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Original Research

Perineural invasion in early-stage cervical cancer: detection and influence on prognosis

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Summary

Purpose: To compare staining methods for detecting perineural invasion (PNI) in early-stage cervical cancer and assess the influence of PNI on survival. *Materials and Methods:* The authors retrospectively analysed data on 300 patients treated between 2010 and 2014 for cervical cancer in Stages Ia2-IIb. Rates of PNI detection using Hematoxylin-Eosin (HE) or anti-S-100 protein staining were compared. Influence of PNI on disease-free survival (DFS) and overall survival (OS) were assessed in all patients and in subgroups based on use of adjuvant therapy and nerve-sparing radical hysterectomy (NSRH). *Results:* Among the 300 patients, HE staining detected PNI in 38 (12.7%) and anti-S-100 staining detected PNI in 45 (15.0%, $p = 0.016$). PNI was associated with significantly shorter DFS ($p = 0.015$) and OS ($p = 0.020$), but it was not an independent risk factor for poor DFS or OS. Significantly higher proportions of patients with PNI received adjuvant radiotherapy or chemo-radiotherapy than patients without PNI ($p < 0.001$), but the two groups of patients showed similar DFS ($p = 0.293$) and OS ($p = 0.329$). *Conclusion:* Patients with PNI showed significantly longer DFS if they received adjuvant therapy ($p = 0.039$). Patients who underwent NSRH showed similar DFS and OS as those who did not, regardless of their PNI status. Anti-S-100 staining detects PNI better than HE in patients with early-stage cervical cancer. PNI is associated with poor survival, which can be improved through adjuvant therapy. NSRH does not appear to adversely affect survival of patients with or without PNI.

Key Words: Cervical cancer; Perineural invasion; Staining method; Prognosis

Introduction

Perineural invasion (PNI) is a pathologic process in which tumours invade nervous structures and spread along nerve sheaths[1]. PNI has been studied extensively in head-and-neck malignant cancer[2], pancreatic cancer [3], prostate cancer [4], and gastrointestinal cancer [5], where it is associated with poor survival [6].

PNI has recently been recognised in cervical cancer, where it is associated with well-known risk factors for poor prognosis [7-11], but its prognostic significance in early-stage cervical cancer remains controversial: some studies have associated it with negative outcomes [12-14], but other studies have not supported this finding [10, 15]. While these discrepancies may simply reflect differences in sample size, reliance on single-center populations, and bias inherent in retrospective studies, the present authors suspect that other confounding factors may be at work, such as differences in staining methods to detect PNI, as well as heterogeneity in whether patients received adjuvant radiotherapy or chemo-radiotherapy, and whether they underwent nerve-sparing surgery.

To begin to address more systematically the potential prognostic significance of PNI for early-stage cervical cancer, the authors examined whether PNI significantly affects patient survival, while taking into account adjuvant therapy and surgical method. They also compared the ability of Hematoxylin-Eosin (HE) staining or anti-S-100 protein staining to detect PNI.

Materials and Methods

Medical records of 300 cervical cancer patients treated between January 2010 and June 2014 at the Affiliated Tumor Hospital of Guangxi Medical University were analysed retrospectively. This study was approved by the Institutional Review Board of Affiliated Tumor Hospital of Guangxi Medical University.

To be enrolled in the study, patients had to (a) have been diagnosed with cervical cancer in Stages Ia2-IIb based on International Federation of Gynecology and Obstetrics criteria, (b) have received nerve-sparing radical hysterectomy (NSRH) or traditional radical hysterectomy combined with pelvic lymphadenectomy with or without para-aortic lymph node dissection, (c) be between 20- and 70-years-old, (d) show normal function of major organs, such that no special treatment was necessary, and (e) have complete follow-up data.

Post-surgical cervical and uterine tissue samples were simultaneously stained using HE and anti-S-100 antibody. Rabbit anti-S-100 polyclonal antibody was used at a dilution of 1:50. Surgical specimens were fixed in formalin, embedded in paraffin and sliced into 3-mm sections, which were then stained by immunohisto-

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Table 1. — *Characteristics of patients with early-stage cervical cancer.*

Characteristic	Value
Age, years	46.57 ± 8.65
FIGO Stage	
Ia2	7 (2.3)
Ib	188 (62.7)
IIa	59 (19.7)
IIb	46 (15.3)
Histology type	
SCC	226 (75.3)
Other	74 (24.7)
Tumour size, cm	
< 4	173 (57.7)
≥ 4	127 (42.3)
Tumour grade	
G1	32 (10.7)
G2	95 (31.6)
G3	173 (57.7)
Depth of invasion	
< 1/2	169 (56.3)
≥ 1/2	131 (43.7)
Lymphovascular space invasion	
No	243 (81.0)
Yes	57 (19.0)
Lymph node metastasis	
No	230 (56.7)
Yes	70 (43.3)
Parametrical invasion	
No	280 (93.3)
Yes	20 (6.3)
Positive vaginal margin	
No	286 (95.3)
Yes	14 (4.7)
Perineural invasion	
No	255 (85.0)
Yes	45 (15.0)

