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Frozen-section evaluation of cervical adenocarcinoma at time of radical trachelectomy: Pathologic pitfalls and the application of an objective scoring system

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Abstract

Objective—To analyze the incidence of diagnostic discrepancy between frozen-section and final diagnosis of the endocervical margin at time of radical trachelectomy and to apply an objective scoring system to non-invasive endocervical glandular atypia to determine its utility in distinguishing benign from malignant lesions.

Methods—Histologic slides from 19 cases of radical trachelectomy performed for invasive endocervical adenocarcinoma were evaluated for correlation between the frozen and permanent sections of the endocervical margin. An objective scoring system for grading non-invasive endocervical glandular lesions proposed by Ioffe et al. was also applied to the frozen and permanent section slides and compared to the final diagnosis.

Results—There was 84% concordance between the frozen-section and final diagnosis using histology alone, vs. 95% concordance using the Ioffe scoring system. One trachelectomy was converted to completion hysterectomy for what was presumed to be adenocarcinoma in situ at the margin, which in retrospect, was a benign lesion and was correctly classified using the Ioffe system. Most of the discrepancies were due to misinterpretation of tubal metaplasia, tubo-endometrioid metaplasia, and atypical tubal metaplasia as adenocarcinoma in situ.

Conclusion—Benign mimics of endocervical adenocarcinoma in situ can be difficult to distinguish from malignant lesions, especially during frozen-section evaluation of the trachelectomy. Correctly diagnosing the margin status intraoperatively has great clinical impact and the application of an objective scoring system, like that proposed by Ioffe et al., can increase diagnostic accuracy when applied to frozen-section slides and better correlates with final diagnosis when compared to histology alone.

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Conflict of interest statement

YS has served on the Speaker's Bureau for Genzyme and as a consultant for Covidien. All other authors have no conflicts of interest to declare.

Keywords

Trachelectomy; Frozen section; Cervix; Adenocarcinoma

Introduction

Adenocarcinoma of the uterine cervix accounts for approximately 20–25% of all cervical carcinomas and has become more prevalent in recent years. While the incidence of squamous cell cancers has declined since the introduction of widespread screening in developed countries, the absolute frequency of adenocarcinoma and the proportion of adenocarcinomas relative to squamous carcinomas has increased [1]. Since cervical cancer is a disease that often affects young women who are still in their childbearing years, fertility-sparing procedures such as the radical trachelectomy have become an increasingly popular option for these patients, and more gynecologic oncologists are performing the procedure when clinically appropriate. The resected cervix is usually sent for frozen-section evaluation of the proximal margin with recommended tumor (invasive and in situ) clearance of 5–10 mm [2]. If this clearance is not achieved, either additional cervical tissue is excised to obtain that clearance or a completion hysterectomy is performed if adequate margin clearance is not possible. Therefore, it is critical that an accurate diagnosis of the margin be reported at the time of intraoperative consultation. We retrospectively collected 19 cases of endocervical adenocarcinoma for which radical trachelectomy was performed with frozen-section analysis of the proximal margin. The aim of the study was to report on the incidence of diagnostic discrepancy between frozen-section and final pathology and to apply an objective scoring system for grading non-invasive glandular cervical lesions proposed by Ioffe et al. [3] to determine whether this could be a useful tool in differentiating benign from malignant lesions on frozen-section slides.

Materials and methods

A retrospective review of all cervical carcinoma patients who underwent fertility-sparing radical trachelectomy and pelvic lymphadenectomy was performed at Memorial Sloan-Kettering Cancer Center from 11/01 to 5/06. All patients had frozen sections of the endocervical resection margins performed at the time of surgery. Only cases of adenocarcinoma were selected for this study, and all trachelectomy slides, including the original frozen section and corresponding permanent sections were retrieved from the pathology files. The number of slides ranged from 10 to 71, with a mean of 35 slides. All original frozen-section slides and their permanent section counterparts were reviewed by two pathologists (KJP, RAS) and independently analyzed using the scoring system for non-invasive glandular lesions proposed by Ioffe et al. [3]. All available slides from the cases were subsequently reviewed by one pathologist to confirm the diagnoses (KJP). The Ioffe scoring system consists of three separate components—nuclear stratification, nuclear atypia, and mitotic/apoptotic score per gland. The first component is scored based on the level of nuclear stratification and is scored as 0 (no stratification), 1 (mild, up to one third of the epithelial thickness), 2 (moderate, up to two thirds of the epithelial thickness), or 3 (severe, up to the luminal surface of the gland). The second component, nuclear atypia, is scored as 0

