Assessing Endometrial Hyperplasia and Carcinoma Treated With Progestin Therapy

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Abstract

The effects of increased amounts of progesterone on the endometrium, including such features as eosinophilic cytoplasmic metaplasia, glandular atrophy, and decidualized stroma, are well-known among surgical pathologists. These changes are typically seen as secondary effects of pregnancy or exogenous hormone therapy for birth control purposes or abnormal bleeding. Treatment with progesterone has become a viable alternative to hysterectomy in some patients with complex atypical hyperplasia (CAH) and well-differentiated endometrial carcinoma (WDC), especially those who are poor surgical candidates or those wishing to preserve fertility. To date, only 1 study has specifically examined the effects of progestin therapy on patients with a previous diagnosis of CAH or WDC. That study proposed a classification scheme for the assessment of treated CAH and WDC. The authors concluded that after 6 months of treatment, endometrial biopsy findings of persistent cytologic atypia and architectural abnormalities were associated with treatment failure. This current study aims to assess the previously proposed criteria in a cohort of 30 patients (18 with a diagnosis of CAH and 12 with a diagnosis of WDC), and determine the usefulness of these criteria in clinical practice. Our study confirms that cytologic atypia after 6 months of therapy is strongly associated with treatment failure, and should be an indication to pursue definitive surgical treatment in these patients.

The exquisite sensitivity of the endometrium to endogenous sex steroid hormones creates a unique environment with a shifting and varied microscopic morphology. Whether it is the balanced cycling of estrogen and progesterone coordinating menstruation in premenopausal women or unopposed estrogen-driving malignant epithelial proliferation, the ultimate clinical and histologic effects of these hormones on the endometrium are well-known among clinicians. Clinically, because of the ability of these hormones to induce known changes, exogenous hormone therapy is used therapeutically in various situations. Specifically, over the past 2 decades, high-dose progesterone therapy has become a viable alternative to hysterectomy in some patients with complex atypical hyperplasia (CAH) and well-differentiated endometrial carcinoma (WDC), and can be delivered systemically with oral progestins or locally with a progesterone-releasing intrauterine device (IUD). The ability to use progesterone therapy for these early lesions is especially useful in premenopausal women who wish to preserve fertility and those who are poor surgical candidates.

The microscopic changes induced by unopposed progesterone on the normal or benign endometrium are commonly seen in specimens obtained during pregnancy, and therefore are familiar to most surgical pathologists. These changes include inactive-appearing glands, stromal decidualization, and various cytologic metaplasias. Yet, despite numerous clinical studies and a reported overall effectiveness of progesterone therapy for the treatment of CAH and WDC ranging from 60% to 80%, the specific histologic changes induced by therapeutic progesterone in these lesions has only been documented sporadically. A consequence of this lack of studies is a paucity of data indicating when treatment
with progesterone should be deemed ineffective and definitive surgical therapy be considered.

Wheeler et al \(^{20}\) examined the effects of progesterone therapy in 44 patients treated for either CAH or WDC. They proposed a classification scheme for histologic assessment of treated CAH and WDC, and concluded that cytologic atypia and architectural abnormalities persisting for more than 6 months of therapy was strongly associated with treatment failure. These results are important because the group was able to show a link between the histologic changes induced by progesterone and eventual patient outcome. The current study attempts to reexamine the criteria proposed by Wheeler et al to determine their clinical usefulness and to assess if these criteria can reliably predict treatment efficacy in a separate patient population.

### Materials and Methods

With institutional review board approval, the surgical pathology archives at the University of Virginia (Charlottesville, VA) and Emory University (Atlanta, GA) were searched for endometrial samples of women treated with progestins. A total of 34 patients (29 from University of Virginia and 5 from Emory University) were identified as having a diagnosis of CAH or WDC, and subsequently received treatment with progesterone. The original, pretreatment biopsy or curettage specimens of 22 patients with CAH and 12 patients with WDC were retrieved, as well as posttreatment follow-up biopsy, curettage, and hysterectomy specimens when available.

All specimens were reviewed individually and classified using the World Health Organization criteria for the diagnosis of endometrial carcinoma and hyperplasia.\(^{22,23}\) Each case was also evaluated using the criteria proposed by Wheeler et al.\(^{20}\) including gland-to-stroma ratio, architectural abnormalities (gland crowding, gland confluency, or cribriforming), papillary architecture, nucleus-to-cytoplasm ratio, presence and prominence of nucleoli, nuclear chromatin quality, mitotic activity, and cytoplasmic metaplasias. Cytologic atypia was defined as the presence of coarse chromatin, irregular nuclear membrane contours, and prominent nucleoli. Mitotic activity was measured using the mean mitotic count per 10 glands. Glandular cellularity was not assessed as in the original study,\(^{20}\) because it was thought to be impractical for routine practice. All posttreatment specimens were compared with the patients’ original diagnostic biopsy specimens.

