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CLINICAL ARTICLE

Lymph node micrometastases in initial stage cervical cancer and tumoral recurrence

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ABSTRACT

Objective: To evaluate the prevalence of micrometastases in lymph node tissue of patients with stage Ib1–IIA cervical cancer, the correlation of micrometastases with tumor recurrence and survival, and the expression of D2-40 in the primary tumor of patients with recurrence and/or micrometastases and its correlation with histopathologic findings. *Methods:* In a retrospective study, the medical records of all patients with cervical cancer treated at a hospital in São Paulo, Brazil, between 2001 and 2007 were reviewed. Patients with no lymph node metastases and treated with radical hysterectomy without adjuvant treatment were included. Tumor sections were reviewed and lymph nodes were analyzed with AE1/AE3. Patients with and without recurrence were compared. The presence of lymph node micrometastasis or isolated tumor cells was also evaluated. *Results:* Of the 83 patients evaluated, 15 (18%) had recurrence. Significant differences between patients with and without recurrence were observed with regard to tumor greatest axis, clinical stage, number of micrometastases, and negative lymph nodes ($P \leq 0.04$). Lymph node micrometastases and isolated tumor cells were significantly different for a stromal invasion depth greater than 2/3 ($P = 0.046$). *Conclusion:* The presence of lymph node micrometastases is an important risk factor for tumor recurrence. These patients should be considered eligible for adjuvant radiochemotherapy treatment.

1. Introduction

Cervical cancer is the third most frequent type of cancer among women worldwide [1]. For 80% of women with cervical cancer, diagnosis occurs in the initial stages (International Federation of Gynecology and Obstetrics [FIGO] grade Ia1–Ib2) [2–5]. Lymphadenectomy is indicated in all initial clinical stages except for microinvasive carcinoma (Ia1) [6] because the lymphatic system is the major route of spread of most carcinomas. In addition to the clinical stage, regional lymph node involvement is the main prognostic factor in most cancers. In cervical cancer, pelvic lymph node involvement is the main criterion for the indication of adjuvant therapy [7–12].

Van Trappen et al. [13] demonstrated that immunohistochemical evaluation with cytokeratin 19 revealed micrometastases in half of lymph nodes previously considered negative following initial histologic evaluation with hematoxylin-eosin (HE). However, only some of them continue to grow and form tumors. Other studies have shown that, among patients with lymph nodes initially considered negative, the recurrence rate and prevalence of micrometastases have both been estimated at approximately 10%–15% [1,14]. Additionally, strong associations have been demonstrated between micrometastases and angiolymphatic invasion [2,13,15], tumor size [2,13], histological grade III [13], stromal invasion depth [5], and intensity of the inflammation secondary to the neoplasia [5], although one study failed to show correlations between these factors [14]. Nevertheless, lymph node micrometastases are not routinely evaluated using immunohistochemistry and the role of lymph node micrometastases in cervical cancer prognosis remains controversial [1].

The objective of the present study was to evaluate the prevalence of micrometastases in lymph node tissue of patients with FIGO stage Ib1–IIA cervical cancer using anti-pan cytokeratin (AE1/AE3) antibody immunostaining, as well as to correlate micrometastases with tumor recurrence and overall survival. Additionally, the immunohistochemical expression of the lymphatic endothelial marker D2-40 and its correlation

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with histopathologic findings by conventional HE staining in the primary tumor of patients with tumoral recurrence and/or lymph node micrometastases were also evaluated.

2. Materials and methods

An observational, retrospective study was performed at the Centro de Referência da Saúde da Mulher do Estado de São Paulo – Hospital Pérola Byington (CRSM), São Paulo, Brazil. Medical charts for consecutive patients with cervical cancer of stages Ib1, Ib2, and IIA, who were surgically treated between January 1, 2001, and December 31, 2007 were retrieved and reviewed. The inclusion criteria were radical hysterectomy with a minimum of 5 years of follow-up, no associated neoplasia, absence of lymph node metastasis as evaluated by initial histopathologic examination with HE staining, and the availability of paraffin-embedded tissue. Patients for whom surgery was not the primary treatment were excluded, as were patients using adjuvant radiotherapy and patients who had not undergone lymphadenectomy. The study protocol was approved by the local ethics committee (Comitê de Ética em Pesquisa do Centro de Referência em Saúde da Mulher do Estado de São Paulo, Hospital Pérola Byington; protocol number 16327). Because the study involved only medical chart review and archived tumoral tissue evaluation, informed consent was waived.

Patients were classified according to cancer recurrence. All histopathology slides were independently reviewed by two authors (L.F.C. and R.C.M.F.), with a consensus meeting in cases of discordance. Seven variables were re-evaluated in the HE-stained slides: histological type and grade, disease stage, risk stage, tumor size, stromal invasion, angiolymphatic invasion, and lymph node status.