(nuclei resembling those of normal endocervical glands), 1 (minimal hyperchromasia and dyspolarity, slight nuclear enlargement and absent nucleoli), 2 (nuclei up to 3 times normal size, anisocytosis or variation in nuclear size, moderate hyperchromasia and dyspolarity, occasional small nucleoli), or 3 (nuclei more than 3 times normal, marked anisocytosis, marked hyperchromasia, loss of polarity and frequent prominent nucleoli). Due to the number of features that need to be evaluated for the atypia score, they state that the lesion should have two features in a category to be included in that category and the worst score must be chosen if nuclear features are shared between two categories. The third component is mitotic/apoptotic score per gland, which is expressed as the average number of mitoses and apoptosis counted in the two most active glands of the lesion. If the sum is equal to 0, the score is 0; 0.5 has a score of 1; 0.6–3 has a score of 2; and >3 has a score of 3. They cite the following as an example: if one of the two glands chosen had no mitoses and three apoptosis and the other has one mitosis and one apoptosis, the score is calculated by the following: $(3+2)/2=2.5$ =score of 2. All scores are recorded in the worst areas or those with the most pronounced diagnostic features. All three scores from each component are then added for a total score equivalent to a diagnostic category (0–3, benign; 4–5, so-called “endocervical glandular dysplasia” [EGD]; and 6–9, adenocarcinoma in situ [AIS]). We scored the most atypical-appearing areas and adhered to Ioffe’s proposed system whereby a score of 6 was considered AIS, and anything short of that benign. This is because the clinical significance of a diagnosis of EGD is unknown and the authors propose combining all lesions with a score <6 as benign/reactive in the clinical practice setting.

Each case had two Ioffe scores, one for the frozen section (IS_{FS}) and one for the permanent section of the tissue examined at frozen section, or the frozen-section control (IS_{FSC}). Each pathologist scored the cases independently without knowledge of the final diagnosis, and any discrepancies were reviewed together for a consensus score. The final scores were then compared to the original frozen-section and final diagnoses.

All gross descriptions of the specimens were retrieved from pathology reports. A retrospective review of all follow-up Pap tests, curettages and biopsies, along with high risk-HPV status, was also performed.

Indications for radical trachelectomy

The general eligibility criteria for radical trachelectomy include the following: confirmed diagnosis of squamous, adenosquamous or adenocarcinoma, women less than 45 years of age who have a strong desire to preserve fertility, lesion size less than or equal to 2 cm, International Federation of Gynecology and Obstetrics (FIGO) stages IA2 or IB1, no evidence of metastasis, and adequate resolution of post-conization acute inflammation [4].

Method for obtaining trachelectomy margin

There are different approaches for submitting sections of the trachelectomy margin for histologic evaluation, but there is no defined consensus on the best sampling method. Some prefer a transverse (en face) section, while others prefer a longitudinal (perpendicular) section. Others only take a perpendicular margin if there is a grossly visible tumor [2]. At our institution, we apply both transverse and perpendicular methods (Fig. 1). First, an en

face shave of the proximal cervical margin is submitted, approximately 2 mm in thickness (Fig. 1A). The most important aspect of this method is examining the entire endocervical mucosa, including endocervical clefts deep within the stroma. This allows us to examine the entire mucosal surface of the proximal margin. If this margin is positive for carcinoma, no other frozen section is performed on the specimen. If this margin is benign, the cervix is opened, usually at the 3 or 9 o'clock position, and the endocervical canal is examined grossly. If a grossly visible tumor is present, its distance to the proximal end plus 2 mm (for the shave that was already taken) is reported as the margin clearance (Fig. 1B). If a grossly visible lesion is present but the nature of the lesion is uncertain (benign vs. malignant), we take a perpendicular section to include the lesion and the margin for frozen-section examination (Fig. 1C). If tumor is present microscopically in this perpendicular section, we measure the distance to the proximal edge and add 2 mm for a final margin distance. If no visible lesion is present within the canal, a second transverse cross section is taken 8 mm distal to the margin (for a total of 10 mm from the original margin) and examined (Fig. 1D). If both margins are clear, we report them as such. If tumor (invasive or in situ) is present in the second transverse section, we report the distance of the tumor from the proximal resection margin as at least 10 mm (2 mm from initial shave+8 mm from second transverse section). Of note, because radical trachelectomy is a relatively new procedure, the method for margin sampling was not uniform in the beginning and there was a learning curve for both surgeons and pathologists. For example, although not performed in the earlier cases, endocervical curettages and endometrial curettages are now routinely performed at the time of trachelectomy.

