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Original Contribution

Value of additional sections: Tissue handling of small biopsies in detecting squamous dysplasia of the uterine cervix

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ABSTRACT

Cervical cancer screening is currently based on high-risk human papillomavirus (HR-HPV) molecular testing, Pap cytology testing, and histologic evaluation of cervical biopsies. As primary HPV screening for cervical cancer becomes widely used, some of the recommended screening guidelines propose colposcopy and biopsies following positivity for HPV16/18 without cytologic triage. In such instances, a biopsy would be the only tissue sample available for informing further management. The use of additional histologic levels on cervical biopsies is commonly employed to achieve a diagnosis, although no set criteria for when to obtain additional levels exist. In this study, we evaluated the value of additional sections in cervical biopsy and endocervical curetting, as well as clinical and histologic features that should be considered when ordering additional levels. Additional levels were obtained for the following scenarios: benign mucosa with Pap discrepancy (HSIL or ASC-H interpretation), size discrepancy with the gross description, suspicious atypia for a high-grade lesion, and long-standing high-risk HPV infection. A change in diagnosis was observed in 21.4% of the cases, with an upgrade to a high-grade squamous intraepithelial lesion (CIN2–3) in 12.1% of cases. An initial impression of atypia significantly correlated with both a change in diagnosis and an upgrade to CIN2–3. In the era of primary HPV screening, when evaluating tissue samples following positive HPV test, small, atypical foci should be followed by additional levels. We recommend six (6) initial levels on all cervical biopsies, particularly if there is no loss of tissue between the levels, to ensure an accurate interpretation. This will be crucial in the timely and accurate identification of HPV-related intraepithelial lesions and proper subsequent management.

1. Introduction

Cervical cancer screening is based on molecular testing for high-risk human papillomavirus (HR-HPV) and Papanicolaou cytology testing (Pap test). Guidelines from the American Society for Colposcopy and Cervical Pathology (ASCCP) may recommend subsequent colposcopy and biopsies based on the screening results.

Despite colposcopic guidance, discrepancies between colposcopic and histologic findings are documented in 10% of cases. While colposcopy provides targeted sampling, lesions may not be readily visible therefore not correctly and easily sampled [1-3].2.3.4.

Treatment of cervical lesions ultimately depends on the results of cervical biopsies. The size of an average cervical biopsy is 0.2–0.5 cm, while endocervical curetting specimens frequently contain only sparse mucoid material. Given the gross (macroscopic) findings, histopathology laboratories carefully handle these delicate samples in order to preserve as much tissue as possible for diagnostic and ancillary studies.

Abbreviations: AGC, atypical glandular cells; ASCCO, American Society for Colposcopy and Cervical Pathology; ASC-H, atypical squamous cells cannot exclude high-grade; ASC-US, atypical squamous cells of uncertain significance; CIN, cervical intraepithelial neoplasia; HR-HPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous cell lesion; NILM, negative for intraepithelial lesion or malignancy; Pap test, Papanicolaou test; SIL, squamous intraepithelial lesion; SOP, standard operative procedures; TAT, turnaround time.

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However, many laboratories have different initial steps and standard operative procedures (SOP) when processing these samples. In order to gain insight into different practices, we performed a survey of eighteen (18) gynecologic pathologists or pathologists that frequently sign out biopsy specimens from the uterine cervix in academic and community practice settings from 17 different laboratories (Supplemental data 1–4). Based on the answers provided by the surveyed pathologists, most laboratories (61%) initially cut three levels, with 33% of the laboratories cutting only one or two levels and 6% laboratories cutting six levels or more (Supplemental data 1). According to the survey, most pathologists have a low threshold for ordering additional tissue sections, with 50% of pathologists order additional levels in 10–25% of the cases (Supplemental data 2). Based on their clinical experience, the surveyed pathologists report that in 10–20% of the cases, there is a significant change in diagnosis following additional levels. However, 65% of the pathologists answered that they do not have well-established criteria for ordering levels (Supplemental data 3). The most common criteria for ordering additional levels are:

1. Suspicious atypia on the initial level. Atypia is best classified as a small focus with nuclear enlargement, hyperchromatic, or irregular nuclear membranes (Fig. 1) but insufficient for the definitive diagnosis of dysplasia. Additionally, small foci of squamous metaplasia with nuclear crowding or enlargement with or without inflammation that is difficult to classify are also characterized as atypia (Fig. 2).
2. A high-risk Pap test results, such as a high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells cannot exclude high-grade (ASC-H), and/or atypical glandular cells (AGC) without histologic correlation on biopsy.
3. Long-standing HPV infection, which is defined as a persistent HPV infection $\geq$ two years [4].
4. Discrepancy between the gross description of the biopsy specimen and size/or the number of tissue fragments on the slide.

