

Glandular Lesions: Mimics and Diagnostic Pitfalls

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Relatively few topics in diagnostic cytopathology evoke such strong reactions as those encountered in cases of glandular lesions on Pap smears. This is quite understandable as diagnosticians must tread a fine line: not over-calling benign entities while simultaneously recognizing uncommon neoplastic lesions. In our own institution, we encounter "Atypical Glandular Cells" quite infrequently, as these cases account for only approximately 0.25% of our Pap test volume (or one out of every four hundred smears). The purpose of this article is to review the basic cytologic features of endocervical adenocarcinoma in situ and well-differentiated endocervical adenocarcinoma, and to briefly discuss some of the more common mimics and diagnostic pitfalls encountered with these lesions.

Endocervical adenocarcinoma in situ was added as a separate diagnostic entity with the implementation of the Bethesda 2001 recommendations. Remarkable progress has been made in our understanding of this lesion, considering it was not widely recognized as a precursor lesion to invasive endocervical adenocarcinoma until the 1980s. The vast majority of endocervical adenocarcinoma in situ cases are HPV-related, with up to 90% of lesions harboring either HPV type 16 or 18 DNA. Realization of this has been reflected in the utilization of HPV-testing in the ASCCP management algorithms for patients diagnosed with atypical glandular cells, not otherwise specified on Pap smears.

The main justification for creating an independent diagnostic category for adenocarcinoma in situ was that the cytomorphologic features of

these lesions are sufficiently reproducible and definable. These features encompass both architectural and cytologic characteristics and include the presence of tightly crowded, three-dimensional, hyperchromatic groups (so called "dark crowded groups"); demonstration of columnar morphology with nuclear pseudostratification;

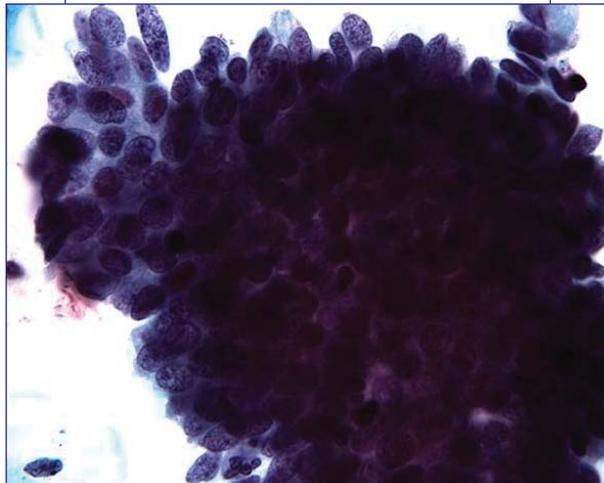


Figure 1a: ThinPrep slide showing a typical dark crowded group with columnar cells showing increased nuclear:cytoplasmic ratios, nuclear hyperchromasia, and altered chromatin, characteristic of endocervical adenocarcinoma in situ.

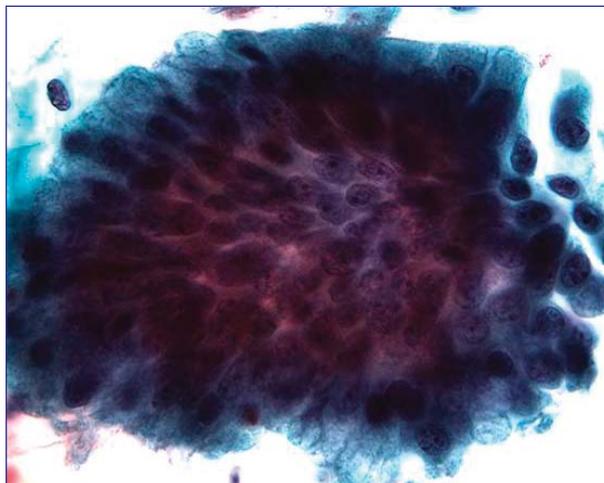


Figure 1b: ThinPrep slide showing reactive endocervical cells.



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tion; feathering, rosette formation, or other features suggestive of glandular differentiation; increased nuclear to cytoplasmic ratios; coarsely textured chromatin; mitoses and apoptotic figures (**Figure 1a**). Figure 1 depicts typical dark crowded groups. Evaluating the cells at the center of these groups is difficult to impossible and should be avoided. Much more information can be obtained by concentrating on the cells at the periphery of the groups. In this case, columnar morphology is evident in the cells with markedly increased nuclear to cytoplasmic ratios (compare to **Figure 1b**, normal endocervical cells) and coarse, speckled chromatin. Along the upper edge of the group is a good example of feathering, in which the nuclei (and sometimes cytoplasmic processes) extend outward in a perpendicular fashion from an imaginary line drawn along the contour of the group. It is worth noting that the presence of feathering is not to be equated with malignancy. However, feathering is a useful feature to suggest glandular (and even endocervical rather than endometrial) differentiation.