Evaluation of frozen sections

Pathologists at our institution are subspecialized by organ systems so that gynecologic pathologists review all gynecologic specimens. However, this subspecialization system was not instituted until July 1, 2004, so that all pathologists reviewed all surgical cases prior to this date. Also, the frozen sections are not evaluated by subspecialists unless specifically requested, and therefore, most of the frozen section diagnoses evaluated in this study were rendered by non-gynecologic pathologists. Additional tissue submitted for frozen section, such as endocervical curettage, endometrial curettage, or additional cervical stump biopsies were also examined, and the scoring of the atypia was performed on the sections with the worst diagnoses (short of invasive adenocarcinoma) that somehow affected the surgeon's decision making process.

Final diagnosis

The gold standard for diagnosing adenocarcinoma in situ was histology, the characteristic cytologic features including nuclear enlargement, hyperchromasia, apoptotic bodies, and mitotic figures. Short of these features, immunohistochemistry was used to supplement the final diagnosis in cases difficult to classify.

Immunohistochemistry

The stains that were used include p16, Mib-1, ER and PR, which were performed in 6 cases at the discretion of the pathologist. Immunohistochemistry was performed using standard protocols (Table 1).

Results

Forty-six consecutive radical trachelectomies for cervical carcinoma were identified, 19 of which were for adenocarcinoma (41%). Four of these showed grossly visible tumors, all of which showed invasive carcinoma. Seven showed no grossly visible lesions, 2 of which had either AIS or minimally invasive adenocarcinoma. The trachelectomy margins were negative for both patients. Five showed non-specific changes, including hemorrhage, fibrosis, and irregular borders correlating microscopically with biopsy site changes. Three cases had no gross description available in the pathology report. The final diagnoses for the 19 patients are included in Table 2 and include 6 adenocarcinomas, 1 AIS and 12 with no residual tumor.

The mean patient age was 31.4 years (range, 25–37). We used the FIGO staging system, which included the following: IA1 (1), IA2 (3), and IB1 (15); lymphovascular invasion was present in 3 cases (16%). All original cervical cancer diagnoses were made on biopsy (1), loop electrical excision procedure (LEEP) (6), or cone biopsy (12). Frozen section of the margins was performed for each trachelectomy and 16 patients also had at least one additional frozen section of post-trachelectomy endocervical biopsies and/or endocervical/endometrial curettages, either as standard procedure or because of the frozen section results of the trachelectomy margin.

Scoring using the Ioffe system was performed independently by two gynecologic pathologists. Interobserver variability only differed by 1 or 2 points in any category and none resulted in different classifications of the lesions (benign vs. malignant). Similar to what was found in the original Ioffe study, the various scores among the three categories somehow seemed to “even out” in the final score tally, allowing us to reach the same diagnosis. The worst diagnoses (short of invasive adenocarcinoma) out of all the frozen sections for each patient are listed (Table 2) and consisted of the following: benign in 11 (58%), AIS in 2 (10%), AIS greater than 1 cm from the margin in 1 (5%), atypical tubal metaplasia (ATM) in 2 (11%), atypical not otherwise specified (NOS) in 2 (11%), and atypical favor benign in 1 (5%). Although patient 15 was diagnosed with adenocarcinoma on a post-trachelectomy endometrial curettage at the time of surgery, we felt that this was not a case of non-invasive glandular lesion for which we could apply the Ioffe criteria and, therefore, only scored the trachelectomy.

Additional surgical intervention was performed in 5 (26%) cases where the frozen sections were abnormal (2 AIS at margin, 1 AIS>1 cm clearance, 1 adenocarcinoma at 1 cm, 1 ANOS). One patient diagnosed with AIS at the margin had immediate completion hysterectomy (patient 5), and the other patient diagnosed with AIS had an additional cervical stump excision (patient 7). Two patients with >1 cm clearance had additional curettings (patients 6 and 15), and 1 of them (patient 15) had additional uterine shave excised due to the presence of carcinoma in the curettage. One patient diagnosed with ANOS on frozen-section analysis also had additional cervical tissue excised (patient 18) (Table 2).