Values are mean ± SD or n (%). FIGO: International Federation of Gynecology and Obstetrics; SCC: squamous cell carcinoma.

chemistry according to the manufacturer's instructions. Sections were examined independently by two pathologists. PNI was defined as the presence of tumour cells within any of the three layers of the nerve sheath, or as the presence of tumour in close proximity to a nerve and involving at least one-third of the nerve's circumference [6].

The following data were extracted for all patients: age, histology subtype, tumour grade and size, depth of stromal invasion, lymphovascular space invasion (LVSI), lymph node metastasis, parametrical invasion, positive vaginal margin, positive detection of PNI by each of the two staining methods, treatment with NSRH, and postoperative treatment with radiotherapy or chemo-radiotherapy. Data were also collected to allow calculation of disease-free survival (DFS) and overall survival (OS) curves.

All patients were followed up until death or September 2016, corresponding to median follow-up of 36 (range, 3-79) months. Follow-up was conducted during routine outpatient visits or via telephone or letter every three months during the first two postoperative years, then every six months for postoperative years three to six, and subsequently once annually. Data were collected on recurrence and survival.

All analyses were performed using SPSS 17.0. Inter-group differences were assessed for significance using the chi-squared test, which was also used to test for possible associations. Survival curves were generated using the Kaplan–Meier method and compared using the log-rank test. Cox proportional hazard regression was performed to identify factors associated with DFS and OS. When appropriate, 95% confidence intervals were calculated. The threshold of significance was defined as $p < 0.05$.

Results

Clinicopathologic characteristics of the entire sample of 300 patients are shown in Table 1. Mean age of the 45 patients with PNI was marginally higher than that of the 255 patients without PNI (48.53±6.80 *vs.* 46.28±8.90 years; $p = 0.09$).

Anti-S-100 staining detected PNI in 45 patients (15.0%), significantly more than the 38 cases (12.7%) detected by HE staining alone ($p = 0.016$, Table 2).

A total of 125 patients with risk factors for poor prognosis received radiotherapy or chemo-radiotherapy after surgery (Table 2), including 37 of the 45 patients with PNI (82.2%). Adjuvant therapy was given to a significantly greater proportion of patients with PNI than patients without PNI ($p < 0.001$). A total of 97 patients underwent NSRH (Table 2), including 12 of the 45 patients with PNI (26.7%). Similar proportions of patients with or without PNI underwent NSRH ($p = 0.378$).

Kaplan-Meier analysis of the entire sample of 300 patients showed that the 45 patients with PNI had significantly shorter DFS ($p = 0.015$) and OS ($p = 0.020$) than the 255 patients without PNI (Figure 1).

Cox regression analysis identified the following independent risk factors for poor DFS and OS (Table 3): histological type, with respective p values of 0.008 and 0.006, depth of invasion $p = 0.013$ and 0.004, lymph node metastasis $p = 0.001$ and $p < 0.001$ parametrical invasion $p = 0.002$ and 0.001, and positive vaginal margin $p = 0.024$ and 0.033. However, PNI was not identified as an independent risk factor for poor DFS ($p = 0.391$) or OS ($p = 0.204$).

Among the 125 patients who received adjuvant radiotherapy or chemo-radiotherapy, the 37 patients with PNI showed similar DFS as the 88 patients without PNI ($p = 0.293$), as well as similar OS ($p = 0.329$) (Figure 2). Among the subgroup of 45 patients with PNI, the 37 who received adjuvant therapy showed longer DFS than the eight patients who did not ($p = 0.039$), while the two groups showed similar OS ($p = 0.06$).

The 97 patients who underwent NSRH showed similar DFS as the 203 patients who did not ($p = 0.474$), as well as similar OS ($p = 0.521$) (Figure 3). Among the subgroup of 45 patients with PNI, the 12 who underwent NSRH showed similar DFS as the 33 patients who did not ($p = 0.351$), as well as similar OS ($p = 0.415$).

