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# CD10 immunohistochemical staining enhances the histological detection of endometriosis

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**Objective:** To determine whether the use of CD10 immunohistochemistry in addition to hematoxylin and eosin (H&E) staining would increase the sensitivity of surgically suspected endometriosis lesions.

**Design:** Retrospective cohort study.

**Setting:** Tertiary care government research hospital.

**Patient(s):** Thirty-one women with chronic pelvic pain.

**Intervention(s):** Immunohistochemical analysis for CD10 was performed on 108 possible endometriotic lesions and in the corresponding endometrial biopsy samples obtained during laparoscopy. When CD10 immunohistochemistry results were positive, the corresponding H&E section was reviewed to determine if the initial diagnosis should be revised.

**Main Outcome Measure(s):** Histologic diagnosis of endometriosis by adjunctive use of CD10 immunohistochemistry in conjunction with H&E-stained specimens.

**Result(s):** In endometrial stroma, CD10 was consistently present. Of the 70 specimens judged negative initially by H&E staining, CD10 staining led to the diagnosis of endometriosis in 11. The addition of CD10 immunohistochemistry detected more positive endometriosis lesions than H&E staining alone (45% vs. 35%). In three women with minimal endometriosis at surgery but initially negative histopathology, CD10 immunohistochemistry changed the histologic diagnosis to endometriosis.

**Conclusion(s):** The adjunctive use of CD10 immunohistochemistry improves diagnostic sensitivity for endometriosis, especially for women with minimal disease. (Fertil Steril® 2004;82:86–92. ©2004 by American Society for Reproductive Medicine.)

**Key Words:** CD10, diagnostic test, endometriosis, hematoxylin and eosin, immunohistochemistry

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The diagnosis of endometriosis may be suspected in the clinical context of pelvic pain or infertility, and suggested by inspection at surgery. The positive predictive value, however, of a visual diagnosis may be as low as 45% (1). Better laparoscopic optics and video monitors, systematic evaluation of pelvic surfaces, and recognition of the variable appearance of endometriosis can improve the surgical detection of endometriosis, but the clinical appearance can mimic endosalpingiosis, cancer, or pelvic infection (2–4). Because the treatment of these conditions differs, it is important to confirm the diagnosis of endometriosis by examination of biopsy samples stained with hematoxylin and eosin (H&E). However, histologic examination may produce unexpected false-negative results, so an improved method for detection of endometriosis would be welcome.

The cell-surface metalloendopeptidase CD10 is expressed in myoepithelial breast cells, normal renal tubular and glomerular cells (5), renal carcinoma (6), hepatocellular carcinoma (7), prostatic glandular epithelium (8), pulmonary alveoli (9), lymphoid cells (10), dermal tumors of mesenchymal origin (11), mesonephric tumors (12), and acute lymphoblastic leukemia and lymphoma (13). Additionally, several small studies suggest that CD10 is present in normal and ectopic endometrial

stroma (14–16), endometrial stromal neoplasms (14, 17), and adenomyosis (18). Thus we hypothesized that CD10 immunohistochemistry (IHC) would increase the sensitivity of the H&E-based histologic diagnosis of endometriosis by improving the recognition of the ectopic stromal cells. To examine this possibility, we compared the diagnostic efficacy of H&E staining with and without adjunctive CD10 IHC in biopsies of surgically diagnosed endometriosis.

## MATERIALS AND METHODS

### Subjects and Clinical Intervention

We evaluated all the biopsies obtained from 31 women with chronic pelvic pain recruited for a clinical trial of a postsurgical treatment for endometriosis. The women were 21 to 46 years old (mean age: 32.9 ± 7.8 years) and their ethnic and racial classifications were African American (n = 3), Caucasian (n = 27), and other (n = 1).

The institutional review boards of the National Institute of Child Health and Human Development and Georgetown University Medical Center approved this study. After providing written informed consent, all women underwent laparoscopy. The goal was to systematically inspect the pelvic peritoneal surfaces and to excise all possible endometriosis lesions with a contact Nd:YAG laser (Surgical Laser Technologies, The Oaks, PA).