Immunohistochemistry was utilized to confirm the final diagnosis in 6 equivocal cases. One patient (patient 16) was diagnosed with atypical tubal metaplasia on frozen-section analysis (Fig. 2A); however, final histology and immunohistochemistry revealed the presence of AIS at the trachelectomy margin (Fig. 2B). The AIS was diffusely and strongly positive for Mib-1, CEA (monoclonal), and p16, while it was negative for vimentin, ER, and PR (Fig. 2C). The patient chose not to undergo further surgical treatment and subsequently became pregnant; her pregnancy resulted in a miscarriage at 18 weeks. She is currently without evidence of disease. Immunohistochemical stains were also performed on patient 5, who had “AIS” at the margin on frozen section and underwent completion hysterectomy. Stains performed on the frozen-section control and hysterectomy were negative for p16 and Mib-1 in all glands, including the atypical areas, which in retrospect was a benign process (Figs. 3A and B). The remaining 4 cases of equivocal glandular atypia all stained in a manner that did not support adenocarcinoma (p16 negative, low Mib-1).

The results of the frozen-section review using Ioffe criteria [3] are shown in Table 2. We reviewed and scored the frozen-section and frozen-section control slides of all 19 patients. Only 1 case (patient 14) had a frozen-section control slide no longer showing the area of interest for scoring. In 17 of the 18 evaluable cases, the IS_{FS} and IS_{FSC} resulted in the same diagnostic category (benign or malignant) equaling an IS_{FS} to IS_{FSC} correlation of 94%. In the one discrepant case, the patient’s IS_{FS} was 4 (benign) but the IS_{FSC} was 6 (malignant). The final diagnosis in this case was malignant (AIS). Sixteen of 18 cases had total IS_{FS} and IS_{FSC} scores of 4 or less, equivalent to a benign diagnosis (patients 1–6,8–13,15,17–19). One patient had IS_{FS} and IS_{FSC} scores of 7 and 8, respectively, equivalent to AIS; however, this was diagnosed as atypical tubal metaplasia on the frozen section. Final pathology showed AIS. Also of note, one case was diagnosed as AIS away from the proximal margin on frozen section (patient 6), which had no clinical implications at the time of surgery. On final review, however, the area of “AIS” was actually reactive atypia. No carcinoma was found in the entire trachelectomy.

The correlation between the IS_{FS} and the original frozen-section diagnosis was 15 (79%) of 19; between the IS_{FS} and final diagnosis, 18 (95%) of 19; between the IS_{FSC} and the original frozen-section diagnosis, 15 (83%) of 18; and between the IS_{FSC} and final diagnosis, 18 (100%) of 18. Without using the Ioffe scoring, the frozen-section and final diagnosis correlation was 16 (84%) of 19. Using the Ioffe scoring system would have allowed us to correctly diagnose AIS in patient 16, benign glands in patient 6, as well as the benign glands, which were called AIS, in patient 5, sparing her an unnecessary completion hysterectomy. However, using the scoring system would have also resulted in an undercall of AIS as benign (patient 7). This patient, even with a positive margin, had only additional cervical tissue excised and did not undergo a hysterectomy.

Ten patients were also followed with subsequent biopsies or curettages at their follow-up visits. All patients have been followed with at least one Pap test, and many have also been tested for reflex HPV. Follow-up ranged from 25 to 78 months, with a mean of 43 months. There was 1 case of recurrence that was detected within 19 months of surgery (patient 15) where the endometrial curettage was positive for tumor on the frozen section at the time of surgery. She did not receive any adjuvant therapy and recurred in the lower uterine segment,

upper vagina and pelvic lymph nodes and died of disease 7 months after her recurrence. Three patients have successfully given birth, all in the third trimester after 34 weeks, 2 had miscarriages at 5 and 18 weeks, 1 had an elective termination, and 1 is currently pregnant.

Discussion

The overall incidence of cervical adenocarcinoma has been steadily increasing over the past several years and currently accounts for approximately 20–25% of all cervical carcinomas [1]. These tumors commonly affect women in their childbearing years, and radical trachelectomy is now a viable option for women who want to preserve fertility. The procedure is considered for patients with stage IA2–IB1 adenocarcinoma and sometimes for IA1 patients with lymphoinvasive adenocarcinoma. The resected cervix is usually sent for frozen-section evaluation of the proximal margin, and the result of the frozen-section evaluation affects the surgeon's decision intraoperatively. Therefore, it is essential that an accurate diagnosis be rendered at that time.