2. Materials and methods

2.1. Case selection and processing

Following institutional review board approval (IRB, number #AAAS8262) and based on the survey results, we used the criteria mentioned above to prospectively evaluate routinely received cervical biopsies between 01/2020 and 04/2020. The biopsies were not split or cut at the grossing bench, due to the small size of the specimen, prior to histologic processing. We applied the above criteria for ordering three (3) additional levels on all our small cervical specimens that included both biopsies and endocervical curettage.

At our institution, all cervical biopsies are initially cut on three hematoxylin and eosin (H&E) levels and six serial sections (each level has two serial sections). Between each level, there is a 40 μm loss of tissue while there is no loss of tissue on the consecutive serial sections. All cervical biopsies were received and evaluated by two gynecologic pathologists. Clinical history, initial impression, reasons for levels, and final interpretations were prospectively recorded.

A change in final diagnosis was correlated with the initial

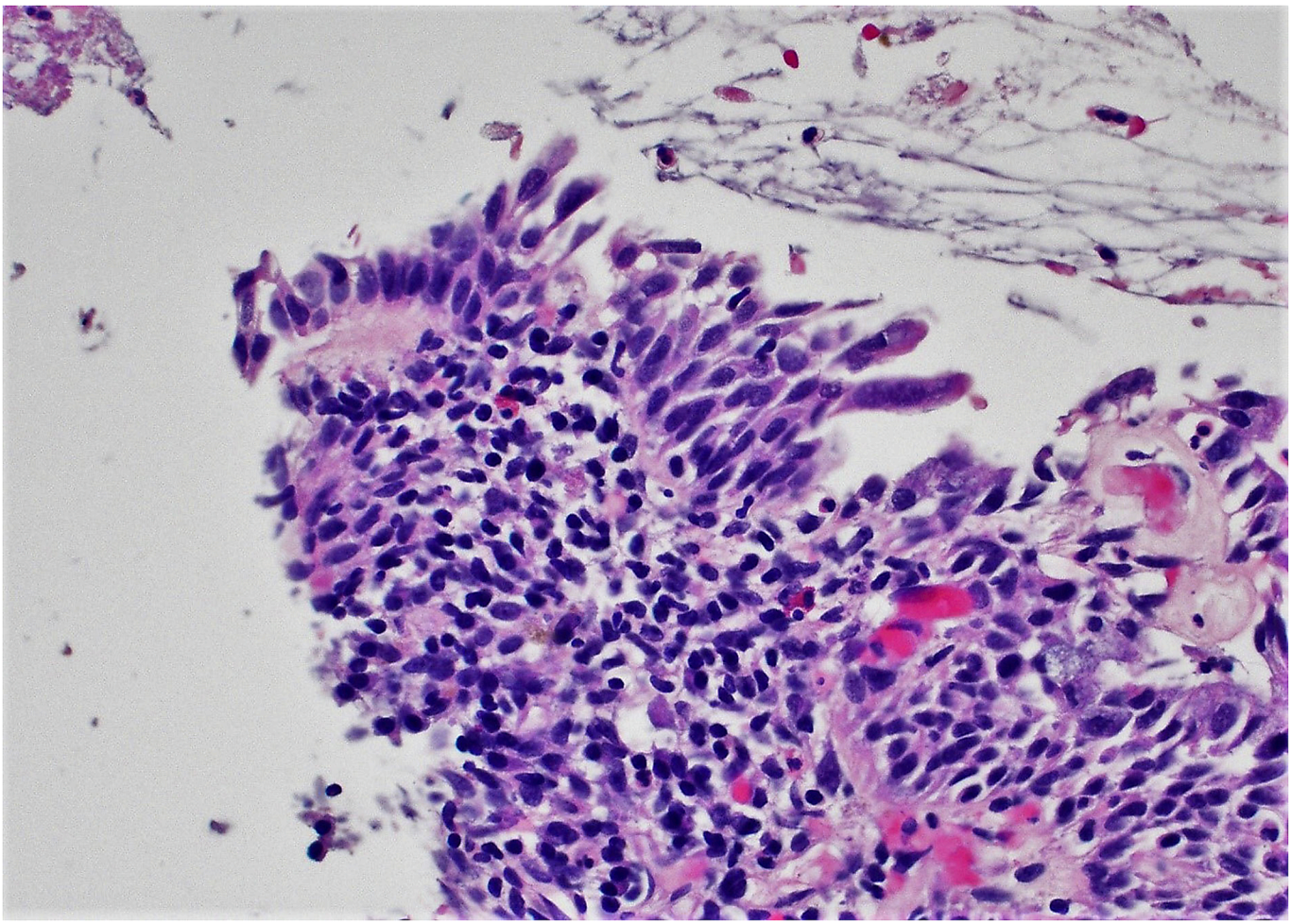

Fig. 1. An excellent example of atypia that requires additional levels. The cells are crowded with hyperchromatic, irregular nuclei. The presence of chronic inflammation sometimes makes it difficult to recognize the lesion on scanning magnification (Olympus BX43, 20×).