Clinical outcomes at the time of last biopsy or hysterectomy was determined for all patients and defined as (1) resolution, if final specimen showed atrophic, proliferative, or secretory endometrium; (2) regression, if final specimen showed hyperplasia without atypia; (3) persistence if CAH or WDC was present in both the original and final specimens; and (4) progression, if pretreatment specimen showed CAH and final specimen showed WDC, or if original specimen showed WDC and final specimen revealed moderate or poorly differentiated carcinoma.

### Results

A median of 4 (range 2 to 10) endometrial samples were retrieved per patient. On second review, 4 patients were excluded from the study because of a review diagnosis of disordered proliferative endometrium based on the original specimen. Hysterectomy material was available for 7 (23%) of 30 patients. Hormone receptor status before therapy was unknown in all cases. Clinical data are summarized in Table 1. Average follow-up time was 20 months, with a median of 12 months (range, 6-59 months). Eight patients (44%) with CAH were premenopausal, with a mean age of 38 years (range, 25-39 years), and 10 (56%) were postmenopausal, with a mean age of 57 years (range, 50-74 years). Six patients (50%) with WDC were premenopausal, with a mean age of 32 years (range, 18-42 years), whereas 6 (50%) were postmenopausal, with a mean age of 64 years (range, 54-78 years). A total of 7 patients (23%) were treated with a levonorgestrel-releasing IUD (Mirena, Bayer, Morristown, NJ), and 23 (77%) were treated with oral progestins (megestrol acetate, Megace, Bristol-Myers Squibb, New York, NY) at varying doses (40-160 mg/day).

### Histologic Features of Progestin-Treated CAH

In general, the overall gland-to-stroma ratio decreased after the initiation of progesterone therapy. Ten (55%) cases showed completely inactive endometrium Image 1, whereas 2 (11%) cases showed focal, residual glandular crowding with treatment effect Image 2. In addition, increased architectural complexity (confluency/cribriforming) not seen in the pretreatment biopsy specimens was noted in 4 (22%) cases Image 3. Subjective decreases in both nuclear size and nucleus-to-cytoplasm ratio were seen in most cases. Irregular...
nuclear contours progressed to oval, round, and eventual pyknosis in inactive cases. Nucleoli were absent in 2 (11%) cases, inconspicuous in 14 (78%), and prominent/conspicuous in 2 (11%) cases. Nuclear chromatin was fine in 15 (83%) cases with smudginess, and coarse and clumped in 3 (17%). Median mitotic rate per gland was decreased after treatment (0.03; range, 0-0.4), compared with pretreatment specimens (0.1-1.0). All cases showed evidence of one to several different cytoplasmic metaplasia, with eosinophilic/pink cell change being present in all 18 cases. Seven cases (39%) showed mucinous metaplasia, whereas squamous metaplasia was present in 5 cases (28%). Squamous metaplasia was also present in 3 (17%) pretreatment specimens, but eosinophilic and mucinous changes were not seen in any of the pretreatment specimens. Stromal cells were decidualized and occasionally spindled after treatment.

**Histologic Features of Progestin-Treated WDC**

Similar to cases of CAH, a decrease in overall gland-to-stroma ratio was seen after the initiation of progesterone therapy. Untreated complex atypical hyperplasia (CAH) compared with a biopsy specimen 3 months after treatment which shows complete resolution of disease with glandular atrophy and stromal decidualization (H&E, ×100).

**Image 1** Untreated complex atypical hyperplasia (A) compared with a biopsy specimen 3 months after treatment which shows complete resolution of disease with glandular atrophy and stromal decidualization (B) (H&E, ×100).

**Image 2** A, B, Retained abnormal architectural patterns (glandular crowding) 4 months after the initiation of therapy in a case of complex atypical hyperplasia (CAH). Both images show evidence of progesterone therapy with eosinophilic cytoplasmic metaplasia and decreased cytologic atypia. These cases would be diagnosed as treated CAH and considered to show persistence of disease (H&E, ×100).
 Increased worrisome patterns following treatment. A, Pretreatment specimen showing complex atypical hyperplasia. Biopsy specimens 3 months after treatment revealed areas suspicious for glandular confluency (B) and cribriforming (C, D). This patient eventually showed complete resolution of disease on biopsy at 7 months, suggesting that these features may be degenerative changes associated with progesterone therapy (H&E, ×100).