Part of the reason this study was undertaken was that cases of glandular atypia were being interpreted as AIS at frozen section, resulting in an unnecessary hysterectomy in one case and undue stress over the possibility of unnecessary hysterectomies in other cases. Although not reflected in the final pathology reports, there were a few occasions in which a gynecologic pathologist was called for consultation because of uncertainty in interpreting the atypia.

Although an accurate diagnosis can be achieved most of the time, frozen-section evaluation is always accompanied by a certain amount of artifactual histologic distortion, regardless of the type of tissue that is being examined. The very nature of flash freezing fresh tissue, in addition to the preparation process, alters nuclear and cytoplasmic characteristics that may confound the diagnosis. In the cervix, this fact may be of particular concern due to some of the mimics of cervical AIS that can occur in the uterus. Invasive cervical adenocarcinoma is not diagnostically difficult with its irregular, architecturally complex, infiltrative glands with enlarged hyperchromatic nuclei, nucleoli, increased mitotic figures, and apoptotic bodies. However, in AIS, these features are not always present, and in fact, only a few histologic hallmarks of malignancy may be present, especially in small samples. In addition, a few well described benign changes that occur in the endocervix and lower uterine segment share some morphologic features with AIS and can be mistaken for malignancy. The confounding factor of artifactual distortion due to freezing the tissue can present a diagnostic challenge for the pathologist.

Tubal metaplasia, tubo-endometrioid metaplasia, atypical tubal metaplasia and endometriosis are benign changes that can be seen in the cervix [5–13]. These changes occur throughout the müllerian tract and can involve the endometrium, ovaries, fallopian tubes, serosal surfaces, and even lymph nodes. Within the uterus itself, these changes can often be seen in the upper endocervix/lower uterine segment, which is the precise area sampled for frozen-section evaluation of the trachelectomy proximal margin. These benign changes can appear cytologically atypical mimicking AIS (Figs. 4 and 5). Conversely, AIS can have apical blebs that may appear to be cilia, thus mimicking a benign process (Fig. 6).

Immunohistochemical staining can help distinguish between benign and malignant processes. Although not available at the time of frozen-section analysis, immunohistochemical stains are useful for final diagnosis, since even on permanent sections, the distinction can be quite difficult. Endocervical adenocarcinoma is usually positive for CEA (monoclonal) and p16INK4a with a high Mib-1 index, while mostly negative for vimentin, ER, and PR [14–17]. Although there is some staining overlap, in general the opposite is true for neoplastic and non-neoplastic endometrial and non-neoplastic endocervical glands [15,18,19]. Since the main differential diagnosis is often between AIS and tubal metaplasia, the stains are usually helpful. One must be careful, however, since tubal metaplasia and other benign endometrial and endocervical processes can be positive for p16 [20–22], although usually less diffusely than in AIS. Tubal metaplasia also should almost always be negative for CEA (monoclonal) and have a low mitotic index with Mib-1, unlike AIS. It is best to use a panel of immunohistochemical stains in conjunction with atypical histomorphology since one stain may show overlapping staining patterns between benign lesions and AIS. In our series, at least one of the above immunohistochemical stains was performed on 6 patients. These stains were useful in those difficult cases in which routine histologic exam alone was insufficient for a definitive diagnosis.

In our experience, the majority of frozen-section diagnoses were not problematic (14/19 [74%]). However, in 5 cases, either a diagnosis of benign was rendered for malignancy (1) or there was enough uncertainty at the time of frozen-section analysis that required the surgeon to excise additional tissue to ensure clear margins (4). There was need for consultation with gynecologic pathologists during frozen-section analysis because other pathologists interpreted atypia and unusual architectural patterns as AIS. This diagnosis would have compelled the surgeon to perform a completion hysterectomy. Instead, with a diagnosis of atypia not reaching the level of AIS, the surgeon was comfortable excising additional margins but not the entire uterus. The final diagnoses on these few cases were all benign. This brings to attention the fact that in our study, the original frozen sections were performed by general surgical pathologists while the scoring was performed by gynecologic pathologists who have a considerable more amount of experience in evaluating these lesions. This underscores the need for a more objective way to evaluate these atypical glands, and the Ioffe scoring system is one that could be utilized by all pathologists regardless of their subspecialty training.

