Accuracy of Papanicolaou Smears in Cervical Cancer Patients Treated With Radiochemotherapy Followed by Radical Surgery

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Key Words: Cervical cancer; Papanicolaou test; Neoadjuvant radiochemotherapy; Morphologic changes; Accuracy; Cytology

DOI: 10.1309/AJCPP1ZWK8EMHBCZ

Abstract

Papanicolaou (Pap) smears are part of the follow-up program for patients with cervical cancer treated with radiochemotherapy. After therapy, residual neoplastic cells may be rare, seldom exfoliate, and may demonstrate many alterations, which creates a risk of inaccurate results. We report a study of 65 patients who received radiochemotherapy before radical surgery. For each patient, we compared smear results with results from histologic examination of the whole cervix. The Pap smear was “negative for cancer” in 50 cases, “positive for cancer” in 6 cases, and detected atypical squamous cells of undetermined significance in 9 cases. Histologic examination revealed a complete pathologic response (pR0) in 26 cases and a partial pathologic response (pR1) in 27. In 12 cases, no pathologic response (pR2) was shown. Comparison of these results showed that the Pap test had a sensitivity of 0.16, a specificity of 0.96, a negative predictive value of 0.83, a positive predictive value of 0.42, and an accuracy of 0.46.

With half a million new cases every year and the second highest incidence rate for any malignancy except breast cancer, cervical cancer is one of the world’s major health problems. As Papanicolaou (Pap) screening has spread, it has become common to detect preinvasive lesions rather than invasive cancer, and incidence rates have fallen steadily. Yet, despite heavy investment in prevention and early diagnosis, 5-year survival rates for patients with advanced disease barely reach 40%. Possible therapies include various combinations of external beam radiotherapy, brachytherapy, chemotherapy, and radical surgery. Today, an important emerging therapeutic option is external beam radiotherapy with concurrent chemotherapy followed by radical surgery.

Pap testing has an important role in the follow-up to this kind of treatment, in which it is used in conjunction with pelvic and digital rectal examination, laboratory tests, and imaging. The test is especially important in patients who do not undergo radical surgery after the completion of the radiochemotherapy program.

Since the introduction of radiotherapy for cervical malignancies, there have been a number of reports that it can induce mucosal alterations of the cervix and upper vagina. These morphologic changes, which affect neoplastic and non-neoplastic epithelial cells, make it extremely difficult to interpret Pap test results. Previous studies of Pap smears obtained after therapy are relatively old with no standardized evaluation criteria and no uniform terminology for the description of reported features. Therefore, the aim of the present study was to estimate the diagnostic accuracy of the Pap smear in patients with cervical cancer who have received radiochemotherapy. To this end, we compared Pap test results with results from a histologic examination of the hysterectomy specimen.
Materials and Methods

The study was based on computerized records held by the Division of Surgical Pathology and Diagnostic Cytopathology of the Policlinico Gemelli, Catholic University, Rome, Italy. A search for the period from January 1997 to December 2007 yielded 124 hysterectomy specimens from patients with cervical cancer who had received treatment with radiochemotherapy. We restricted our study to 65 patients who had received a histologic diagnosis of cervical cancer followed by radiochemotherapy before hysterectomy and who had a Pap smear dating from after the last course of radiochemotherapy.

All patients received the same treatment consisting of radiotherapy administered to the whole pelvic region in 22 fractions (1.8 Gy/d) totaling 39.6 Gy. Radiotherapy was accompanied by concomitant chemotherapy according to the following schedule: 2-hour intravenous infusion of 20 mg of cisplatin/m² body surface area on days 1 through 4 and days 27 through 30; 24-hour continuous intravenous infusion of 1,000 mg of 5-fluorouracil/m² (>1,500 mg/d) on days 1 through 4 and 27 through 30. At the end of the last course of radiochemotherapy, all patients were subjected to pelvic and digital rectal examinations, laboratory tests, and imaging studies. The clinical examination was completed with a Pap test.

Cervical smears were wet fixed in 95% ethanol and conventionally stained using the Papanicolaou method. All patients then underwent hysterectomy using the procedure previously described by Jurado et al. The mean ± SD time between clinical examination and surgery was 28.07 ± 22.90 days (maximum, 125 days; minimum, 3 days). Nearly 90% of the study population underwent hysterectomy between 10 and 40 days after the Pap smear was obtained. In a small number of cases, the surgery took place more than 50 days after the smear.