The extent of endometriosis was described using the revised American Society for Reproductive Medicine (revised ASRM) classification system (19). Subtle lesions that might represent endometriosis were excised even if endometriosis was not suspected and the revised ASRM stage was 0. One to nine biopsy samples were collected from each woman. Specimens of 3 mm diameter or larger were divided only if they had a visible lesion, and a portion of each lesion was reserved for research purposes. A sample considered representative of endometriosis was submitted for pathologic examination. An endometrial biopsy sample was obtained during surgery in 30 women.

### Histologic Examination

After formalin fixation and paraffin embedding, sections taken from three different levels of the lesion were stained with H&E and evaluated for evidence of endometriosis by two pathologists (MM and AF). Criteria for H&E diagnosis required the identification of endometrial glands and stroma. Positive staining for CD10 was interpreted as positive endometrial stroma and was considered to be consistent with endometriosis. All slides were reviewed by both pathologists and characterized as positive or negative for CD10.

When CD10 IHC was positive, the pathologists reexamined the corresponding initial H&E slide to consider whether the previous diagnosis should be revised based on identification of endometrial morphology of glands and stroma. In specimens for which CD10 staining could not distinguish lymphocytes from endometrial stroma, adjacent sections were stained with leucocyte common antigen (LCA) IHC to distinguish between the two cell types.

To test whether CD10 IHC might improve diagnostic sensitivity, we retrospectively chose to examine a disproportionately higher number of negative H&E specimens. Of the 108 lesions tested, 70 were negative and 38 were positive for endometriosis on H&E.

The immunohistochemical staining for CD10 was performed using a CD10 monoclonal antibody (CD10-270, Novocastra, Newcastle upon Tyne, United Kingdom) at a 1:40 dilution. The LCA staining was performed using monoclonal mouse anti-human leucocyte common antigen clones PD7/26 and 2B11 (DAKO-LCA, DAKO Corporation, Carpinteria, CA) at a 1:200 dilution. Except where noted, IHC reagents were purchased from Vector Laboratories (Burlingame, CA). Sections were rehydrated through a xylene and graded alcohol series, endogenous peroxidase activity was blocked with 1.5% hydrogen peroxide (CD10) or Biotin A and B Ventana Blocker (LCA; Ventana Medical Systems, Inc, Tucson, AZ), and antigen retrieval was accomplished by boiling the slides in 0.01M citrate buffer (pH 6.0) in a pressure cooker.

For CD10 IHC, sections were incubated with normal horse serum to reduce nonspecific binding, followed by CD10 primary antibody at room temperature overnight. Slides were then incubated with biotinylated universal secondary antibody (1:50 dilution, Vectastain Elite kit) for 30 minutes. For LCA IHC, staining was performed in the Ventana 320 machine with incubation of primary antibody at 40°C for 32 minutes, followed by the Ventana secondary antibody for 30 minutes. Slides were stained with 3′3-diaminobenzidine and counterstained with hematoxylin. The endometrial biopsy served as a control tissue, with stroma predicted to be positive, and epithelium negative.