We retrospectively scored the frozen-section and frozen-section control slides using the Ioffe scoring system [3], comparing the two scores with each other, as well as with the original frozen-section and final diagnoses for diagnostic correlation. In applying the Ioffe system, we encountered a few issues that may pose problems in its application. One of these was the presence of cilia. Tubal metaplasia and atypical tubal metaplasia are often extremely stratified with nucleomegaly, cellular crowding, and occasionally mitoses [9] and are often associated with cilia on the luminal surface. The Ioffe system does not specifically address how to score the stratification in glands and clefts lined by obviously ciliated cells. Counting apoptotic bodies in the presence of intraepithelial lymphocytes was also a challenge. Especially on the frozen-section slide, individual lymphocytes can mimic the karyorrhectic debris of apoptosis. We found that counting only figures that have multiple, small, dark, debris-like granules and disregarding single, small, dark nuclei was helpful in distinguishing

the two, although it was sometimes very difficult. The Ioffe system also relies on counting mitoses/apoptoses per gland; however, it does not address how to apply this to surface epithelium where there is a single continuous stretch of atypical cells without the formation of individual glands. Since most cervical glands, including those of AIS, are at least within a 400× field, this may be a substitute method for counting mitoses/apoptoses involving surface epithelium.

Results show that using the scoring system had good correlation with final diagnosis (95%) compared to routine histologic examination alone (84%). This shows that using an objective system for scoring glandular atypia, such as that proposed by Ioffe et al. [3], is helpful in classifying difficult cases, especially at the time of frozen-section analysis.

Radical trachelectomy is a viable and increasingly utilized option for women with early invasive cervical carcinoma of childbearing age. The correct frozen-section diagnosis of the trachelectomy margin is crucial to appropriate patient management, and the pathologist and surgeon should be aware of the diagnostic dilemmas that can be encountered to avoid incorrect diagnoses. The Ioffe scoring system [3] for non-invasive glandular lesions of the cervix has high concordance with the final diagnosis and can be utilized at the time of frozen-section analysis to assist in difficult cases.

References

1. Smith HO, Tiffany MF, Qualls CR, Key CR. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States—a 24-year population-based study. *Gynecol Oncol.* 2000; 78:97–105. [PubMed: 10926787]
2. Tanguay C, Plante M, Renaud MC, Roy M, Tetu B. Vaginal radical trachelectomy in the treatment of cervical cancer: the role of frozen section. *Int J Gynecol Pathol.* 2004; 23:170–5. [PubMed: 15084846]
3. Ioffe OB, Sagae S, Moritani S, Dahmouch L, Chen TT, Silverberg SG. Proposal of a new scoring scheme for the diagnosis of noninvasive endocervical glandular lesions. *Am J Surg Pathol.* 2003; 27:452–60. [PubMed: 12657929]
4. Roy M, Plante M. Pregnancies after radical vaginal trachelectomy for early-stage cervical cancer. *Am J Obstet Gynecol.* 1998 Dec; 179(6 Pt 1):1491–6. [PubMed: 9855586]
5. Babkowski RC, Wilbur DC, Rutkowski MA, Facik MS, Bonfiglio TA. The effects of endocervical canal topography, tubal metaplasia, and high canal sampling on the cytologic presentation of nonneoplastic endocervical cells. *Am J Clin Pathol.* 1996; 105:403–10. [PubMed: 8604682]
6. Baker PM, Clement PB, Bell DA, Young RH. Superficial endometriosis of the uterine cervix: a report of 20 cases of a process that may be confused with endocervical glandular dysplasia or adenocarcinoma in situ. *Int J Gynecol Pathol.* 1999; 18:198–205. [PubMed: 12090586]
7. Jonasson JG, Wang HH, Antonioli DA, Ducatman BS. Tubal metaplasia of the uterine cervix: a prevalence study in patients with gynecologic pathologic findings. *Int J Gynecol Pathol.* 1992; 11:89–95. [PubMed: 1582750]
8. Oliva E, Clement PB, Young RH. Tubal and tubo-endometrioid metaplasia of the uterine cervix. Unemphasized features that may cause problems in differential diagnosis: a report of 25 cases. *Am J Clin Pathol.* 1995; 103:618–23. [PubMed: 7741110]
9. Schlesinger C, Silverberg SG. Endocervical adenocarcinoma in situ of tubal type and its relation to atypical tubal metaplasia. *Int J Gynecol Pathol.* 1999; 18:1–4. [PubMed: 9891235]
10. Suh KS, Silverberg SG. Tubal metaplasia of the uterine cervix. *Int J Gynecol Pathol.* 1990; 9:122–8. [PubMed: 2332270]
11. Yeh IT, Bronner M, LiVolsi VA. Endometrial metaplasia of the uterine endocervix. *Arch Pathol Lab Med.* 1993; 117:734–5. [PubMed: 8323440]