Pretreatment biopsy specimens and hysterectomy specimens were fixed in 4% buffered formalin for 18 to 24 hours. The cervix was sectioned clockwise into at least 12 blocks, which were completely embedded in paraffin. Two 3- to 4-μm sections were cut from different levels in each block and stained with H&E. To differentiate reactive giant cells from neoplastic giant cells, additional sections were prepared for immunohistochemical analysis. These slides were deparaffinized in xylene, rehydrated, and treated with 0.3% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase activity. The avidin-biotin streptavidin-biotin peroxidase complex (DAKO, Glostrup, Denmark). The slides were deparaffinized in xylene, rehydrated, and treated with 0.3% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase activity. The avidin-biotin streptavidin-biotin peroxidase complex (DAKO, Glostrup, Denmark).

Results

Pretreatment Biopsy Specimens

Pretreatment biopsy specimens were available for all patients: 58 specimens (89%) showed squamous cell carcinoma, 3 (5%) adenosquamous carcinoma, 3 (5%) adenocarcinoma, and 1 undifferentiated large cells (2%). The squamous cell carcinoma group comprised 2 grade 1, 34 grade 2, and 22 grade 3 cancers, classified by the modified Broder method. Two of the adenocarcinomas were mucinous; 1 was moderately differentiated (grade 2), and 1 was poorly differentiated. The remaining adenocarcinoma was endometrioid and poorly differentiated (grade 3). Two adenosquamous carcinomas were moderately differentiated (grade 2). The remaining carcinoma was poorly differentiated (grade 3).

Posttherapy Pap Smears

All except 1 of the Pap smears were adequate. In 1 smear, epithelial cells were too scant to be satisfactory. This smear was successfully repeated 3 days later. The majority of smears contained relatively few cells, but enough for adequacy. Of the 65 smears, 13 (20%) showed clear signs of atrophy. Of the 65 smears, 13 (20%) showed clear signs of atrophy. In 30 cases (46%), we observed enlargement of the cell nucleus and the cytoplasm, multinucleation, and cytoplasmic vacuolization.

Another large group of 20 smears (31%) displayed few therapy-dependent features. These smears were reported as normal. In just 8 cases we encountered naked nuclei and...
**Image 1**

A. Atrophic cells with inflammatory background.  
B. Nuclear and cytoplasmic vacuolization in nonneoplastic squamous cells.  
C. Multinucleated giant cell.  
D. Tridimensional cluster of neoplastic squamous cells.  
E. Cluster of neoplastic glandular cells with surrounding atrophic squamous cells and inflammatory background.  
F. Atypical squamous cells of indeterminate significance (A-F, Papanicolaou, ×200).
karyorrhexis. In 6 cases, we detected severe inflammation and/or a bloody background. In 2, we found multinucleated giant cells Image 1C. Despite these alterations, no neoplastic cells were detected in either group. All of these smears were considered negative for cancer.

Smears positive for cancer were rare (6 cases [9%]). In 5 cases of squamous carcinoma Image 1D and 1 of adeno-carcinoma Image 1E, cellular features were similar to those characterizing nonirradiated cancer cells. These cells coexisted with small numbers of epithelial cells showing radiation-induced alterations.

In 9 cases (14%), we detected cytologic changes of uncertain significance Image 1F. We classified these specimens as ASCUS. Seven cases were originally classified as ASC-US, and 2 were classified as ASC-H. However, in both populations, the mean area of the cell nucleus, while smaller than in frankly malignant cells, was larger than in benign cells. We also observed variable levels of nuclear hyperchromasia, irregular distribution of chromatin, and unusually shaped nuclei. Nuclear/cytoplasmic ratios were higher than in untreated high-grade SIL. Considering the fact that these smears came from patients with a previous histologic diagnosis of cancer, it was unclear whether these features were reactions to therapy or the result of residual neoplasia. We thus merged these specimens into a single category (ASCUS).

**Posttherapy Hysterectomy Specimens**

In 20 (31%) of 65 cases, hysterectomy specimens showed a complete pathologic response to therapy (no neoplastic cells detected). In the area previously occupied by the tumor, we detected fibrous tissue, foamy macrophages, foreign body–like giant cells, cholesterol clefts, and cellular debris Image 2A. These cases were restaged as ypT0.

In 6 cases (9%), we detected moderate (cervical intraepithelial neoplasia [CIN] 2, 3 cases) or severe (CIN 3, 3 cases) epithelial dysplasia at the squamous-columnar junction. The significance of these cells is doubtful. They could represent a surviving subpopulation from the original tumor. However, it is also possible that the squamous epithelium was previously noncancerous and that the observed dysplasia was a de novo phenomenon produced by therapy. These cells showed no sign of invasiveness. Cases in this category were restaged as ypT1. Given the absence of invasive carcinoma, these were considered as cases of complete pathologic response (pR0).

In 4 cases (6%), we detected a single isolated microscopic cluster of neoplastic cells in the cervical stroma Image 2B. In 23 cases (35%), multiple clusters were detected. These were considered cases of partial pathologic response (pR1).

