Perineural invasion in early-stage cervical cancer and its relevance following surgery

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Abstract. Perineural invasion (PNI) is the neoplastic invasion of nerves by cancer cells, a process that has attracted attention as a novel prognostic factor for cancer. The present study aimed to investigate the prognostic value of PNI in patients with early-stage cervical cancer (International Federation of Gynecology and Obstetrics stage IA2-IIA). A total of 210 patients who underwent radical hysterectomy and pelvic lymphadenectomy between 2007 and 2012 were included in the current study, of whom 8.57% (18/210) exhibited PNI. Patients with PNI were more likely to exhibit adverse histopathological features, such as increased tumor size, depth of stromal invasion, parametrial invasion, lymphovascular space invasion and lymph node metastases (all P<0.05). Patients with PNI exhibited shorter disease-free and overall survival (P=0.002 and P=0.017, respectively). However, PNI was not identified as an independent risk factor for either recurrence or death by multivariate analysis. Furthermore, 88.9% (16/18) of patients with PNI received adjuvant therapy following surgery. PNI was significantly associated with well-established indicators for adjuvant therapy. In conclusion, PNI was associated with multiple high-risk factors and its presence was indicative of a poor outcome in patients with early-stage cervical cancer, which may influence management decisions regarding adjuvant therapy.

Introduction

Malignant cells dissociate from their environment in the extracellular matrix and establish metastatic deposits at distant sites. The generally recognized route of metastatic spread occurs directly as well as through vascular and lymphatic channels; however, whether tumor cells can spread in and along nerves is not yet widely accepted. Perineural invasion (PNI), also called neurotropic carcinomatous spread or perineural spread, is the neoplastic invasion of nerves, which may prove to be another metastatic route (1). As described in literature since the mid-1800s, PNI has since emerged as a pathological feature of ~90% of surgical specimens in cancer of the head and neck, prostate, pancreas, colon and rectum; for a number of these malignancies, PNI is independently associated with high recurrence rates and poor survival (2-7).

Early-stage cervical cancer is treated by radical hysterectomy and pelvic lymphadenectomy, with or without sampling of the paraaortic lymph nodes (8). This surgical approach allows for the preservation of ovarian endocrine function and offers greater protection of sexual function. It also makes resection of enlarged lymph nodes possible, and facilitates the histopathological evaluation of the involvement of the parametria and lymph nodes (9). Postoperative adjuvant therapy for cervical cancer should be considered, depending on whether pathological examination reveals the presence of risk factors for disease recurrence (10). Although parametrial and lymph node involvement, and lymphovascular space invasion are well-known poor prognostic indicators in cervical cancer, the histological details of disease status within the parametria and any PNI are often overlooked and rarely reported in histopathological evaluations of radical hysterectomy specimens. The objective of the present study was to investigate the incidence...
of PNI in early-stage (IA2-IIA) cervical cancer and evaluate its prognostic impact.

Materials and methods

**Clinical characteristics of patients.** Data from a total of 210 women (median age, 44 years, range 27-72 years) who underwent radical hysterectomy and pelvic lymphadenectomy between January 2007 and December 2012 at the Sichuan Cancer Hospital (Chengdu, China) were assessed in the present study. All patients with International Federation of Gynaecology and Obstetrics (FIGO) stage IA2 to IIA2 squamous cell carcinoma, adenosquamous carcinoma or adenocarcinoma of the cervix were included, with other histological subtypes, such as neuroendocrine or small cell tumors, excluded. Those with incomplete local tumor resection were excluded from the study. Patients with preoperative tumors >4 cm underwent platinum-based neoadjuvant chemotherapy (NACT). In total, 134 patients that had at least one high-risk factor or more than two intermediate-risk factors received adjuvant therapy with radiation therapy (RT) and/or 3-7 cycles of concurrent chemoradiation therapy (with a platinum-based regimen) following surgery (11,12). High-risk factors for recurrence of cervical cancer included lymph nodes metastasis, parametrial invasion and resection margin involvement (11,13). Intermediate-risk factors included tumor size, depth of invasion according to the guidelines of Elshawi et al (14), and lymphovascular space invasion (LVSI) (11,13). The recurrence of disease was identified radiologically by computed tomography (CT) or magnetic resonance imaging (MRI). The study was approved by the Research Ethics Committee of the Sichuan Cancer Hospital. Each patient gave written informed consent prior to enrollment in the study.

**Histopathological analysis.** The pathological features were diagnosed and recorded by two pathologists. PNI was defined as occurring if cancer cells were present in or immediately surrounding nerve bundles, as identified using hematoxylin and eosin staining (15). The tissue in each case was formalin-fixed and embedded in paraffin. Paraffin sections (5 µm thickness) were used for hematoxylin and eosin staining. Table I summarizes patient demographics (age) and tumor features assessed in the study population, including histological subtype, tumor dimensions, depth of invasion, parametrial invasion, lymph nodes metastasis, surgical resection margin status, LVSI and PNI.

