We found PNI+ b was an independent prognostic indicator when adjusted with GS and PSM. This finding suggested that the number of PNI focus indicated not only the possible sign of the symbiotic interaction between nerves and malignant cells but also the scale of cancer survival, invasion, and spread which may directly impact the oncologic outcomes.

Previously, using a 265 RP cases cohort with a median 45 months follow-up, Sun et al. [23] reported that 91 (46.4%) cases with PNI+ (74% were unifocal [=1], and 26% were multifocal [>1]), and the presence of multifocal PNI was strongly associated with increasing incidence of BCR independently. Our results confirmed the conclusion that multifocal PNI could be an independent prognostic factor of BCR. But different from their results, in our study, PNI+ frequency was much higher (73.5%), and unifocal (n = 1) rate of PNI+ was at 15.5%, which is much lower than their rate of 74%. Given the lower prognostic power of PNI+ cases with 2 or 3 foci, multifocal (n > 1) was not an independent prognostic indicator by subgrouping into unifocal (n = 1) and multifocal (n > 1) (data not shown). Furthermore, with assistance of S100 immunostaining, Lubig et al. [24] evaluated overall survival (OS) prognosis of PNI in 114 RP cases with a median 94 months follow-up, and they identified 61.4% PNI+ cases from their whole cohort. Although no significant difference in OS was found on Kaplan−Meier analysis ($P = 0.19$), they found that cases with less than 1 PNI-positive nerve in 5 high power fields had significantly longer survival times than those cases with more than one. Their results indicated that the intensity of PNI+ may correlate with worse OS outcome. Recently, after pathologic reevaluation of 314 patients with T1-T2 oral squamous cell carcinoma, Wei et al. [38] identified 83 PNI+ cases (26.4%).With a 5 PNI foci as cut-off value, they found that the number of PNI focus was significantly predictive for cervical lymph node metastasis, poor disease-specific survival, and poor OS in multivariate analysis. Taking all the above evidences and together with our own results, we think PNI quantification by counting PNI focus number in RP specimens could be a novel prognostic factor which can significantly improve the

predictive power of PNI. The optimal cut-off value needs be verified in future studies.

It has been well documented in the literature that 80% of PCa arises in the peripheral zone (PZ) of the prostate [39], and only 10% of PZ PCa were found at the anterior horns of the PZ [40]. Furthermore, the neurovascular bundles are situated posterolaterally in the prostate [41]. Our data confirmed that posterior-dominant PCa carried higher frequency of PNI+ than anterior-dominant PCa without reaching a statistical significance (29.6% versus 11.3%, $P = 0.060$). However, posterior-dominant PCa showed significantly higher PNI+ b frequency than PNI+ a subgroups (30.0% versus 6.2%, $P < 0.001$). Nondominant PCa of anterior-posterior carried the highest frequency of PNI+ and PNI+ b subgroup, and it also showed significantly higher PNI+ ($P < 0.001$) but similar to PNI+ b than posterior-dominant PCa. These data suggested that PNI+ was correlated with higher tumor volume as well as posterior-dominant PCa. The posterior-dominant PCa appeared to have more PNI foci (PNI+b) than the anterior-dominant PCa. These results could also explain the finding that PNI, especially the increased PNI+ foci are strongly correlated with the extraprostatic extension. In our study, PNI status was not significantly different between right- and left-dominant PCa; however, we found left-dominant PCa carried much higher PNI+ b frequency ($P = 0.006$). This finding will warrant a further study.

Except the density of PNI foci discussed in our study, PNI diameter has been suggested as an important factor to measure the scale of PCa invasion. Previously, Maru et al. [25] reported that PNI was detected in 75.0% of 640 RP cases. They found the mere presence of PNI was not an independent BCR predictor in multivariate analysis which is similar to our finding; however, they found that the increasing diameter of the largest focus of PNI was strongly associated with other established prognostic factors and was an independent predictor of BCR prognosis. When comparing the 5-year BCR-free survival rate (70% ± 3% for patients with PNI), they found that cases with PNI diameter<0.25 mm were at 93%, cases with PNI diameter range from 0.5 to 0.75 mm were at 36% and cases with PNI ≥ 0.75 mm were at 14%, which is a dramatical decrease when comparing those with PNI < 0.25 mm. They suggested that measuring PNI diameter could add important information to the prognosis of PCa patients. In our study, we also observed a trend that a larger PNI diameter usually found in cases with increasing number of PNI foci. It could be speculated that cases with more PNI foci (>3) or larger PNI diameter (≥0.5 mm) might suggest a high volume of tumor spread and could be considered as a high-risk indicator when combining with other established risk factors.