impression, reasons for levels, HPV status, and Pap test results. Confirmatory immunohistochemistry for p16 (clone E6H4, Ventana Medical Systems) was performed at the discretion of the pathologist on a case-by-case basis. High-risk HPV (HR-HPV) genotyping was performed using the Cobas 4800 HR-HPV test (Roche Diagnostics, Rotkreuz, Switzerland).

2.2. Statistical methods

The Chi-square test and Fisher's exact test were used to explore the association of levels with change in the final diagnosis. p-Values < 0.05 were considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) IBM Version 27 (SPSS) (UNICOM Systems, Inc.).

3. Results

One-hundred and forty (140) cases met the criteria for additional levels, which represents 16.2% of all cervical biopsies during the four-month study period. Of the 140 cases, 58 (41.4%) were endocervical curettings, and 82 (58.6%) were cervical biopsies.

Patients' ages ranged from 22 to 90 years, with a median of 42 years. The majority (90%) of the cases were HR-HPV positive, with only 10% of the cases negative for HR-HPV by PCR testing. Notably, of all HR-HPV positive cases, 28.6% were positive for HR-HPV16/18 (Table 1).

An initial impression of benign cervical mucosa or epithelium was established in 101 (72.1%) of the cases, low-grade squamous intraepithelial lesion (CIN1) was present in 27 (19.2%) of the cases, while atypia was present in 12 (8.6%) of the cases (Figs. 3A–D, 4, and 5A–C). The most common reason for ordering additional levels was atypia on initial morphologic evaluation (37.9%), followed by high-risk Pap-test (35%), long-standing HR-HPV (20.7%), and a discrepancy between the gross specimen size and the number/size of the tissue fragments on the slide (6.4%) (Fig. 6) [1–15].

A history of abnormal Pap test interpretation that prompted the pathologist to order additional sections included atypical squamous cells

Table 1
Distribution of HPV and Pap test results.

Pap test result (n)	HPV molecular result		
	Negative (%)	Positive (HPV 16/18)	Positive (other HPV)
NILM (23)	0 (0%)	12 (52%)	11 (48%)
ASC-US (28)	2 (7%)	11 (39%)	15 (54%)
LSIL (40)	6 (15%)	7 (17.5%)	27 (67.5%)
ASC-H (34)	2 (5.9%)	6 (17.6%)	26 (76.5%)
HSIL (8)	0 (0%)	1 (12.5%)	7 (87.5%)
AGC (7)	4 (57.1%)	3 (42.9%)	0 (0%)

NILM – Negative for intraepithelial lesion or malignancy.
ASC-US – Atypical squamous cells of uncertain significance.
LSIL – Low-grade squamous cell lesion.
ASC-H – Atypical squamous cells, cannot exclude a high-grade lesion.
HSIL – High-grade squamous cell lesion.
AGC – Atypical glandular cells.
HPV – Human papillomavirus.