**Statistical analysis.** Disease-free survival (DFS) was defined as the interval between the day that surgery was performed and the day that recurrence was observed radiologically by CT or MRI. If recurrence was not detected, the date of death or of the last follow-up was used. Overall survival (OS) was defined as the interval between the dates of surgery and death. The follow-up period following the initial operation for the primary lesion was 5 years for DFS and OS. DFS and OS were estimated by Kaplan-Meier curves and were compared using the log-rank test. Time to relapse and death was analyzed using the Cox proportional hazards model for univariate and multivariate analyses. Associations between nominal factors were assessed with χ² tests, with the application of a two-tailed Fisher's exact test where appropriate. P<0.05 was considered to indicate statistical significance. Analysis was performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA).

**Results**

Cases in which PNI or parametrial invasion was described in the original pathology report were reviewed. A total of 18/210 patients (8.57%) presented with PNI; 15 patients exhibited PNI in the cervix, 3 in the cervix and the parametrium. An example of PNI is presented in Fig. 1.

The mean age of subjects with PNI was 47 (±7) (range 32-62) years compared with 44 (±8) (range 27-72) years in subjects without PNI (P=0.908). The association between PNI and known risk factors for recurrence of cervical cancer is presented in Table I. A total of 13 patients with PNI had tumors >4 cm vs. 93 subjects without PNI (P=0.045). Patients with deeper cervical invasion reported significantly more PNI than those with more superficial invasion (P<0.001). PNI was significantly associated with parametrial invasion (P<0.001), lymph node metastasis (P=0.001) and the increased occurrence of LVSI (P=0.005). However, there was no significant difference in FIGO stage, tumor histology or extension to the vaginal margin between the two groups (Table I).

Cox proportional hazard regression modeling was performed to determine a predictive association of the clinicopathological variables with recurrence and death-associated with cervical cancer (Table II). At the 5-year follow-up, FIGO stage, tumor size, depth of invasion, parametrial invasion, LVSI, lymph node metastasis and positive vaginal margin were identified to be associated with recurrence by univariate analysis. Depth of invasion, parametrial invasion and positive vaginal margin were risk factors for death in this population (Table II). Models that include all histopathological variables and tumor markers found to have significant prognostic value in univariate analysis were then analyzed by multivariate analysis (Table II). Depth of invasion and parametrial invasion were significantly associated with DFS.
Lymph node metastasis was associated with a risk of recurrence only ($P=0.04$; Table II). Early-stage cervical cancer patients without PNI had significantly longer DFS times (Fig. 2A) and OS (Fig. 2B) than patients who had evidence of PNI ($P=0.002$ and $P=0.017$, respectively). However, PNI was not identified as an independent risk factor for either recurrence [hazard ratio (HR), 0.26; 95% confidence interval (CI), 0.07-0.91; $P=0.05$] or death (HR, 0.28; 95% CI, 0.05-1.59; $P=0.15$).

The presence of risk factors for recurrence was indicated for postoperative adjuvant treatment. A total of 63.8% patients (134/210) underwent adjuvant therapy such as RT or concurrent chemoradiation therapy; 88.9% patients (16/18) with PNI received adjuvant therapy. Patients with PNI received significantly more adjuvant therapy than those without PNI. PNI was significantly associated with adjuvant therapy (88.9% sensitivity; 62.7% specificity; odds ratio, 5.0; $P=0.021$).

Discussion

In 1835, PNI was first reported as a notable pathological feature in head and neck cancers that exhibited a predilection for growth along nerves as they made their way toward the intracranial fossa (2). A precise definition of PNI has not yet been established in the literature. In 2009, Liebig et al (16) advocated a broad definition of PNI, characterizing it as a tumor in close proximity to a nerve and involving $\geq33\%$
of its circumference or tumor cell invasion in, around and through any of the three layers (the epineurium, perineurium and endoneurium) of the nerve sheath. Various tumor-nerve growth patterns have been described, including complete and incomplete encirclement, concentric lamination and tangential contact (17). A much more frequent finding in PNI is tumor-nerve contact within the perineurum without tumor cells invasion of the sheath (16). A scientific and comprehensive definition of PNI would aid further research and clinical applications.