### Statistical Analysis

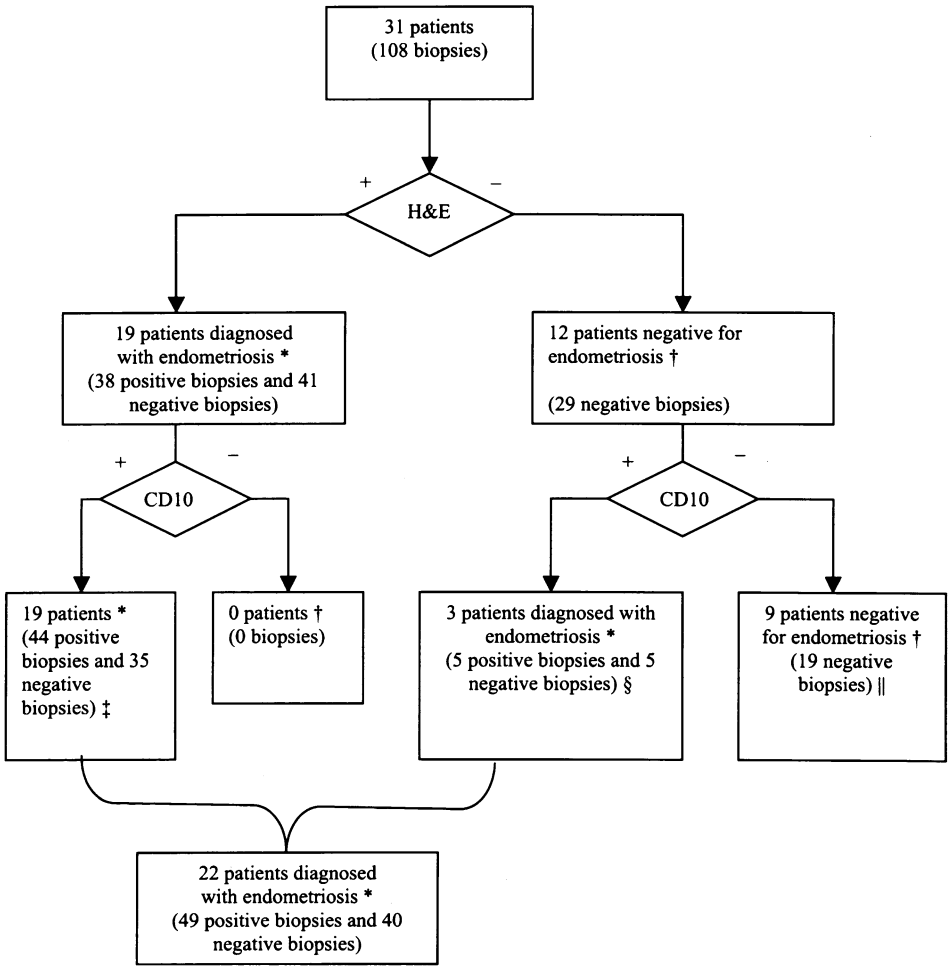
The pathologic diagnosis of endometriosis was assigned to a patient if at least one excised lesion was histologically positive. We used McNemar's test, calculated using SAS software (SAS Version 8.2, SAS Institute, Cary, NC), to compare paired testing of biopsy samples for H&E with and without CD10 IHC for the detection of endometriosis. A two-tailed $P < .05$ was considered statistically significant.

## RESULTS

By the revised ASRM classification, the women had no (n = 3), minimal (n = 16), mild (n = 6), moderate (n = 4), or severe (n = 2) endometriosis. Of the 108 lesions, 38 (35%) were histologically positive for endometriosis by H&E staining and 70 (65%) were negative. Of the 70 specimens initially judged negative by H&E staining, reexamination of all samples that were CD10 positive led the pathologist to change the diagnosis of 11 specimens to endometriosis. Nine of these lesions were initially considered to be fibrotic; in

## FIGURE 1

Diagnostic results of H&E staining with and without the adjunctive use of CD10 immunohistochemistry, in 108 suspected endometriosis lesions obtained from 31 women. *At least one biopsy sample for each patient was positive. †All the biopsy samples from each patient were negative. ‡Three biopsy samples (each one from a different patient): one was confirmed as positive after CD10 and leucocyte common antigen (LCA) staining, and two negative biopsy samples were inconclusive after CD10 and LCA staining. §Two negative biopsy samples (from the same patient) were inconclusive after CD10 and LCA staining. ‖One negative biopsy sample was inconclusive after CD10 and LCA staining.

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each, CD10 staining was focal, and did not appear to stain fibroblasts. Two other lesions had the initial diagnosis of stromal tissue with hemosiderin, and chronic hemorrhage. A total of 49 (45%) lesions were considered to be positive. Thus, the adjunctive use of CD10 IHC detected more positive endometriosis lesions than H&E staining alone (45% vs. 35%, $P < .001$; Fig. 1). The stroma of all endometrial biopsy samples were found to contain CD10 (Fig. 2).