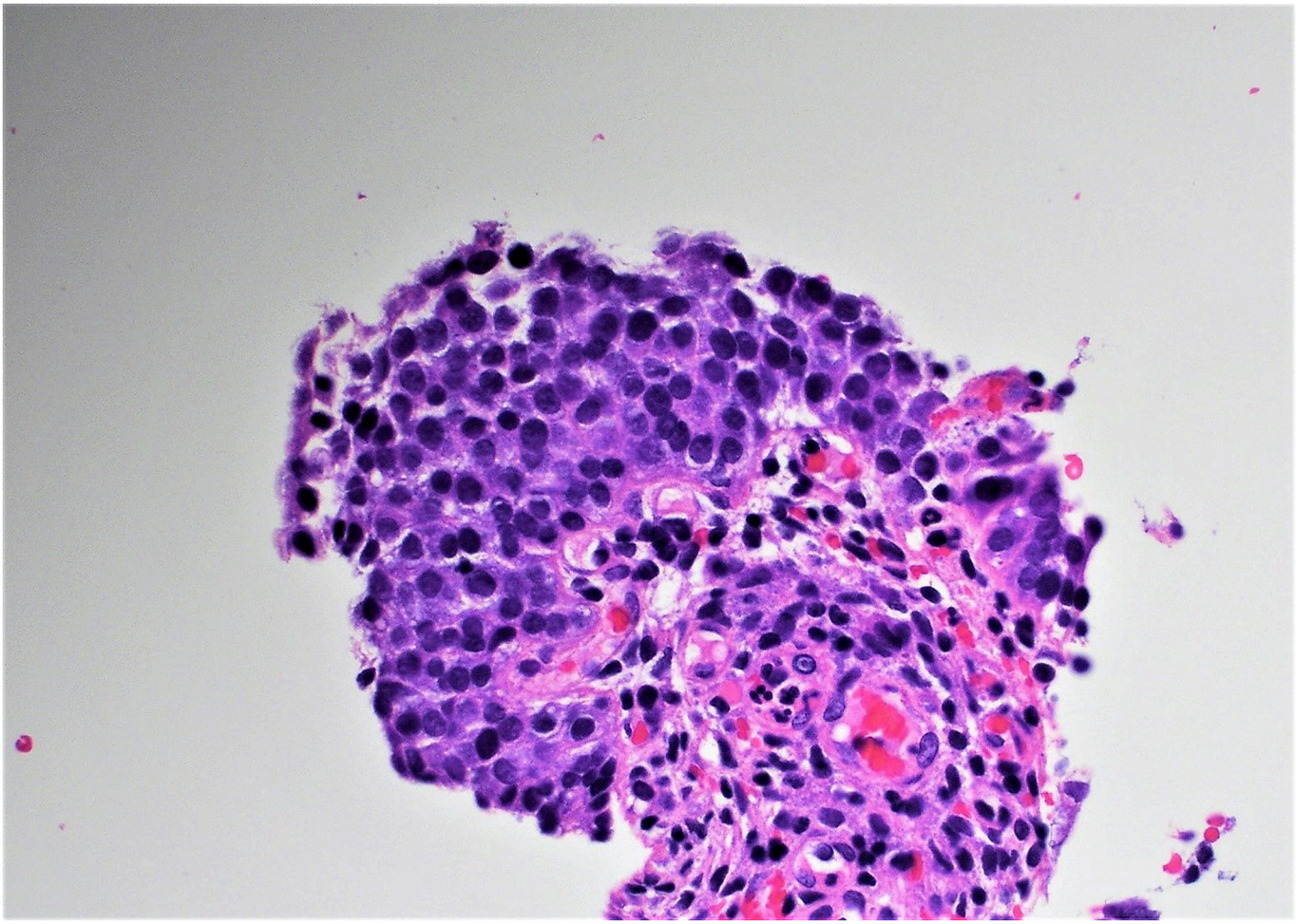

Fig. 2. Hyperchromatic and crowded cells with metaplastic features. Small lesions like this one almost always require additional sections. Immunohistochemistry for p16 is frequently used for lesions like this one at our institution (Olympus BX43, 40×).