12. Young RH, Clement PB. Pseudoneoplastic glandular lesions of the uterine cervix. *Semin Diagn Pathol.* 1991; 8:234–49. [PubMed: 1759058]
13. Zaino RJ. Glandular lesions of the uterine cervix. *Mod Pathol.* 2000; 13:261–74. [PubMed: 10757337]
14. Liang J, Mittal KR, Wei JJ, Yee H, Chiriboga L, Shukla P. Utility of p16INK4a, CEA, Ki67, P53 and ER/PR in the differential diagnosis of benign, premalignant, and malignant glandular lesions of the uterine cervix and their relationship with Silverberg scoring system for endocervical glandular lesions. *Int J Gynecol Pathol.* 2007; 26:71–5. [PubMed: 17197900]
15. McCluggage WG, Maxwell P, McBride HA, Hamilton PW, Bharucha H. Monoclonal antibodies Ki-67 and MIB1 in the distinction of tuboendometrial metaplasia from endocervical adenocarcinoma and adenocarcinoma in situ in formalin-fixed material. *Int J Gynecol Pathol.* 1995; 14:209–16. [PubMed: 8600071]
16. Negri G, Egarter-Vigl E, Kasal A, Romano F, Haitel A, Mian C. p16INK4a is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immunocytochemical correlations. *Am J Surg Pathol.* 2003; 27:187–93. [PubMed: 12548164]
17. Pavlakis K, Messini I, Athanassiadou S, Kyrodinou E, Pandazopoulou A, Vrekoussis T, et al. Endocervical glandular lesions: a diagnostic approach combining a semi-quantitative scoring method to the expression of CEA, MIB-1 and p16. *Gynecol Oncol.* 2006; 103:971–6. [PubMed: 16876235]
18. Cina SJ, Richardson MS, Austin RM, Kurman RJ. Immunohistochemical staining for Ki-67 antigen, carcinoembryonic antigen, and p53 in the differential diagnosis of glandular lesions of the cervix. *Mod Pathol.* 1997; 10:176–80. [PubMed: 9071723]
19. McCluggage WG, Sumathi VP, McBride HA, Patterson A. A panel of immunohistochemical stains, including carcinoembryonic antigen, vimentin, and estrogen receptor, aids the distinction between primary endometrial and endocervical adenocarcinomas. *Int J Gynecol Pathol.* 2002; 21:11–5. [PubMed: 11781517]
20. Li C, Rock KL, Woda BA, Jiang Z, Fraire AE, Dresser K. IMP3 is a novel biomarker for adenocarcinoma in situ of the uterine cervix: an immunohistochemical study in comparison with p16(INK4a) expression. *Mod Pathol.* 2007; 20:242–7. [PubMed: 17192788]
21. Tringler B, Gup CJ, Singh M, Groshong S, Shroyer AL, Heinz DE, et al. Evaluation of p16INK4a and pRb expression in cervical squamous and glandular neoplasia. *Hum Pathol.* 2004; 35:689–96. [PubMed: 15188135]
22. Murphy N, Heffron CC, King B, Ganuguapati UG, Ring M, McGuinness E, Sheils O, O’Leary JJ. p16INK4A positivity in benign, premalignant and malignant cervical glandular lesions: a potential diagnostic problem. *Virchows Arch.* 2004; 445(6):610–5. [PubMed: 15378361]

Fig. 1.

Schematic representation of how to submit trachelectomy margin at the time of frozen-section analysis. A. Shave of proximal margin, approximately 2 mm in thickness. B. Trachelectomy opened after shave showing grossly visible tumor; distance to margin is measured grossly. C. Trachelectomy opened with grossly visible lesion, indefinite for tumor; perpendicular section including lesion is submitted for microscopic evaluation. D. Trachelectomy opened without gross lesion; transverse section is submitted 8 mm distal to first transverse section.

Fig. 2.
A. Adenocarcinoma in situ interpreted as atypical tubal metaplasia on frozen-section analysis. B. Adenocarcinoma in situ frozen-section control. C. Mib-1.

Fig. 3.
A. Tubal metaplasia diagnosed as adenocarcinoma in situ at frozen section. B. Mib-1 staining of permanent section showing no increase in proliferation.

Fig. 4.
Atypical tubal metaplasia with irregular, cystically dilated glands with altered stroma.

Fig. 5.
Atypical tubal metaplasia with nuclear stratification, nucleomegaly, and mitotic figure.