PNI is associated with pain and tumor metastasis, independent of lymphatic or vascular involvement (18). The mechanism by which PNI occurs remains unclear. For the last 40 years, the predominant theory behind the pathogenesis of PNI has been that cancer cells are marauders that invade nerves through the path of least resistance. However, novel data challenge the conventional theories, suggesting that prostaglandin E2 promotes cancer invasion and, in a positive-feedback mechanism, galanin released by cancer induces neuritogenesis, facilitating PNI (19). PNI may involve reciprocal signaling interactions between tumor cells and nerves, with the invading tumor potentially acquiring the ability to respond to pro-inflammatory signals within the peripheral nerve milieu. In vitro PNI models of prostate cancer and pancreatic cancer provide evidence for this interaction, suggesting that the signaling mechanisms behind PNI are likely to involve at least three different cellular elements, including tumor cells, nerve cells, and stromal cells, and may include autocrine and paracrine mechanisms (16,20). Other evidence suggests the presence of a complex signaling interaction that involves the exchange of neurotrophic growth factors between nerves and invading tumor cells to drive pathogenesis, factors including neurotropin, granulocyte colony-stimulating factor, and cytokines (21-23). Another study investigated the mechanism of PNI in other aspects, including the role of gene defects and the tumor suppressor TP73 (24).

Patients with early-stage or locally advanced-stage cervical cancer are preferentially treated with radical hysterectomy and pelvic lymph node dissection or NACT followed by radical hysterectomy, achieving 5-year survival rates of ~85% (25-27). Additional adjuvant treatment may be pursued depending on the presence or absence of risk factors for recurrence. Lymph node metastasis, parametrial invasion and resection margin involvement are known to be high-risk factors that increase the recurrence rate up to 40% in postoperative cervical cancer (28,29). The significance of parametrial resection in the operative management of patients with cervical cancer has led to the evolution of the surgical techniques used to manage disease in these patients. However, thus far, large prognostic studies that deal with morphologic parameters do not even refer to PNI (30-35), and histopathological descriptions of parametria usually ignore the existence of PNI altogether.

### Table II. Univariate and multivariate analysis of clinicopathological features and patient survival.

<table>
<thead>
<tr>
<th>Variable</th>
<th>5-year DFS</th>
<th>5-year OS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
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<tr>
<td>Univariate analysis</td>
<td></td>
<td></td>
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<tr>
<td>Age</td>
<td>1.00</td>
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<td>FIGO stage</td>
<td>1.43</td>
<td>1.06-1.93</td>
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<tr>
<td>Pathology</td>
<td>1.66</td>
<td>0.74-3.69</td>
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<tr>
<td>Histological grade</td>
<td>1.49</td>
<td>0.88-2.51</td>
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<tr>
<td>Tumor size (≤4 vs. &gt;4 cm)</td>
<td>2.71</td>
<td>1.26-5.87</td>
</tr>
<tr>
<td>Depth of invasion (&lt;2/3 vs. ≥2/3)</td>
<td>5.58</td>
<td>2.29-13.56</td>
</tr>
<tr>
<td>Parametrial invasion</td>
<td>13.79</td>
<td>6.13-31.04</td>
</tr>
<tr>
<td>LVSI</td>
<td>3.31</td>
<td>1.51-7.21</td>
</tr>
<tr>
<td>Lymph nodes metastases</td>
<td>4.30</td>
<td>2.15-8.60</td>
</tr>
<tr>
<td>Positive vaginal margin</td>
<td>2.96</td>
<td>0.90-19.72</td>
</tr>
<tr>
<td>PNI</td>
<td>3.56</td>
<td>1.53-8.29</td>
</tr>
<tr>
<td>Multivariate analysis</td>
<td></td>
<td></td>
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<tr>
<td>FIGO stage</td>
<td>1.21</td>
<td>0.83-1.75</td>
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<tr>
<td>Tumor size (≤4 vs. &gt;4 cm)</td>
<td>1.26</td>
<td>0.49-3.26</td>
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<tr>
<td>Depth of invasion (&lt;2/3 vs. ≥2/3)</td>
<td>3.04</td>
<td>1.17-7.88</td>
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<tr>
<td>Parametrial invasion</td>
<td>16.74</td>
<td>4.63-60.50</td>
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<tr>
<td>LVSI</td>
<td>2.22</td>
<td>0.98-5.04</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>2.24</td>
<td>1.06-4.75</td>
</tr>
<tr>
<td>Positive vaginal margin</td>
<td>-</td>
<td>-</td>
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<tr>
<td>PNI</td>
<td>0.26</td>
<td>0.07-0.91</td>
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DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; OS, overall survival; FIGO, International Federation of Gynecology and Obstetrics; PNI, perineural invasion; LVSI, lymphovascular space invasion.
For certain human malignancies, PNI has been found to occur with a high incidence and to be an indicator of poor outcome. However, the knowledge concerning the frequency and the impact of PNI in cervical cancer is limited (14,36-39). In the present study, PNI occurred in 8.57% (18/210) of patients with cervical cancer (FIGO stages IA2-IIA). PNI was detected in the parametrium in only three cases, and in the cervix in all 18 cases. A consensus regarding a standardized definition and reporting of PNI in cervical cancer histopathology reports has not yet been reached. Compared with results of the present study, the incidence of PNI (8.57%, 18/210) was significantly lower than that reported by Horn et al (38) (P=0.002). Patients that received NACT therapy were excluded from the present study; thus, NACT may reduce the incidence of PNI.