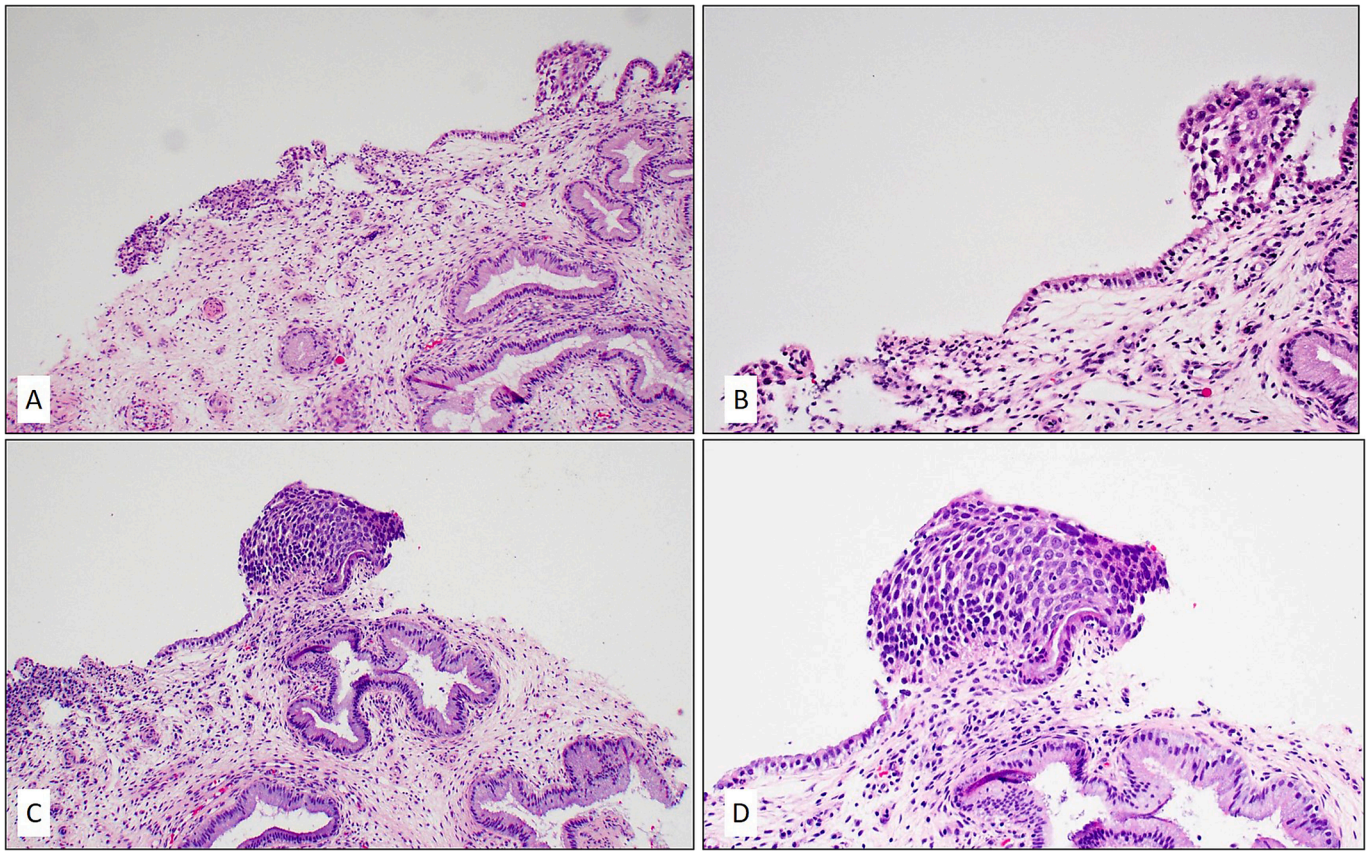

Fig. 3. A and B: Cervical biopsy low-power shows two small foci suspicious but not diagnostic of high-grade dysplasia) (Olympus BX43, 4× and 20×). C and D: Additional levels clearly show high-grade dysplasia (arrow); the focus with an asterisk is lost on additional levels (Olympus BX43, 10×, and 20×).