 Progesterone-treated complex atypical hyperplasia (CAH). A, High-power view of pretreatment CAH with prominent cytologic atypia. B, Biopsy at 3-month follow-up revealed loss of atypical nuclear features and rounded nuclear contours, eosinophilic cytoplasmic metaplasia, and conspicuous nucleoli. Mitotic figures were also decreased (not shown) (H&E, ×400).
cases (75%) showed residual architectural complexity, including glandular confluence and cribriforming. These features were focal in 3 such cases of architectural complexity (33%). In addition, 3 (33%) of these cases showed papillary architecture that was not present in pretreatment specimens. In general, nuclear size decreased with treatment, and nuclear contours progressed from irregular to round. Nucleoli were absent in 1 case (8%), inconspicuous in 7 cases (58%), and prominent/conspicuous in 4 (33%) cases. Nuclear chromatin pattern was fine in 9 cases (75%), and coarse and clumped in 3 (25%). Median mitotic rate per gland was decreased after treatment (0.09; range, 0-0.3) compared with pretreatment specimens (0.5; range, 0.2-1.6). All cases showed evidence of one to several different cytoplasmic metaplasias, with eosinophilic/pink cell change being present in 12 cases (100%). Seven cases (58%) showed mucinous metaplasia, and 9 cases (75%) showed squamous metaplasia. Squamous metaplasia was also present in 6 pretreatment specimens (50%), but no eosinophilic and mucinous changes were noted. Stromal cells were decidualized and spindled after treatment.

Outcomes

Table 2 summarizes outcomes in patients with CAH and Table 3 in patients with WDC. Overall, resolution occurred in 21 cases (70%), regression in 2 cases (7%), persistence in 3 cases (10%), and progression in 4 cases (13%). The mean time to resolution was 7 months (range,
No significant difference was noted in resolution status between pre- and postmenopausal women, with 9 (64%) of 14 premenopausal women and 12 (75%) of 16 postmenopausal women showing resolution of the original lesions on the final specimen (P = .6874). The mean time to regression was 7.5 months in premenopausal women (range, 4-16 months) and 6.8 months in postmenopausal women (range, 4-10 months). Age did not seem to contribute to final outcome, with essentially equal numbers of patients younger than 45 years (n = 3; age range, 26-44 years) and older than 50 years (n = 4; age range, 52-79 years) failing to show resolution. All nonresolved cases occurred in women treated with oral progestins (9 [39%] of 23); in all 7 patients treated with levonorgestrel-releasing IUDs, the original lesions resolved. The dose of oral progestins did not appear to play a role in final outcome, because the majority of patients in the group showing resolution of disease (8/14 patients) were treated with 80 mg/day of megestrol, whereas the majority of patients showing progression or persistence of disease (4/7 patients) were treated with a higher dose (160 mg/day).

Outcomes of Women With CAH and WDC

The frequency of the 4 potential outcomes at various time points throughout the study is summarized in Table 4. Of 18 patients diagnosed with CAH based on the original specimen, 13 (72%) resolved completely, showing inactive or secretory endometrium with progesterone effect on the final specimens. Two cases (11%) showed regression of CAH to complex hyperplasia without atypia; the 2 patients showing regression of CAH to complex hyperplasia without atypia had limited follow-up (6 months each).
WDC. Mean follow-up was 15 months. Both cases showing regression revealed crowded glands, rounded nuclei with inconspicuous nucleoli, and eosinophilic metaplasia, consistent with progestin-treated hyperplasia without atypia. Follow-up in both these cases was limited, however, with 1 patient having a hysterectomy at 5 months, and the other having had 3 biopsies over a 6-month period.

Three cases showing progression to WDC showed evidence of progesterone therapy, with eosinophilic metaplasia and stromal decidualization; however, they all continued to show evidence of cytologic atypia (conspicuous/prominent nucleoli, coarse chromatin, atypical nuclear contour) in addition to glandular confluency. One case had areas of prominent papillary architecture (Image 5C). Two of the 3 cases showed progression of disease at 4 to 6 months after initiation of therapy. The third case actually showed regression of CAH at the 4- to 6-month biopsy, but progression to WDC was seen 12 months later, presumably after progesterone therapy was discontinued.