Before the diagnosis of endocervical adenocarcinoma in situ is rendered, a number of benign and neoplastic mimics must first be excluded. The most commonly encountered of these mimics are high grade squa-

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mous intraepithelial lesions (particularly those involving endocervical glands). It is useful to keep in mind that the Pap smear was optimized to detect squamous lesions and that, from a purely statistical standpoint, these squamous lesions are much more prevalent than glandular lesions. The practical application of this is that before making the diagnosis of a glandular lesion, one should work very hard to both convince him or herself that there is true, bona fide glandular differentiation (by finding either convincing columnar morphology, extensive feathering, or true rosette formation) and that there is no evidence of squamous differentiation. This distinction can be difficult because some cases of HSIL (particularly the syncytial pattern) seem to exhibit morphologic and architectural features that overlap with adenocarcinoma in situ. Both entities may display significant crowding, nuclear hyperchromasia, increased nuclear:cytoplasmic ratios, and mitoses. Squamous lesions may even show so-called “partial feathering” in which one or two cells can extend perpendicularly outward from the contour of the group.

By requiring the presence of extensive feathering, in concert with other strong evidence of glandular differentiation, one can try to minimize the misclassification of squamous lesions as glandular lesions. Even though making the distinction between squamous and glandular lesions can be very challenging, the management of patients with the two types of lesion can be significantly different, so every attempt should be made to correctly classify the lesions in question. The following “rules of thumb” have proven useful when faced with difficult-to-classify lesions. If one thinks the lesion is glandular, then it is probably squamous. If one is fairly sure the lesion is glandular, then it is probably squamous. The take home message is that one should be very certain before making a diagnosis of “adenocarcinoma in situ.”

With the increasing usage of liquid based cytology, a number of ancillary techniques have become available to aid the cytopathologist in resolving diagnostic dilemmas. We have found that preparing cell blocks from residual material has been extremely helpful in many difficult glandular lesion cases. Often the cellularity of these cell blocks compares favorably with that of endocervical biopsies or curettage specimens. Once the material has been embedded in paraffin, it can be treated much like a surgical specimen: immunohistochemistry, additional levels, and other special stains can be performed. These additional steps can make seemingly difficult diagnostic problems (squamous vs. glandular and

endocervical vs. endometrial) much more straight-forward.

Once one is convinced of the glandular nature of the lesion, the most commonly encountered benign mimic is tubular (tubo-endometrioid) metaplasia. The degree of nuclear hyperchromasia, enlargement, and cell crowding can be quite striking (**Figure 2**). Some helpful features distinguishing tubal metaplasia from adenocarcinoma in situ include, of course, the presence of cilia and terminal bars—very helpful in unambiguously identifying tubal metaplasia. Mitotic figures are not typically seen in tubal metaplasia. Likewise, prominent feathering and rosette formation also point away from the diagnosis of tubal metaplasia.

With the advent of improved sampling devices such as endocervical brushes and cervical brooms, the ability to more thoroughly sample the transformation zone became a reality. This has likely improved the diagnostic yield of Pap tests for glandular lesions, but it has also increased the frequency with which these preparations contain directly sampled endometrium, which is another benign mimic of adenocarcinoma in situ. Directly sampled endometrium (typically from the lower uterine segment) generally consists of two components: spindled endometrial stromal cells which have a hap-hazard arrangement, very little cytoplasm, and often, hyperchromatic nuclei; and endometrial glandular cells, which may form flat or folded sheets with monomorphic, often hyperchromatic nuclei. Depending upon the phase of the endometrial cycle, mitoses may be seen in both the glandular and stromal components, which may make the distinction from adenocarcinoma in situ difficult. Helpful features for distinguishing between adenocarcinoma in situ and directly sampled endometrium include the lack of feathering in endometrial cells and identification of the randomly arranged fragments of endometrial stromal cells (**Figure 3**).

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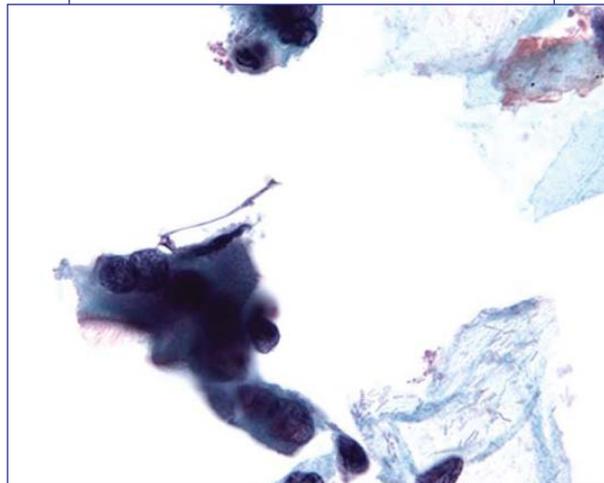


Figure 2: ThinPrep slide showing tubal metaplasia with distinct cilia and terminal bars. There is conspicuous nuclear irregularity.