Previous evidences have established that the perineural space is a distinct route of cancer spread and metastatic tumor dissemination [6]. In our cohort, all the cases with metastasis progression were PNI+ and patients of PNI+ b group had a significantly higher metastasis risk than those of PNI+ a group. These findings reaffirm that perineural space are the major route of PCa metastasis and suggest that the number of PNI focus could be associated with tumor volume in the perineural space and tumor metastasis. Previously, Stone et al. [42] reported that the presence of PNI in biopsy specimens could predict pelvic lymph node metastasis in men with localized carcinoma of the prostate. Furthermore, Ciftci et al. [43] reported that biopsy PNI was associated with increased bone metastasis in PCa. In addition, Zhao et al. [44] found that multifocal PNI in biopsy specimen was an independent adverse prognosticator for both castration-resistant PCa-free survival and OS in patients with favorable/intermediate-risk metastatic PCa patients when compared with PNI− patients. However, because of the present under-reporting of PNI in clinical practice which hampered the precise evaluation of its true clinical significance, it was reported that only 43% of all the urologists would consider biopsy PNI in pathology reports to have an influence on their decisions when selecting treatment options for PCa patients [45]. Active surveillance (AS) has been widely accepted as an observational strategy in response to the overtreatment of men with low-risk PCa [46]. The clinical-oncological significance of PNI on biopsy specimen remains a matter of debated and has not been included in the current enrollment criteria of AS. Our finding on RP PNI may help future investigation on the importance of biopsy PNI because an increased number of RP PNI may correlate with higher incidence of PNI detected in biopsy specimens from the same PCa patient.

Our study supports the ongoing efforts to target PNI in treating PCa [47]. In our study, we showed that the higher number of PNI foci correlated with an increased risk of tumor recurrence, indicating the importance of the neural microenvironment. Our study results give weight to treatments targeting this route of cancer progression which may represent a therapeutic approach for the treatment of PCa. Previously, intraprostatic Botox injections before RP induced prostate denervation and apoptosis of PCa [48]. Future studies will be needed to find out in which settings such therapeutic strategy can be used.

Our study is limited by several factors. First, our study is limited by its retrospective and nonrandomized nature. Second, without the possibility to review patient's prostate biopsy slides, more information on the association of biopsy PNI and RP PNI quantification could not be carried out. Furthermore, our cohort was established from a single tertiary referral institution, and further external validation will be needed for generalization. Finally, we did not exam the prognostic impact of RP PNI on metastasis and OS because of the low incidence in the current cohort.

5. Conclusion

Quantification of PNI focus in addition to recording its dichotomized present status will not only provide more

detailed information but also can be used as a novel prognostic indicator for risk stratification. Further external validation will be needed for identifying the optimal cut-off value. Our findings will encourage further evaluation on the relevance of PNI in the pretreatment setting and support ongoing efforts to target this pathway of cancer progression.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA A Cancer J Clin 2019;69:7–34.

[2] Sooriakumaran P, Nyberg T, Akre O, Haendler L, Heus I, Olsson M, et al. Comparative effectiveness of radical prostatectomy and radiotherapy in prostate cancer: observational study of mortality outcomes. BMJ 2014;348:g1502.

[3] Kattan MW, Wheeler TM, Scardino PT. Postoperative nomogram for disease recurrence after radical prostatectomy for prostate cancer. J Clin Oncol 1999;17:1499–507.

[4] Kupelian P, Katcher J, Levin H, Zippe C, Klein E. Correlation of clinical and pathologic factors with rising prostate-specific antigen profiles after radical prostatectomy alone for clinically localized prostate cancer. Urology 1996;48:249–60.

[5] Thompson I, Thrasher JB, Aus G, Burnett AL, Canby-Hagino ED, Cookson MS, et al. Panel AUAPCCGU. Guideline for the management of clinically localized prostate cancer: 2007 update. J Urol 2007;177:2106–31.

[6] Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: a review of the literature. Cancer 2009;115:3379–91.

[7] Chen SH, Zhang BY, Zhou B, Zhu CZ, Sun LQ, Feng YJ. Perineural invasion of cancer: a complex crosstalk between cells and molecules in the perineural niche. Am J Cancer Res 2019;9:1–21.

[8] Zhang LJ, Wu B, Zha ZL, Qu W, Zhao H, Yuan J, et al. Perineural invasion as an independent predictor of biochemical recurrence in prostate cancer following radical prostatectomy or radiotherapy: a systematic review and meta-analysis. BMC Urol 2018;18:5.