In 12 cases (18%), we detected cervical masses larger than 0.3 cm (macroscopic residual, mean ± SD width, 1.89 ± 0.71 cm; maximum, 3 cm; minimum, 0.7 cm) Image 2C. These were considered cases of no pathologic response (pR2).

Histologically, the morphologic features of cervical cancer treated with radiotherapy and chemotherapy resembled those of cancers in other organs, displaying alterations to the cell nucleus and to cytoplasm and modifications of the stroma such as swelling, fibrosis, and hyalinization. In some cases, residual neoplastic cells formed symptomatic giant cells, which we sometimes found together with reactive foreign body giant cells, surrounding the same area of keratin debris. Immunophenotyping with CAM 5.2 and AE1/AE3 for neoplastic giant cells and CD68 for reactive giant cells can help to distinguish these cells.

The partial pathologic response (pR1) group occupies an intermediate position between complete pathologic response (pR0) and no pathologic response (pR2). This heterogeneous group includes all patients in whom we detected single or multiple clusters of neoplastic cells with a maximum radius up to 0.3 cm.

In 2 cases, we observed features similar to those of in situ dysplasia but accompanied by focal microinvasion of the cervical stroma. These cases were restaged as ypT1a1. In most cases, the residual tumor was confined to the cervical wall (ypT1a2 and ypT1b1). In a few cases, we found residual clusters of neoplastic cells beyond the cervix (ypT2a and T2b).

In a few cases, preoperative radiochemotherapy achieved only very partial success, and examination of the surgical specimen showed the presence of a deeply infiltrating macroscopic residual tumor. None of the tumors detected were larger than 3 cm. However, their microscopic features were the same as those observed in pretreatment biopsy specimens, with variable amounts of fibrosis and inflammation. In the majority of patients in the pR2 group, the tumor was confined to the cervical wall (ypT1b1). In a few cases, it had invaded the parametria (ypT2b). In all cases, the extension of the residual tumor was restaged using the classification in the *AJCC Cancer Staging Manual*, (ypT) Table 1I.

**Cytohistologic Comparison**

**Table 1I** compares the results of the Pap smears with those from the hysterectomy specimens. In 21 cases in which the Pap tests had been classified as negative for cancer, hysterectomy specimens showed a complete pathologic response (pR0). These cases represent the group of true-negatives.

In 5 cases classified as positive for cancer, we detected a neoplastic residual in hysterectomy specimens. Of these 5 cases, 2 were classified as partial pathologic response (pR1) and 3 as no pathologic response (pR2). These cases represent the group of true-positives.

A single case classified as positive for cancer yielded a hysterectomy specimen showing a complete pathologic response (pR0). This was the only case classified as false-positive.
In 9 cases with Pap tests classified as negative for cancer, hysterectomy specimens showed no pathologic response (pR2). In 20 cases with negative Pap tests, hysterectomy specimens showed a partial pathologic response (pR1). These should be considered as false-negatives.

The 9 cases of ASCUS were more difficult to interpret. The 14% ASCUS rate found in our study was very high in comparison with the rate of ASCUS in screening Pap tests. To understand the significance of this group, we compared pathologic response in patients with ASCUS smear results.

<table>
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<tr>
<th>ypT</th>
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<th>pR1</th>
<th>pR2</th>
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<td>1 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>ypT2b</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>3 (6)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (40)</td>
<td>27 (42)</td>
<td>12 (18)</td>
<td>65 (100)</td>
</tr>
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</table>

* Data are given as number (percentage).
with the response in patients with smears classified as negative for cancer Table 3.

These data show that, compared with cases classified as negative for cancer, cases with ASCUS smears had a relative risk of residual cancer of 1.05 and an odds ratio of 1.1. The difference between the 2 groups was not statistically significant.

To measure the accuracy of the Pap test in detecting residual cancer, we compared the results from the Pap tests with those from histologic examination of hysterectomy specimens. In view of the results from the analysis of relative risk, ASCUS smears were reclassified as negative for cancer. Histologic specimens classified as pR1 and pR2 were regrouped into a single category (persistence of disease) Table 4.

The analysis of these data shows that Pap tests have a sensitivity of 0.16 (16.66%), a specificity of 0.96 (96.15%), a positive predictive value of 0.83 (83.33%), and a negative predictive value of 0.42 (42.37%). The accuracy of the test was 0.46 (46.15%).

Discussion

The Pap test has a critical role in cancer prevention programs and makes an important contribution to follow-up programs for patients who have received radiochemotherapy for advanced cancer. It is also paramount in the follow-up of women treated for preinvasive disease. Ever since the present therapy was introduced, it has been recognized that it can produce a wide range of morphologic alterations in neoplastic and nonneoplastic cells in the epithelium and the stroma.