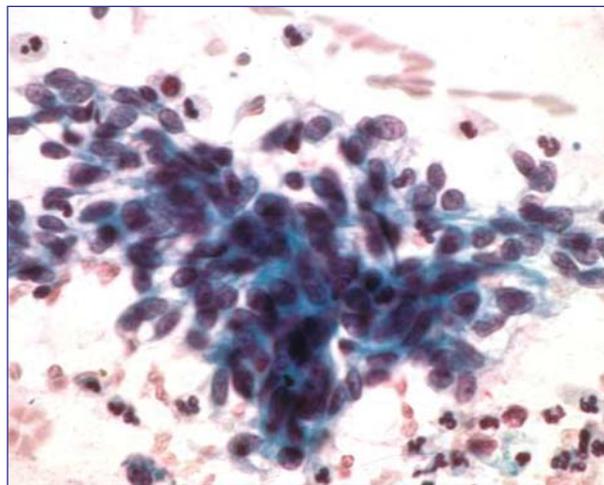


Figure 3: Conventional smear showing stromal cells from directly sampled lower uterine segment endometrium.

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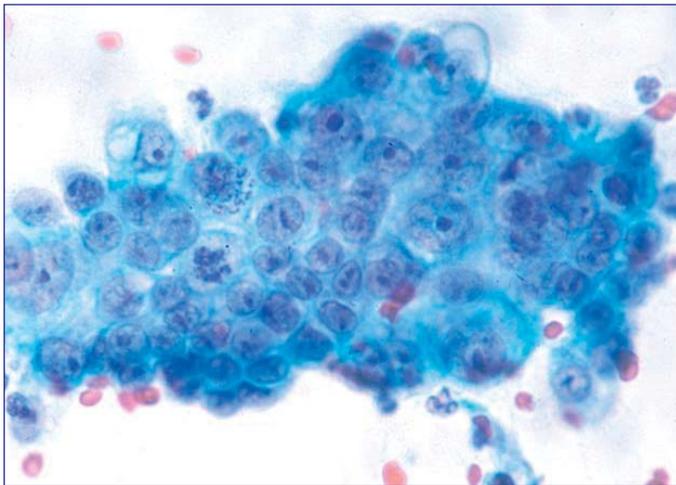


Figure 4: Conventional smear showing invasive endocervical adenocarcinoma. Note the macronucleoli, numerous mitoses, and markedly altered chromatin.

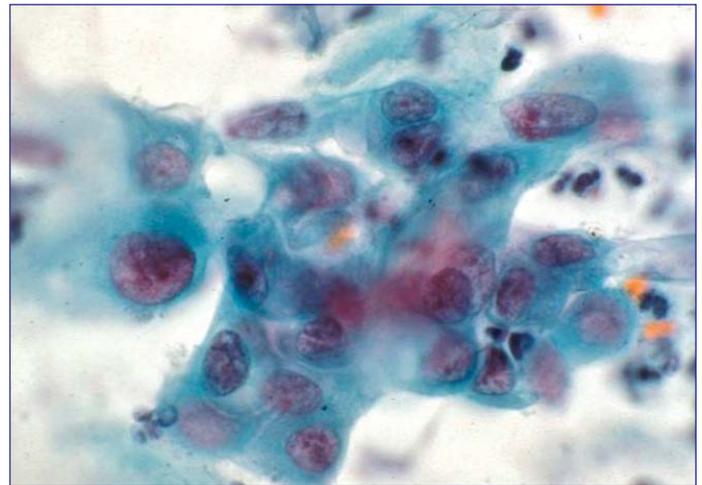


Figure 5: Conventional smear showing reactive endocervical cells associated with an endocervical polyp. Note the pale chromatin, polymorphic appearance of the cells, and thin nuclear membranes.

Well-differentiated endocervical adenocarcinoma also shares some morphologic features with endocervical adenocarcinoma in situ. Both lesions may exhibit nuclear enlargement with increased nuclear:cytoplasmic ratios; however, some features, which may be indicative of adenocarcinoma, are the presence of macronucleoli in tumor cells, and markedly abnormal chromatin distribution. Some adenocarcinomas present with vesicular nuclei showing prominent margination of the chromatin and thickened nuclear membranes, while others show coarse chromatin (**Figure 4**). Numerous reactive processes including severe cervicitis, ischemic changes at the surface of endocervical polyps, and even endocervical microglandular change can give rise to significant reactive nuclear atypia which can mimic endocervical adenocarcinoma (**Figure 5**). Useful features for distinguishing these reactive entities from

well-differentiated adenocarcinoma include the polymorphic population of cells which often accompanies reactive conditions. Well-differentiated carcinomas characteristically display a monomorphic quality to the cells (a “clonal look”). The cells of reactive processes also tend to have thin nuclear membranes and fine or smudgy chromatin.

Glandular lesions in cervical cytology specimens present significant diagnostic challenges. By developing strategies to carefully analyze hyperchromatic, crowded groups—focusing on the cells at the periphery of the groups, by maintaining strict criteria for diagnosing a lesion as truly glandular (unambiguous columnar differentiation, prominent feathering/rosette formation), and by becoming familiar with the diagnostic features of endocervical adenocarcinoma in situ and its mimics, one can approach glandular lesions with increased confidence.

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