[9] Wu S, Lin X, Lin SX, Lu M, Deng T, Wang Z, et al. Impact of biopsy perineural invasion on the outcomes of patients who underwent radical prostatectomy: a systematic review and meta-analysis. Scand J Urol 2019:1–8.

[10] Fromont G, Godet J, Pires C, Yacoub M, Dore B, Irani J. Biological significance of perineural invasion (PNI) in prostate cancer. Prostate 2012;72:542–8.

[11] Kraus RD, Barsky A, Ji L, Garcia Santos PM, Cheng N, Groshen S, et al. The perineural invasion paradox: is perineural invasion an independent prognostic indicator of biochemical recurrence risk in patients with pT2N0R0 prostate cancer? A multi-institutional study. Adv Radiat Oncol 2019;4:96–102.

[12] Kang M, Oh JJ, Lee S, Hong SK, Lee SE, Byun SS. Perineural invasion and lymphovascular invasion are associated with increased risk of biochemical recurrence in patients undergoing radical prostatectomy. Ann Surg Oncol 2016;23:2699–706.

[13] Reeves F, Hovens CM, Harewood L, Battye S, Peters JS, Costello AJ, et al. Does perineural invasion in a radical prostatectomy specimen predict biochemical recurrence in men with prostate cancer? Can Urol Assoc J 2015;9:E252–5.

[14] Andersen S, Richardsen E, Nordby Y, Ness N, Storkersen O, Al-Shibli K, et al. Disease-specific outcomes of radical prostatectomies in Northern Norway; a case for the impact of perineural infiltration and postoperative PSA-doubling time. BMC Urol 2014;14:49.

[15] Jung JH, Lee JW, Arkoncel FRP, Cho NH, Yusoff NAM, Kim KJ, et al. Significance of perineural invasion, lymphovascular invasion, and high-grade prostatic intraepithelial neoplasia in robot-assisted laparoscopic radical prostatectomy. Ann Surg Oncol 2011;18:3828–32.

[16] Masieri L, Lanciotti M, Nesi G, Lanzi F, Tosi N, Minervini A, et al. Prognostic role of perineural invasion in 239 consecutive patients with pathologically organ-confined prostate cancer. Urol Int 2010;85:396–400.

[17] Lee JT, Lee S, Yun CJ, Jeon BJ, Kim JM, Ha HK, et al. Prediction of perineural invasion and its prognostic value in patients with prostate cancer. Korean J Urol 2010;51:745–51.

[18] Jeon HG, Bae J, Yi JS, Hwang IS, Lee SE, Lee E. Perineural invasion is a prognostic factor for biochemical failure after radical prostatectomy. Int J Urol 2009;16:682–6.

[19] Merrilees AD, Bethwaite PB, Russell GL, Robinson RG, Delahunt B. Parameters of perineural invasion in radical prostatectomy specimens lack prognostic significance. Mod Pathol 2008;21:1095–100.

[20] Miyake H, Sakai I, Harada K, Eto H, Hara I. Limited value of perineural invasion in radical prostatectomy specimens as a predictor of biochemical recurrence in Japanese men with clinically localized prostate cancer. Hinyokika Kiyo 2005;51:241–6.

[21] Ozcan F. Correlation of perineural invasion on radical prostatectomy specimens with other pathologic prognostic factors and PSA failure. Eur Urol 2001;40:308–12.

[22] D'Amico AV, Wu YH, Chen MH, Nash M, Renshaw AA, Richie JP. Perineural invasion as a predictor of biochemical outcome following radical prostatectomy for select men with clinically localized prostate cancer. J Urol 2001;165:126–9.

[23] Sun G, Huang R, Zhang X, Shen P, Gong J, Zhao J, et al. The impact of multifocal perineural invasion on biochemical recurrence and timing of adjuvant androgen-deprivation therapy in high-risk prostate cancer following radical prostatectomy. Prostate 2017;77:1279–87.

[24] Lubig S, Thiesler T, Muller S, Vorreuther R, Leipner N, Kristiansen G. Quantitative perineural invasion is a prognostic marker in prostate cancer. Pathology 2018;50:298–304.

[25] Maru N, Ohori M, Kattan MW, Scardino PT, Wheeler TM. Prognostic significance of the diameter of perineural invasion in radical prostatectomy specimens. Hum Pathol 2001;32:828–33.

[26] Reeves FA, Battye S, Roth H, Peters JS, Hovens C, Costello AJ, et al. Prostatic nerve subtypes independently predict biochemical recurrence in prostate cancer. J Clin Neurosci 2019;63:213–9.