Table 3

|Persistence of Disease and Noncancerous Smears in 59 Cases of Cervical Cancer |
|---------------------------------|-------------------------------|---|
|pR0 (No Persistence of Disease)  | pR1 + pR2 (Persistence of Disease) |
|Negative for cancer              | 29                             | 21 |
|ASCUS                            | 5                              | 4  |
|Total                            | 34                             | 25 |

ASCUS, atypical squamous cells of undetermined significance.

Table 4

|Persistence of Disease and Papanicolaou Test Result in 65 Cases of Cervical Cancer |
|---------------------------------|-------------------------------|---|
|Class                            | pR0 (No Persistence of Disease) | pR1 + pR2 (Persistence of Disease) |
|Negative for cancer              | 25                             | 34 |
|Positive for cancer              | 1                              | 6  |
|Total                            | 26                             | 39 |

During diagnosis, it is difficult to distinguish these alterations from residual neoplastic disease.

Many previous studies have investigated the significance of therapy-induced cellular alterations and their impact on prognosis. However, these studies are relatively old, many of them preceding the introduction of the Bethesda System (even in older versions than those used today). These studies do not use uniform terminology for the classification of lesions or uniform criteria for the selection of patients, which are key requirements for comparison with Pap test results. In some studies, smear results are compared with clinically diagnosed cases of recurrent cancer. Other histology-based studies consider only samples from patients with a positive Pap test result.15,18

In the study reported herein, we compared a complete set of Pap test results with results from histologic examination of the whole cervix, embedding the whole cervix in paraffin and taking multiple sections. It is reasonable to assume that this method guarantees sensitivity and specificity close to 100%. The method applied made it possible to evaluate the real effectiveness of radiochemotherapy in reducing tumor bulk. In brief, it can be considered a “gold standard.”29 The short interval (a few weeks) between the Pap test and examination of the hysterectomy specimen eliminated many time-related biases.

As reported earlier, hysterectomy specimens frequently showed marked morphologic alterations due to radiation therapy. These included severe narrowing of the lumen of the vaginal stump, obliteration of the vaginal sinus, adhesions between the cervix and the walls of the vagina, stenosis and even obliteration of the cervical canal, and ulceration of the cervix and vagina. These alterations meant that it was often technically difficult to obtain a representative smear. They also explain why many smears from patients treated with radiochemotherapy are hypocellular. Even on histologic examination, neoplastic residuals were scant and were often segregated inside the cervical stroma. In many cases, they were covered by reactive or reparative epithelium and stroma. This means that the residual tumor rarely reached the cervical surface where it could be sampled by spatula or brush and explains why we found only 5 smears that were true-positives for cancer. The cancer cells that were present on smears displayed morphologic features very similar to those of non-treated cells and were easily recognized.

The comparison between Pap test results and those from histologic examination of the whole cervix showed that the Pap test had a very low sensitivity but a very high specificity and positive predictive value. Almost half of all cases were correctly diagnosed as positive or negative for cancer.

Radiochemotherapy induces marked morphologic changes in nonneoplastic squamous and glandular epithelium. This suggests that the false-positives detected in our study were due to misinterpretation of these features as malignant. Given
that cancer risk in patients with ASCUS smears is similar to the risk in patients whose smears are negative for cancer, we recommend that morphologic features of uncertain significance be considered benign.

The Pap tests in our study failed to detect in situ dysplasia and microinvasive residual cancer, probably because therapy-induced narrowing of the cervical canal made these cells inaccessible to the brush or spatula. The clinical and biologic significance of in situ dysplasia is still in doubt.

Considering the short time between radiochemotherapy and surgery, we favor the hypothesis of a noninvasive surviving subpopulation from the original tumor rather than a de novo phenomenon.

In general terms, radiochemotherapy seemed to be effective, producing a 40% rate of complete pathologic response (pR0). In the remaining cases, the therapy successfully reduced tumor mass to a level where it could be treated by radical surgery. When response to treatment was incomplete, the residual tumor often comprised sparse clusters of neoplastic cells, each of which included no more than a few dozen neoplastic cells. In the pR1 and pR2 groups, the residual tumor (cell clusters or larger masses) was mainly confined to the cervical wall, although in a few unfortunate cases, neoplastic cells had invaded the parametria.

Pap testing should continue to have an important role in the follow-up of patients treated with radiochemotherapy. This is especially important in centers that do not remove the uterus at the conclusion of the therapy. Even when Pap tests are negative, patients should undergo periodic instrumental monitoring. Clinicians should be aware that positive results imply a significant risk of persistent disease and should use more sensitive examinations to test for this possibility.

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