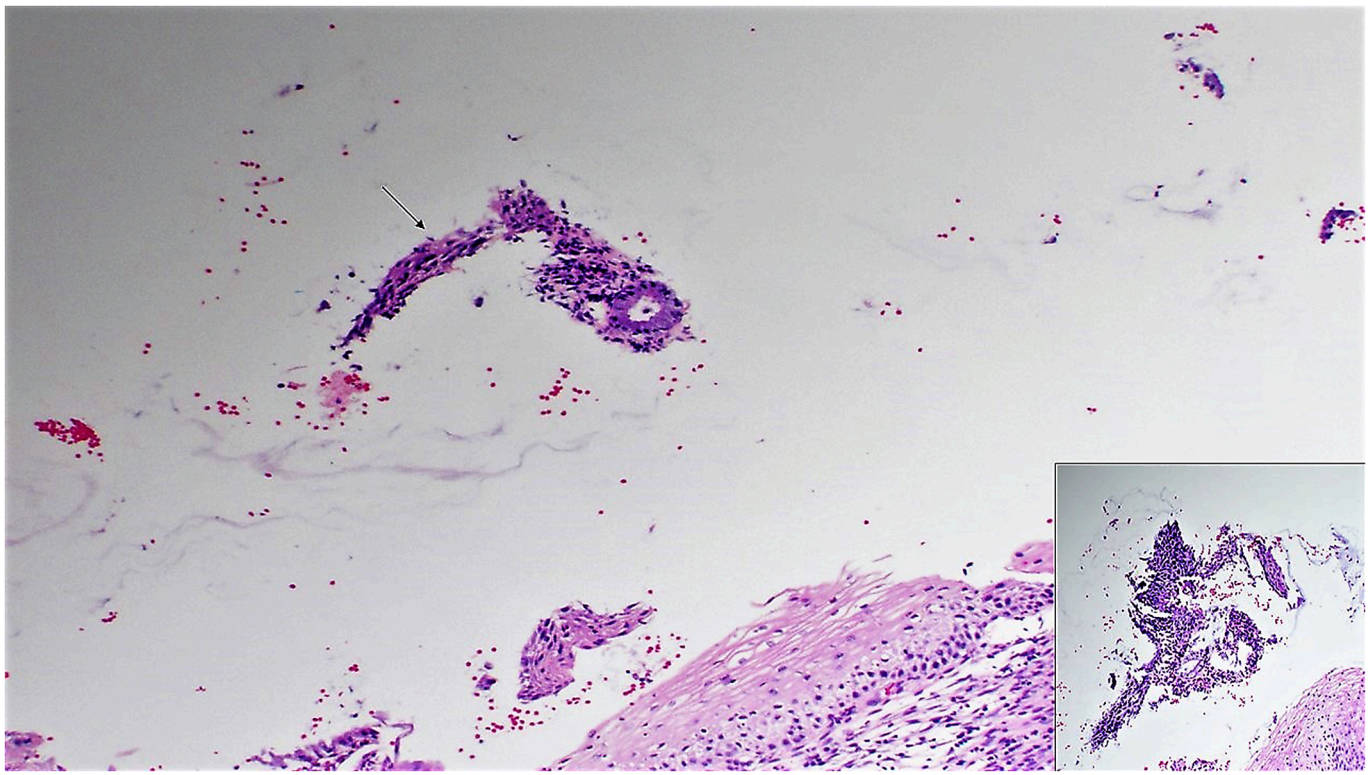

Fig. 4. Another example of a free-floating small fragment of atypical squamous epithelium (Olympus BX43, 4×). The inserted picture is level 6. The small fragment is much larger and consistent with high-grade dysplasia (Olympus BX43, 10×).

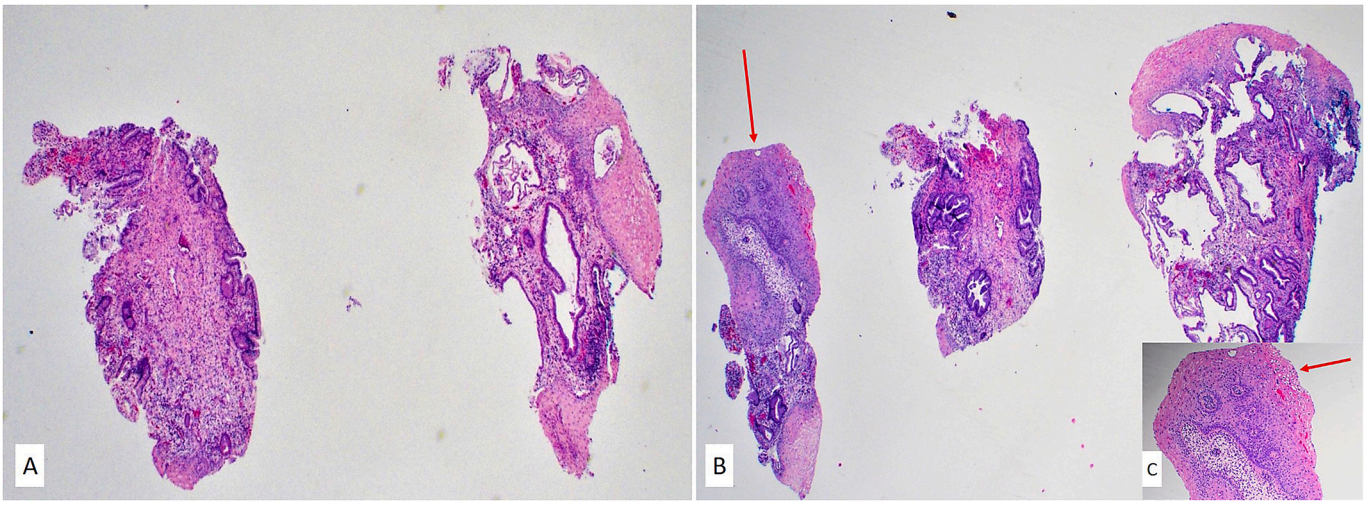

Fig. 5. A: Rare example of a missing fragment on the initial three levels. The discrepancy with gross description prompted additional sections. The fragment that is missing on the initial sections (red arrow) shows low-grade dysplasia (B and C) (Olympus BX43, 2× and 10×). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