Of 12 patients diagnosed with WDC on the original specimen, 8 (66%) cases showed complete resolution (Image 6A, Image 6B, and Image 6C). Three cases showed persistence of WDC with evidence of retained cytologic atypia and glandular confluency despite evidence of progesterone therapy (Image 6D, Image 6E, and Image 6F). One case showed progression of WDC to high-grade endometrioid carcinoma (International Federation of Gynecology and Obstetrics stage 3) 21 months after initiation of progesterone therapy. Hysterectomy performed at this time revealed stage IIIa endometrial carcinoma with invasion of the serosal surface. No evidence of metastatic disease was seen at last follow-up. Table 5 summarizes the specific diagnoses made at various time points for all patients with persistence or progression of disease.

**Prediction of Outcomes With Progesterone-Induced Histologic Features**

No significant difference was found in the effectiveness of progesterone therapy in patients with CAH vs those with WDC ($P = 1.000$). As in the original study, persistence of cytologic atypia and architectural abnormalities were considered markers of treatment failure. Cytologic atypia was defined as prominent nucleoli, atypical nuclear contours, and coarse nuclear chromatin. Architectural abnormalities included glandular crowding, confluency or cribriforming, and papillary patterns. The presence of cytologic atypia and architectural abnormalities at various intervals and correlation with ultimate patient outcomes are summarized in Table 6.

Independent of architectural patterns, cases with cytologic atypia noted on biopsy specimens from 1 to 3 months or 4 to 6 months ultimately showed resolution of disease in 9 (64%) of 14 cases. However, none of the patients with atypia after 6 months of progesterone treatment showed evidence of disease resolution on final biopsy (0 of 7 cases) (Table 6). These findings reached statistical significance on the Fisher exact test ($P = .0071$). When architectural abnormalities were examined independent of cytologic atypia, glandular crowding or confluency noted 1 to 3 months or 4 to 6 months
features of progesterone-treated CAH or WDC were assessed, and persistence of cytologic atypia and architectural abnormalities after 6 months of therapy was correlated with disease persistence and ultimately treatment failure. The current study largely confirms the validity of the previous findings and adds to the growing literature on the histologic effects of progesterone on the endometrium. The combined findings of the 2 studies are important, because they clarify the gray area that previously existed between the histologic features seen on posttreatment pathology specimens and the clinical decisions necessary for continuing pharmacologic therapy or recommending surgical intervention.

Discussion

The goal of this study was to determine if the criteria proposed by Wheeler et al\(^{20}\) can effectively predict the clinical outcomes of patients diagnosed with CAH or WDC and treated with progesterone. In the original study, the histologic features of progesterone-treated CAH or WDC were assessed, and persistence of cytologic atypia and architectural abnormalities after 6 months of therapy was correlated with disease persistence and ultimately treatment failure. The current study largely confirms the validity of the previous findings and adds to the growing literature on the histologic effects of progesterone on the endometrium. The combined findings of the 2 studies are important, because they clarify the gray area that previously existed between the histologic features seen on posttreatment pathology specimens and the clinical decisions necessary for continuing pharmacologic therapy or recommending surgical intervention.

C, 7-month biopsy specimen showing complete regression of original WDC with decidualized endometrium and atrophic glands. D-F, Patient 4, showing persistence of WDC despite 12 months of progesterone therapy. D, Initial biopsy showing WDC with secretory features. E, 6-month posttreatment biopsy specimen showing treated WDC with eosinophilic metaplasia, with retained atypical nuclear features and glandular crowding. F, Hysterectomy specimen showing residual foci of treated WDC (arrow) adjacent to benign-appearing endometrial glands (A-F, H&E, ×100).
The microscopic findings common to all endometrial tissue exposed to increased levels of progesterone include nuclear membrane rounding, condensation of chromatin, and a loss of nucleoli. Cytoplasmic metaplasias are prevalent as well, especially eosinophilic and mucinous, or occasionally squamous; stromal decidualization is also common. Additional features seen in patients treated specifically for premalignant and low-grade malignant lesions such as CAH and WDC consist of decreasing cytologic atypia and architectural abnormalities, with eventual resolution of the original lesion in the majority of cases. Mitotic figures also become less frequent with treatment.