Fig. 6.
Adenocarcinoma in situ.

Table 1

	Clone	Vendor	Location	Antigen retrieval ^a	Dilution	Pattern
p16	16P04	Neo Markers/Lab Vision	Fremont, CA	Citrate, pH 6.00	1:400	C, N
ER	ERID5	Beckman Coulter	Miami, FL	Citrate, pH 6.00	1:100	N
PR	10A9	Beckman Coulter	Miami, FL	–	1:200	N
mCEA	A5B7	DAKO	Carpinteria, CA	Citrate, pH 6.00	1:500	C, M
Vimentin	V9	DAKO	Carpinteria, CA	Citrate, pH 6.00	1:4000	C

ER, estrogen receptor; PR, progesterone receptor; mCEA, monoclonal carcinoembryonic antigen; C, cytoplasmic; N, nuclear; M, membrane.

^aHeat-induced antigen retrieval using a microwave.

Table 2

Patient	Stratification	Atypia (0–3)	Mitotic/apoptotic (0–3)	Total score (0–9)	FS diagnosis <i>a</i> (specimen)	Final margin diagnosis	Final diagnosis
1	IS _{FS} 3	0	0	3	Benign	Benign	No residual
	IS _{FSC} 2	1	0	3			
2	IS _{FS} 1	2	0	3	ANOS (ECC)	Benign	No residual
	IS _{FSC} 0	1	0	1			
3	IS _{FS} 1	1	0	2	Benign	Benign	No residual
	IS _{FSC} 0	0	0	0			
4	IS _{FS} 2	2	0	4	Benign	Benign	ACA
	IS _{FSC} 1	2	0	3			
5	IS _{FS} 1	2	0	3	AlS (Trach)	Benign	No residual
	IS _{FSC} 1	2	0	3			
6	IS _{FS} 2	0	0	2	AlS <i>b</i> (Trach)	Benign	No residual
	IS _{FSC} 0	0	0	0			
7	IS _{FS} 2	2	0	4	AlS (Trach)	AlS	ACA
	IS _{FSC} 2	2	2	6			
8	IS _{FS} 1	1	0	2	Benign	Benign	No residual
	IS _{FSC} 1	0	0	1			
9	IS _{FS} 1	2	0	3	Benign	Benign	No residual
	IS _{FSC} 2	1	0	3	ATM (Cx)	Benign	No residual
10	IS _{FS} 2	1	0	3			
	IS _{FSC} 1	1	1	3	Benign	Benign	No residual
11	IS _{FS} 1	0	0	1			
	IS _{FSC} 1	0	0	1	AFB (ECC)	Benign	AlS
12	IS _{FS} 1	2	0	3			
	IS _{FSC} 0	0	0	0	Benign	Benign	ACA
13	IS _{FS} 1	0	0	1			
	IS _{FSC} 0	0	0	0	Benign	Benign	No residual
14	IS _{FS} 2	3	0	0	Benign	Benign	No residual

Patient	Stratification (0–3)	Atypia (0–3)	Mitotic/apoptotic (0–3)	Total score (0–9)	FS diagnosis ^a (specimen)	Final margin diagnosis	Final diagnosis
15	IS _{FSC}	–	–	–			
	IS _{FS}	3	1	0	4	Benign	ACA
	IS _{FSC}	2	1	0	3		
16	IS _{FS}	3	3	1	7	ATM (Trach)	ACA
	IS _{FSC}	3	3	2	8		
17	IS _{FS}	1	0	0	1	Benign	No residual
	IS _{FSC}	1	2	0	3		
18	IS _{FS}	1	2	0	3	ANOS (Trach)	No residual
	IS _{FSC}	1	0	0	1		
19	IS _{FS}	1	0	0	1	Benign	ACA
	IS _{FSC}	1	1	0	2		

IS_{FS} — Ioffe Score for frozen section; IS_{FSC} — Ioffe Score for frozen section control; ATM — atypical tubal metaplasia; AIS — adenocarcinoma in situ; ANOS — atypical not otherwise specified; AFB — atypical favor benign; ACA — adenocarcinoma; ECC — endocervical curettage; Cx — cervical biopsy; Trach — trachelectomy.

Key for total Ioffe score: 0–3 benign; 4–5 atypical; 6–9 malignant.

^aDiagnosis rendered at the time of frozen section.

^bAIS>1 cm margin clearance.

^cAdenocarcinoma in endometrial curettage.