[27] Aumayr K, Breitegger M, Mazal PR, Koller A, Marberger M, Susani M, et al. Quantification of extraprostatic perineural spread and its prognostic value in pT3a pN0 M0 R0 prostate cancer patients. Prostate 2011;71:1790–5.

[28] Bostwick DG, Grignon DJ, Hammond ME, Amin MB, Cohen M, Crawford D, et al. Prognostic factors in prostate cancer. College of American pathologists consensus statement 1999. Arch Pathol Lab Med 2000;124:995–1000.

[29] Zareba P, Flavin R, Isikbay M, Rider JR, Gerke TA, Finn S, et al. Transdisciplinary prostate canc P. Perineural invasion and risk of lethal prostate cancer. Cancer Epidemiol Biomark Prev 2017;26:719–26.

[30] Ng JC, Koch MO, Daggy JK, Cheng L. Perineural invasion in radical prostatectomy specimens: lack of prognostic significance. J Urol 2004;172:2249–51.

[31] McNeal JE, Redwine EA, Freiha FS, Stamey TA. Zonal distribution of prostatic adenocarcinoma. Correlation with histologic pattern and direction of spread. Am J Surg Pathol 1988;12:897–906.

[32] Egevad L, Srigley JR, Delahunt B. International Society of Urological Pathology (ISUP) consensus conference on handling and staging of radical prostatectomy specimens: rationale and organization. Mod Pathol 2011;24:1–5.

[33] Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA, et al. The 2014 international society of urological pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma: definition of grading patterns and proposal for a new grading system. Am J Surg Pathol 2016;40:244–52.

[34] Bründl J, Schneider S, Weber F, Zeman F, Wieland WF, Ganzer R. Computerized quantification and planimetry of prostatic capsular nerves in relation to adjacent prostate cancer foci. Eur Urol 2014;65:802−8.
[35] Entschladen F, Palm D, Lang K, Drell TLt, Zaenker KS. Neoneurogenesis: tumors may initiate their own innervation by the release of neurotrophic factors in analogy to lymphangiogenesis and neoangiogenesis. Med Hypotheses 2006;67:33−5.
[36] Ayala GE, Dai H, Ittmann M, Li R, Powell M, Frolov A, et al. Growth and survival mechanisms associated with perineural invasion in prostate cancer. Cancer Res 2004;64:6082−90.
[37] Magnon C, Hall SJ, Lin J, Xue X, Gerber L, Freedland SJ, et al. Autonomic nerve development contributes to prostate cancer progression. Science 2013;341:1236361.
[38] Wei PY, Li WY, Tai SK. Discrete perineural invasion focus number in quantification for T1-T2 oral squamous cell carcinoma. Otolaryngol Head Neck Surg 2019;160:635−41.
[39] McNeal JE. The zonal anatomy of the prostate. Prostate 1981;2:35−49.
[40] Haffner J, Potiron E, Bouye S, Puech P, Leroy X, Lemaitre L, et al. Peripheral zone prostate cancers: location and intraprostatic patterns of spread at histopathology. Prostate 2009;69:276−82.
[41] Walsh PC, Donker PJ. Impotence following radical prostatectomy: insight into etiology and prevention. J Urol 1982;128:492−7.
[42] Stone NN, Stock RG, Parikh D, Yeghiayan P, Unger P. Perineural invasion and seminal vesicle involvement predict pelvic lymph node metastasis in men with localized carcinoma of the prostate. J Urol 1998;160:1722−6.
[43] Ciftci S, Yilmaz H, Ciftci E, Simsek E, Ustuner M, Yavuz U, et al. Perineural invasion in prostate biopsy specimens is associated with increased bone metastasis in prostate cancer. Prostate 2015;75:1783−9.
[44] Zhao J, Chen J, Zhang M, Tang X, Sun G, Zhu S, et al. The clinical significance of perineural invasion in patients with de novo metastatic prostate cancer. Andrology 2019;7:184−92.
[45] Rubin MA, Bismar TA, Curtis S, Montie JE. Prostate needle biopsy reporting: how are the surgical members of the Society of Urologic Oncology using pathology reports to guide treatment of prostate cancer patients? Am J Surg Pathol 2004;28:946−52.
[46] Cooperberg MR, Carroll PR. Trends in management for patients with localized prostate cancer, 1990-2013. J Am Med Assoc 2015;314:80−2.
[47] Bakst RL, Wong RJ. Mechanisms of perineural invasion. J Neurol Surg B Skull Base 2016;77:96−106.
[48] Coarfa C, Florentin D, Putluri N, Ding Y, Au J, He D, et al. Influence of the neural microenvironment on prostate cancer. Prostate 2018;78:128−39.