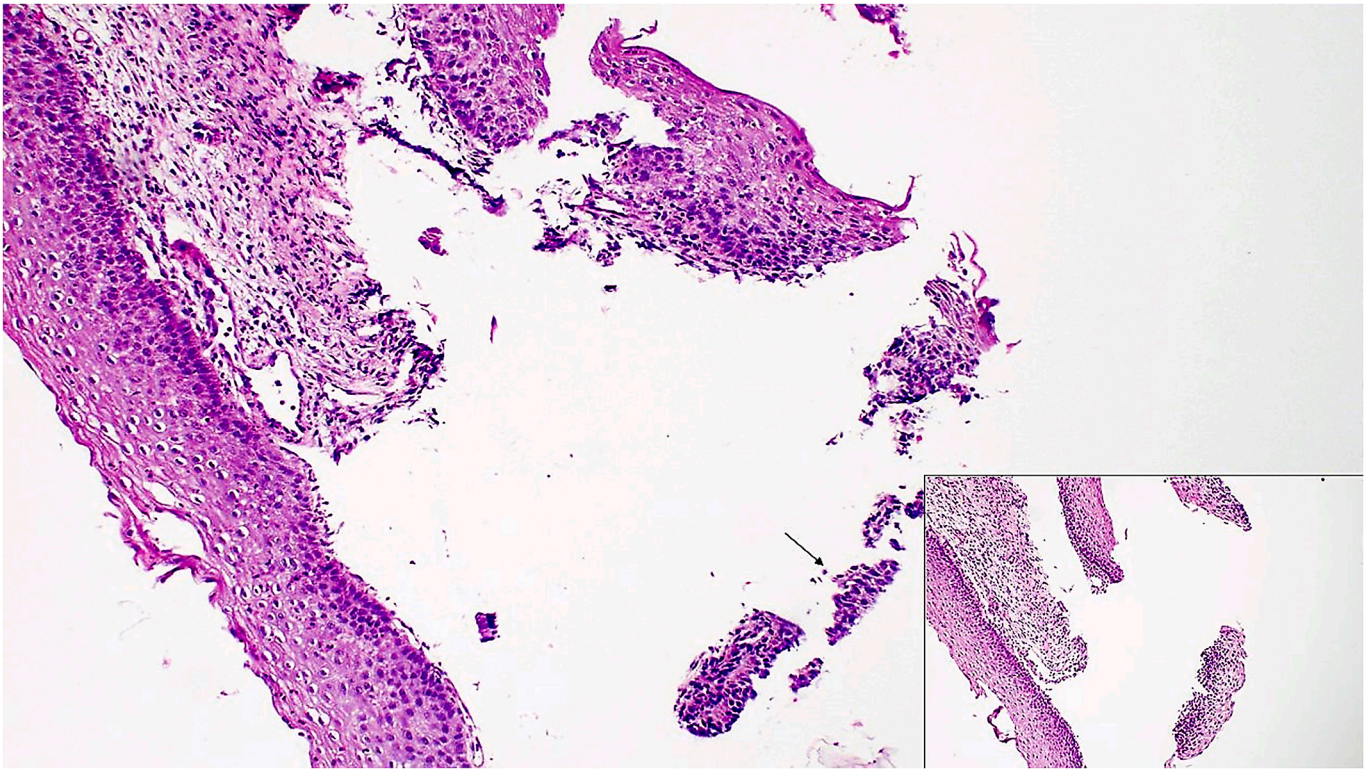

Fig. 6. Another example of a free-floating fragment. It is difficult to interpret if the fragment is the only basal layer of squamous epithelium or a truly atypical fragment. The levels (inset) are very useful and epithelial maturation is easily appreciated (Olympus BX43, 10×).

of undetermined significance cannot exclude high-grade (ASC-H) in 24.3% of the cases and high-grade squamous intraepithelial lesion (HSIL) (5.7%) (Table 1). Immunohistochemistry for p16 was performed in 79 (56%) cases, and 21 (15%) were positive. However, the interpretation on dysplasia was based on morphologic findings, and immunohistochemistry results were only for confirmatory purposes.

A change in final diagnosis was observed in 30 cases (21.4%), with an upgrade to a high-grade squamous intraepithelial lesion (CIN2–3) in 17 cases (12.1%).

An initial impression of atypia and atypia as a reason for ordering additional levels significantly correlated with change in diagnosis ($p = 0.02$) and upgrading to a high-grade (CIN2–3) lesion ($p = 0.03$) (Figs. 3 and 4, Table 2). The other reasons for obtaining additional levels did not significantly correlate with change in diagnosis ($p > 0.05$).

Change in diagnosis was observed in 21 (25.6%) cervical biopsies and 9 (15.5%) endocervical curettings. Change to clinically relevant CIN2–3 was observed in 12 (14.6%) cervical biopsies and 5 (8.6%) endocervical curettings. There was no statistical difference between the type of the specimen and a change in diagnosis ($p = 0.27$).

Table 2
Summary of the reasons for additional levels in cases with final diagnosis upgrade to high-grade squamous intraepithelial lesion (CIN2/3).

Changes in diagnosis to high-grade squamous intraepithelial lesion (CIN2/3)		No	Yes	Total
Reason for levels	HPV	28 (97%)	1 (3%)	29
	Atypia	41 (77%)	12 (23%)	53
	Pap smear	45 (92%)	4 (8%)	49
	Gross appearance	9 (100%)	0 (0%)	9
Total		123	17	140

CIN – Cervical intraepithelial neoplasia; HPV – Human papillomavirus.

4. Discussion

Currently, there are no recommended guidelines for the number of optimal initial levels for cervical biopsies. Due to differences in staffing, turnaround time (TAT), and daily number of specimens, laboratories have different practices for the number of levels and serial sections for cervical specimens.