Additionally, immunohistochemical evaluation was performed to identify the presence of pelvic lymph node micrometastases. According to the definition proposed by the Union for International Cancer Control [16], lymph node micrometastases are tumor cells smaller than 2 mm in diameter in patients with stromal reaction and cellular proliferation. When these signs are absent and yet tumor cells are observed, these are termed isolated tumor cells (ITCs) [16]. In the present study, ITCs were considered as lymph node micrometastases, because only few cases of ITC were found. Immunohistochemical evaluation was performed using the primary antibody AE1/AE3 (Dako, Carpinteria, CA, USA) diluted at 1:2000, as in previous studies [2,5,14,15]. Tissue blocks containing lymph nodes were sectioned to 3-μm thick slices using a LUPE MRP-03 microtome; these slices were thinner than those used in previous studies [2,5,14,15], meaning that the risk of loss of material decreased.

Tumor tissue blocks of patients with no angiolymphatic invasion, as detected by the HE staining evaluation, but who were at risk of undiagnosed angiolymphatic invasion (e.g. in cases of tumor recurrence and lymph node micrometastases), were re-evaluated using the lymphatic endothelial marker D2-40 (Dako), which is a reliable method for evaluation of endothelial lymph vessels, at 1:200 dilution. These findings were compared with the HE evaluation results and classified as positive or negative for angiolymphatic invasion.

Lymph node micrometastases were measured with a melanoma ruler reticle (Holtermann, Osasco, Brazil), with a 0–10 mm scale and 0.05-mm precision. Each lymph node was classified as being either without metastasis, with ITCs, or with one micrometastasis of up to 2 mm. The differentiation between micrometastasis and ITC was made according to the criteria by Hermanek et al. [16]. The distinction between benign epithelial inclusions and metastases was made using the morphological criteria proposed by Reich et al. [17].

An optical microscope (Nykon E200, Higashida-cho, Japan), with 10× and 4× magnifications in the optical and objective lenses, respectively, was used in HE and immunohistochemistry evaluations.

Qualitative variables were expressed by frequencies and percentages and the quantitative variables by mean, standard deviation, median, range, and number of valid observations. To compare groups in relation to qualitative variables, the χ^2 test, Fisher exact test, or the likelihood ratio were used. For quantitative variables, the parametric Student t test for independent groups was used. To calculate the odds ratio of lymph node micrometastases to tumor recurrence, a univariate logistic regression model was used. To examine the relation between independent variables, multivariate logistic regression was used. By calculating the sensitivity and specificity of lymph node micrometastases in relation to recurrence, the number needed to diagnose was calculated. A significance level of 5% was used ($P \leq 0.05$). Statistical analyses were performed using SPSS for Windows, version 15.0 (SPSS Inc, Chicago, IL, USA).

3. Results

Of the 628 consecutive patient records reviewed, 545 were not eligible for inclusion: 150 had initially undergone nonsurgical treatment, 185 had undergone surgery and radiotherapy, 22 had associated neoplasia, 33 had no archived paraffin-embedded samples, 90 had not undergone lymphadenectomy, and 65 had a follow-up of less than 5 years. Of the 83 remaining patients, 15 had recurrence (13 in the pelvis, 1 in lung and liver, and 1 in the lungs) and 68 had no recurrence. Among the 15 patients with recurrence, 12 (80%) survived to 5 years.

Univariate analysis comparing demographic and clinical variables between the two groups is shown in Table 1. The total number of pelvic lymph nodes dissected was 1138. The average length of the longest axis of the tumor, the frequencies of staging Ib2 and IIA, and the prevalence of lymph node micrometastasis were significantly greater among patients with recurrence than among those without ($P \leq 0.030$). The mean number of negative lymph nodes was significantly lower in patients with recurrence ($P < 0.001$).

Patients with suspected risk of angiolymphatic invasion undiagnosed by HE staining underwent D2-40 evaluation. However, only one case had a positive result (Fig. 1).

Using AE1/AE3 staining, 6 (7%) patients were found to have pelvic lymph node micrometastases. Table 2 presents a comparison of the demographic and clinical variables between patients with and without pelvic lymph node micrometastases; Fig. 2 shows an example of a positive result. One (1%) patient had benign epithelial inclusion associated with pelvic lymph node micrometastases and 5 (6%) patients showed expression of AE1/AE3 for benign epithelial inclusion. The number of cases with stromal invasion to a depth greater than two-thirds was significantly higher in the group of patients with lymph node micrometastases than in the group without ($P = 0.046$).

Multivariate regression analysis results (Table 3) showed significant associations between recurrence and the presence of lymph node micrometastases and between recurrence and tumor size: patients with micrometastases had an 11.73-times higher risk of recurrence than did those without micrometastases, and patients with tumors measuring 2 cm or more had a 4.42-times higher risk of recurrence than did those with smaller tumors. The number needed to diagnose was 4.215 (95% confidence interval 2.703–28.633), considering a sensitivity of 27% and specificity of 97% of AE1/AE3 immunohistochemical evaluation in relation to recurrence.

After 5 years of follow-up, 1 (17%) patient with micrometastases and 2 (3%) patients without micrometastases had died, although the difference was not significant ($P = 0.204$).

4. Discussion

In the present study, cervical cancer tumoral recurrence in association with pelvic lymph node micrometastases was the main outcome analyzed. AE1/AE3 staining detected micrometastases in 7% of patients. Additionally, as expected, patients with micrometastases had a much