As in the original study, the presence of cytologic atypia on 6-month posttreatment follow-up biopsy was strongly associated with treatment failure. In the current study, 7 cases showed progression or persistence of disease on the final available specimen. The only cases showing retained cytologic atypia after at least 6 months of treatment were these aforementioned 7 cases which showed disease progression or persistence; this indicates that persistence of cytologic atypia is strongly linked to treatment failure. In general, the evaluation of cytologic atypia in all patients treated with progesterone is straightforward, because most patients respond to therapy. However, the criteria used to classify these features are prone to subjectivity, so it is recommended that all of a patient’s previous specimens be examined concurrently with the most recent one. This way a patient’s current specimen can be more objectively evaluated for true loss or persistence of cytologic atypia.

Assessment of architectural abnormalities in treated cases can occasionally be challenging. The most common effect of progesterone on glandular architecture is to decrease both overall glandular cellularity and the gland-to-stroma ratio. However, as noted before, with treatment of CAH especially, an increase in worrisome architectural patterns can be noticed after initiation of therapy. Specifically, in cases of CAH, areas of glandular confluency and cribriforming are sometimes seen, but often with a concomitant loss of cytologic atypia. This increase in gland complexity should not be considered progression of disease but rather a feature of progestin-treated CAH, and could merely be a degenerative effect of the progesterone on the initial lesion. In addition, persistent architectural abnormalities alone do not appear to be as strongly associated with treatment failure as does retained cytologic atypia. In fact, in many of the cases that were treated effectively, abnormal architectural features resolved after cytologic atypia.

One intriguing finding in this study was the lack of disease persistence or progression in patients treated with progestin-releasing IUDs. Although the number of individuals treated with an IUD in the current group was limited (n = 7), this finding inevitably raises issues of compliance among

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CAH, complex atypical hyperplasia; FIGO, International Federation of Gynecology and Obstetrics; Prog Tx, presence of progesterone therapy–related changes, including cytoplasmic metaplasias; WDC, well-differentiated endometrial carcinoma.

* Four of 7 patients underwent hysterectomy.

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<td>Correlation of Outcome With Presence of Cytologic Atypia and Architectural Abnormalities at Given Biopsy Intervals</td>
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the oral progesterone–treated patient group. In several cases in the current study, either a lack of compliance or a premature termination of progesterone therapy seemed to lead to disease progression or recurrence. For example, 1 patient originally diagnosed with CAH showed resolution of disease after 6 months of oral progestin therapy; however, follow-up biopsy 12 months later revealed progression of disease to WDC, presumably after progesterone therapy had been discontinued. In another case, initial treatment with medroxyprogesterone contraceptive injection (Depo-Provera, Pfizer, New York, NY) led to resolution of disease at the 6-month follow-up biopsy; however, the lesion recurred when the medroxyprogesterone injection was discontinued and replaced with oral progesterone therapy. Thus it is difficult to ascertain whether disease progression in any patient treated with oral progesterone truly represents pathologic progression and thus treatment failure, or if it is simply related to a lack of treatment compliance or insufficient duration of therapy. Either way, these findings suggest that the use of a progestin-releasing IUD as primary therapy is likely more effective at treating CAH and WDC in these cases, and is of course less prone to issues regarding patient compliance.

Overall, the current study validates the features proposed by Wheeler et al. in cases of CAH and WDC after progestin therapy, and confirms that persistence of cytologic atypia after at least 6 months of therapy is strongly associated with treatment failure. Despite a limited number of subjects, these 2 studies argue strongly for consideration of surgical management in patients with nuclear atypia lasting more than 6 months while receiving progesterone therapy. How long a patient needs to continue therapy after disease resolution is still largely unclear; as evidenced by 2 patients in this study, the disease can progress after apparent regression if therapy is discontinued or if the patient is noncompliant. In fact, another general question that remains is whether resolution of cytologic atypia in a successfully treated case truly represents resolution of disease on a genetic level. Future studies examining the molecular changes induced by progesterone therapy will be needed to perhaps more definitively classify the phenotypic features seen.

Regardless, patient compliance is a factor that will always play a role in the ultimate outcome of these patients; because compliance is difficult to ensure and control for, clinical physicians and surgical pathologists should remember the following 2 points when evaluating the efficacy of progesterone therapy in these patients. First, patients should have a careful follow-up, undergo routine biopsies at regular intervals, and be encouraged to comply with their therapy. Second, a pathologist must have access to all of a patient’s previous specimens when assessing treatment effect, to diminish the role of subjectivity with regard to cytologic atypia. Only with regular clinical follow-up and appropriate pathologic review can therapeutic efficacy be truly assessed.

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