PNI has been reported to be associated with a variety of well-established risk factors (35-39). In the present study, the presence of PNI was significantly associated with higher FIGO stage, larger tumors (≥4 cm), greater depth of invasion, increased parametrial invasion, LVI, and lymph nodes metastasis (P<0.05), which are factors that have important roles in deciding adjuvant therapy. The 5-year DFS and OS rates were significantly decreased in early-stage patients presenting with PNI, compared with those that did not (P=0.002 and P=0.011, respectively). This indicated that PNI was associated with more aggressive disease; however, when subjected to multivariate analysis, PNI was not found to be an independent high-risk factor that predicted either recurrence (HR, 1.50; 95% CI, 0.42-5.39) or death (HR, 0.69; 95% CI, 0.06-8.19). Horn et al (38) reported the 5-year OS rate was significantly decreased in patients with PNI, whereas other studies reported no difference in DFS or OS (38,39). A meta-analysis demonstrated that cervical cancer with PNI was associated with a lower OS rate (40). However, in the present study, association of PNI with more established markers of poor prognosis, or as a particular histological characteristic, was limited, as adjuvant therapy had an impact on OS and DFS. Radiotherapy may be beneficial for patients with PNI and has no marked effect on the quality of life. Fully determining the impact of PNI on the prognosis of patients with cervical cancer requires a large sample study to elucidate further information. In the present study, PNI was demonstrated to be markedly associated with risk factors for recurrence in cervical cancer. Surgeons, radiotherapists and oncologists should therefore be aware of the presence of PNI and should note it in surgical pathology reports.

Nerve-sparing radical hysterectomy (NSRH) has been reported to provide similar cure rates for cervical cancer to strategies using the conventional, non-nerve-sparing technique, with decreased vesical, rectal, and, to a lesser degree, sexual dysfunction (41-43). However, the postoperative recurrence rate of patients that undergo this surgical procedure remains unclear and there is no standardized research methodology. There are a number of issues that require clarification by an in-depth study. Since the presence of PNI is associated with risk factors for recurrence in cervical cancer, clinicians may wish to strictly monitor patients who have undergone NSRH. Therefore, the present study set limits on patient eligibility, with patients only eligible if imaging examination revealed no high-risk factors in early-stage patients who did not receive NACT (which may mask the occurrence of PNI). Furthermore, PNI may present in the parametrium in early-stage cervical cancer, so NSRH could retain a risk of recurrence (or residual tumor). PNI can be detected by preoperative imaging studies (CT or MRI) and histological evaluation (17,44). PNI evaluation by 3.0T high-field MRI can map the exact tumor location and improve critical surgical and treatment planning (45). Previous studies have reported that the risk of parametrial involvement is <1% for patients with a tumor <2 cm in size, limited depth of invasion and no lymphovascular invasion (46,47). This knowledge could be used to aid selection of patients who would most benefit from NSRH without requiring postoperative radiation.

In conclusion, PNI may represent a novel prognostic risk factor for cervical cancer, which is expected to assist in determining patient adjuvant treatment. Further study is required to provide for a deeper insight into the pathogenic mechanisms of PNI. PNI is a novel clinicopathological topic in gynecological oncology and pathology, and required further investigation to aid in reducing disease recurrence and improving DFS and OS in patients with cervical cancer.

Acknowledgements
Not applicable.

Figure 2. Kaplan-Meier survival curves of DFS and OS in patients with cervical cancer according to PNI expression. (A) The presence of PNI was significantly associated with lower DFS (P=0.002). (B) The presence of PNI in cervical cancer was significantly associated with lower OS (P=0.017). 18 subjects with PNI vs. 192 subjects without PNI. DFS, disease-free survival; OS, overall survival; PNI, perineural invasion.
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Availability of data and materials
The datasets generated and analyzed in the present study are included in this published article.

Authors' contributions
YZ carried out study design and manuscript editing. GNZ carried out study conception and manuscript review. YY and LC carried out quality control of data and algorithms. SJJ performed data analysis and interpretation, and manuscript preparation. YS carried out statistical analysis. SQS and SQX performed data analysis and interpretation, and manuscript preparation. LC carried out quality control of data and algorithms. SJJ carried out data acquisition. All authors reviewed the manuscript.

Ethics and consent to participate
The study was approved by the Research Ethics Committee of the Sichuan Cancer Hospital. Each patient gave written informed consent prior to enrollment in the study.

Consent for publication
All the study participants gave consent for the data to be published.

Competing interests
The authors declare that they have no competing interests.

References