Table 2. — *Detection of perineural invasion (PNI) with different staining methods and presence of perineural invasion in patients stratified by adjuvant therapy and surgery method.*

	No. (%) of patients			
	Total	PNI+	PNI-	*p*
Staining method				0.016
Anti-S-100 staining	300	45 (15.0)	255 (85.0)	
Hematoxylin-Eosin staining	300	38 (12.7)	262 (87.3)	
Adjuvant radiotherapy or chemo-radiotherapy				
No	175	8 (17.8)	167 (65.5)	< 0.001
Yes	125	37 (82.2)	88 (34.5)	
Nerve-sparing radical hysterectomy				
No	203	33 (73.3)	170 (66.7)	0.378
Yes	97	12 (26.7)	85 (33.3)	

Table 3. — *Multivariate analysis to identify factors affecting disease-free survival and overall survival.*

	Disease-free survival			Overall survival		
	HR	95%Cl	*p*	HR	95%Cl	*p*
Age	0.975	0.395-410	0.956	0.836	0.334-2.090	0.701
FIGO stage	0.636	0.254-1.593	0.334	0.816	0.314-2.124	0.678
Histology type	0.284	0.112-0.722	0.008	0.263	0.101-0.688	0.006
Tumour size	0.630	0.241-1.645	0.345	0.690	0.258-1.843	0.690
Tumour grade	0.865	0.256-2.923	0.815	0.537	0.152-1.891	0.333
Depth of invasion	0.267	0.094-0.754	0.013	0.206	0.070-0.604	0.004
Lymphovascular space invasion	0.482	0.147-1.577	0.228	0.467	0.147-1.483	0.196
Lymph node metastasis	0.228	0.092-0.567	0.001	0.188	0.074-0.472	< 0.001
Parametrical invasion	0.148	0.045-0.486	0.002	0.127	0.036-0.445	0.001
Positive vaginal margin	0.217	0.057-0.821	0.024	0.210	0.050-0.881	0.033
Perineural invasion	1.703	0.504-5.760	0.391	2.251	0.644-7.865	0.204

FIGO, International Federation of Gynecology and Obstetrics.

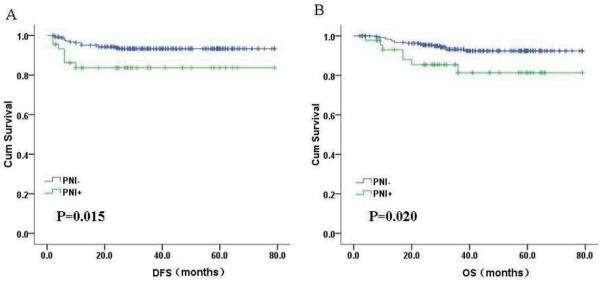

Figure 1. — Kaplan-Meier survival analysis of (a) disease-free survival (DFS) and (b) overall survival (OS) of patients with or without perineural invasion (PNI), based on the entire sample of 300 patients.

Discussion

The PNI incidence of 15.0% in the present cohort falls within the reported range of 7-35.1% [7-10, 15, 16]. The authors found that although PNI was associated with significantly shorter DFS and OS, it did not emerge as an independent risk factor for poor DFS or OS in Cox proportional hazard regression. PNI did not significantly affect DFS or OS when they analyzed separate subgroups based on adjuvant treatment. At the same time, patients with PNI

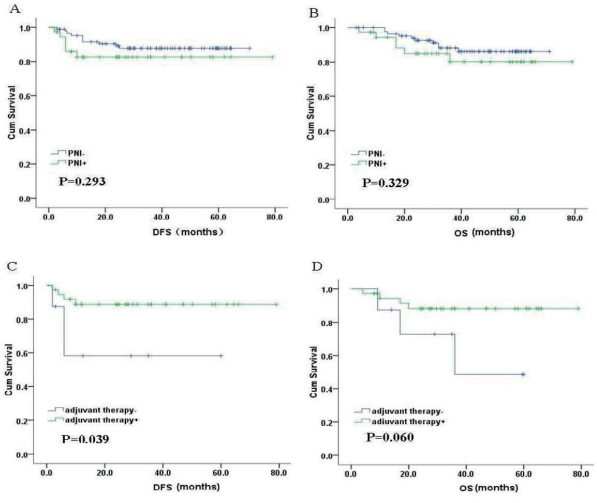

Figure 2. — Kaplan-Meier survival analysis based on adjuvant therapy. (a) Disease-free survival (DFS) and (b) overall survival (OS) of 125 patients who received adjuvant therapy, stratified by whether they had perineural invasion (PNI) or not. (c) DFS and (d) OS of 45 patients with PNI, stratified by whether they received adjuvant therapy or not.

showed significantly better DFS and marginally better OS if they received adjuvant treatment. The use of NSRH did not significantly affect DFS or OS, regardless of whether