To date, few studies have examined the value of additional sections in cervical biopsies. Our results confirm previous results, revealing that 24.3% of the cases with a squamous intraepithelial lesion (SIL) on Pap test but initially negative biopsies demonstrated SIL on additional three levels [5]. While atypia on initial levels had the highest correlation with SIL on subsequent levels, other examined features on initial specimens, including acute and chronic cervicitis and mucosal erosion, did not correlate with subsequent SIL [5].

The literature shows that discrepancies between biopsy and Pap test range from 11 to 30% [6,7]. Although sampling errors will always account for some cytology-histology discrepancies, up to 30% of cases will have a change in diagnosis after deeper levels [6,7]. There is currently no consensus on the appropriate initial number of levels for accurate diagnosis in cervical specimens. One retrospective study concluded that with five initial levels, no lesions or only occasional lesions would be missed [8], and another implied that the first level usually does not carry diagnostic information [9]. Studies from other organ systems similarly concluded that the additional levels tend to improve accuracy, including other factors such as the size of the specimen, orientation toward microtome blade, and the depth of paraffin block sectioning [10-12].

Based on the findings in this study that the final diagnosis was changed in 21.4% of cases after three (3) additional levels, we agree that six (6) initial sections would likely be sufficient to diagnose all squamous lesions of the uterine cervix. From a cost-effective perspective, each additional level should not cost more than 10$ [8]. However, the cost of storage of heavy glass slides has not been included in this calculation. If the levels are subsequently ordered, the histotechnologist's time to locate the blocks and to cut and stain the sections adds an “unseen and unaccounted” cost. Another issue with subsequent ordering is delayed turnaround time (TAT). The aim of this study was to detect the most predictive feature of biopsy for the subsequent change of diagnosis, in particular, change to the high-grade lesion (CIN2 or CIN3). In our study, that feature is atypia recognized by the pathologist on one of the three initial levels. It is important to note that in our laboratory, the paraffin blocks are cut at least 80 μm into the block in comparison with some other laboratories without any loss of tissue [8]. Additionally, all biopsies are signed out by subspecialized, fellowship-trained gynecologic pathologists that are familiar with the treacherous nature of small foci of cervical dysplasia. While in our study there was no statistical correlation with high-risk Pap tests (ASC-H, HSIL, and AGC), we would always encourage leveling the tissue in such circumstances. Any change in diagnosis, particularly to CIN2/CIN3, is significant on the individual level and will often lead to the decision to treat the patient, usually by some form of cervical excision. Failure to identify existing CIN2/CIN3 lesions may allow the high-grade lesion to go untreated and potentially progress to an invasive cancer. It is important to note that some cytologic interpretations are highly subjective, and some cytology laboratories may have more liberal use of ASC-H interpretation than others. We did not have enough AGCs interpretation for adequate statistical analysis. Also, we found that a long-standing HPV alone is least likely to yield a significant change in diagnosis. It is important to emphasize that we had no invasive squamous cell or adenocarcinoma on subsequent levels in our cohort.

Ideally, all cervical biopsies should be examined on six (6) levels, including the first level, particularly if no loss of tissue protocol is used in the laboratory. We advise that if the laboratory is using only three initial levels, our protocol with loss of tissue between the levels and a careful examination of these initial levels by the pathologist is the most critical first step in establishing an accurate diagnosis. Clinical correlation and Pap test results, if available, are excellent additional tools to ensure accuracy. Developing a system within the pathology lab to cut all biopsies with high-risk Pap test (ASC-H, HSIL, or AGC) on 5–6 levels upfront may be something that each pathology laboratory can consider to save histotechnologist's time and shorten TAT. Correlation with a number of fragments in gross description is judicious, and we show a single case where a small additional fragment was present in the subsequent levels changing the diagnosis into the low-grade squamous intraepithelial lesion (CIN1).

As many places around the world are transitioning toward primary HPV screening for cervical cancer, some of the screening guidelines propose colposcopy and biopsies followed by positivity for HPV16/18 without cytologic triage. In those circumstances where biopsy would be the only sampling available, correctly identifying the lesion will be crucial [13]. Lastly, the technology for slide-free histology may help pathology labs with the conundrum of additional levels and accuracy in small biopsies [14,15]. Additional research on its utility for small specimens such as uterine cervical biopsies is warranted.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.anndiagpath.2021.151872.

CRediT authorship contribution statement

Conceptualization: AC
Data curation: AC, SV
Formal analysis: AC, MCS, FKC, XLJ, SV
Methodology: AC
Supervision: SV
Validation: AC, MCS, XLJ, SV
Roles/writing - original draft: AC, SV
Writing - review & editing: AC, MCS, FKC, XLJ, SV.

Declaration of competing interest

The authors declare no conflict of interest.

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