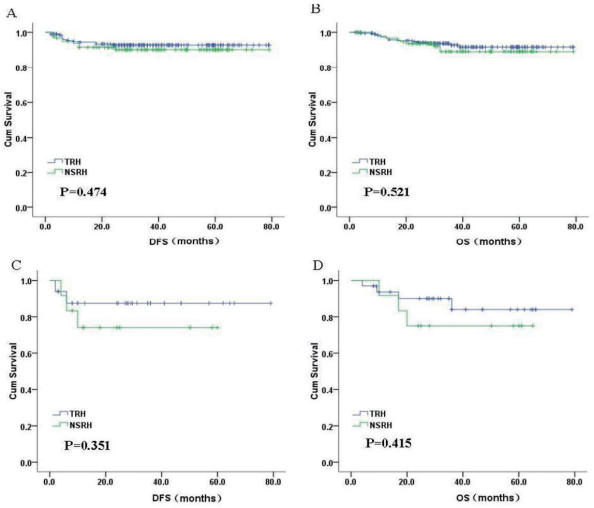

Figure 3. — Kaplan-Meier survival analysis of patients who underwent nerve-sparing radical hysterectomy (NSRH) or traditional radical hysterectomy (TRH). (a) Disease-free survival (DFS) and (b) overall survival (OS) in the entire sample of 300 patients. (c) DFS and (d) OS among the subset of 45 patients with perineural invasion (PNI).

PNI was present.

Previous work has associated PNI with more advanced tumour stage, larger tumours, LVSI, lymph node metastasis, and parametrical invasion [7-10, 15, 16]. The present group showed with multivariate analysis that PNI was associated independently with lymph node metastasis and LVSI [11]. Nevertheless, the results in the present study suggest that PNI is not a significant risk factor for poor DFS or OS. Similar to these findings, Tian *et al.* [13] reported a significantly lower five-year recurrence-free-survival (RFS) in patients with PNI than in patients without it, yet PNI did not emerge as an independent risk factor for poor RFS. Other studies have failed to find any differences between patients with or without PNI [8, 10, 15]. These findings contrast with studies suggesting significantly shorter OS in the presence of PNI [12, 14]. The fact that the present authors confirmed a lack of prognostic impact after stratifying patients based on adjuvant therapy and use of NSRH suggests that these results are real. Nevertheless, these retrospective findings from a single center should be verified and extended in larger studies, preferably from multiple sites.

The present authors found that anti-S-100 staining detected more cases of PNI than HE staining. These results are consistent with previous reports suggesting that anti-S-100 staining is superior to HE staining for detecting PNI in head-and-neck squamous cell carcinoma [17] as well as colorectal cancer [18].

Patients with certain risk factors for poor prognosis are typically treated with adjuvant radiotherapy or chemo-radiotherapy in order to reduce the risk of recurrence and increase survival [19, 20]. The present authors found that these patients showed similar DFS and OS regardless of whether they had PNI or not, but that patients with PNI who received adjuvant treatment showed better survival than patients with PNI who did not receive such treatment. These findings suggest that adjuvant therapy can improve the prognosis of patients with PNI, though the results must be verified in larger studies, especially since the observed improvement in DFS and OS was close to the cut-off for statistical significance.

The present authors found that NSRH did not significantly affect DFS or OS either in the total sample of 300 patients, or in the subset of 45 patients with PNI. NSRH is a popular treatment for cervical cancer because it can reduce injury to the pelvic nerve, improve recovery of bladder and rectal function, and shorten hospital stay relative to traditional surgery [21, 22]. On the other hand, some researchers have suggested that preserving the pelvic nerves may increase risk of cervical cancer recurrence [23, 24]. In fact, some have suggested that NSRH should not be conducted in patients with PNI [15].

The present results with a small cohort suggest that this guidance may not be well-founded. These results may reflect the fact that the authors cut the uterine branches approximately at the initial branch point of the pelvic plexus; this should result in removal of tumour-invaded nerve tissue in patients in which PNI is confined to uterine branches. The safety of NSRH for patients with PNI or other risk factors for poor prognosis remains poorly understood, so future work should explore this question in greater detail with larger samples.

Conclusions

The present retrospective study of patients with early-stage cervical cancer provides evidence that anti-S-100 staining detects PNI better than HE staining, and that PNI is associated with poor DFS and OS, although it does not appear to be a significant independent risk factor for poor survival. Patients with PNI are more likely to receive radiotherapy or chemo-radiotherapy after surgery, and adjuvant treatment may improve survival for patients with PNI. NSRH does not appear to affect DFS or OS in patients with early-stage cervical cancer, regardless of whether they have PNI or not.

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