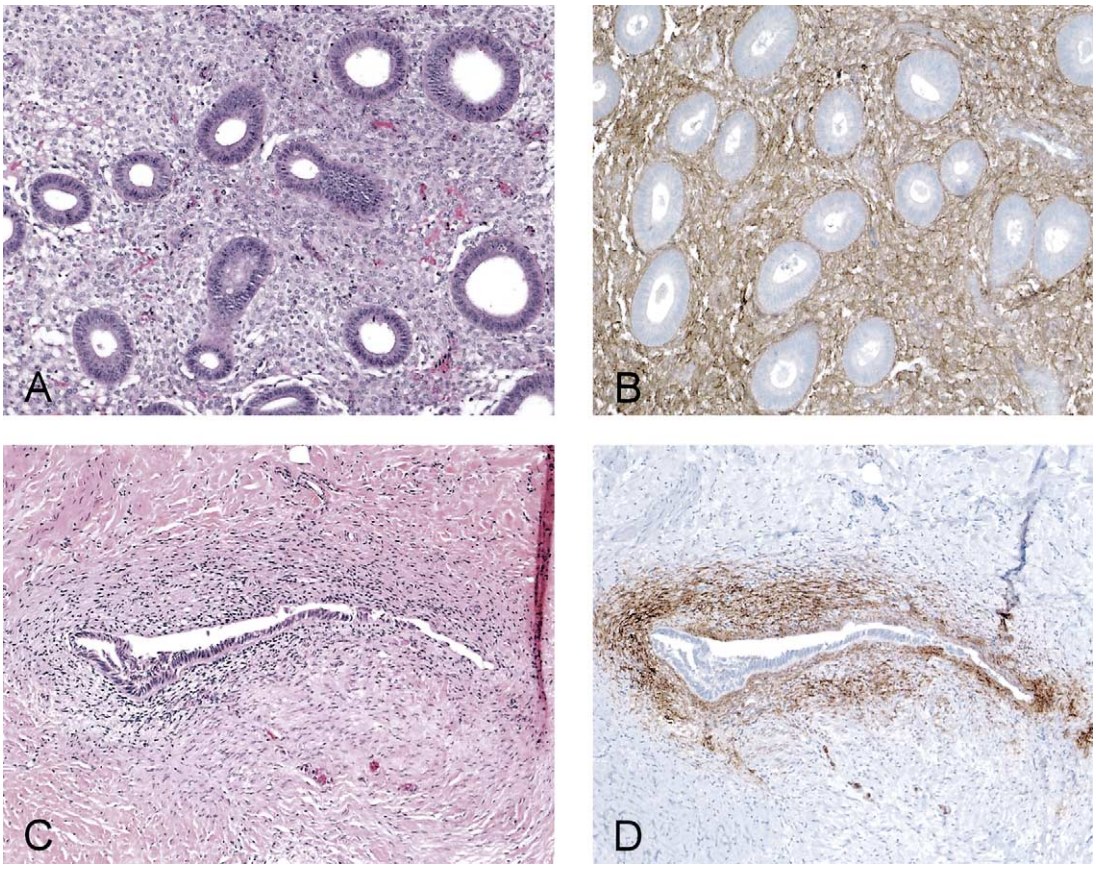
In six specimens (from five women), LCA staining was performed. In two specimens, because LCA staining was positive but did not discriminate between inflammatory cells and stroma, the negative H&E diagnosis was not changed. In three specimens, LCA IHC was performed on adjacent sections that did not show the same area as the CD10 slide, and the negative H&E diagnosis also remained unchanged.

The LCA IHC also was performed on one endometriosis specimen in which inflammatory cells were suspected within the endometriosis stroma. In that case, LCA stained a minority of the CD10-positive cells, confirming the presence of inflammatory cells within stroma (Fig. 3). Thus, LCA staining may aid in discrimination of cell types in some cases.

Four of these women had a positive histologic diagnosis of endometriosis based on another specimen(s), and one had only negative results (Fig. 1). The CD10 and H&E stainings

## FIGURE 2

Light microscopy (magnification ×10) of endometrium and endometriosis lesions. (**A**) H&E staining of normal endometrium. (**B**) On an adjacent section, CD10 immunohistochemistry stains the stroma brown and does not stain the glands. (**C**) H&E staining of a cul-de-sac biopsy considered diagnostic of endometriosis. (**D**) CD10 IHC in an adjacent section confirmed the diagnosis, showing brown staining in the stroma but not the glands.

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were concordant in the remaining 54 negative and 38 positive specimens (Table 1).

Nineteen women diagnosed with endometriosis by H&E staining alone had minimal (n = 9), mild (n = 5), moderate (n = 3), or severe (n = 2) endometriosis by revised ASRM criteria. The CD10 IHC result was positive in each biopsy sample judged to show endometriosis by H&E staining. The 12 women with a clinical diagnosis of no disease (n = 3), or minimal (n = 7), mild (n = 1), or moderate (n = 1) endometriosis by revised ASRM criteria, had no diagnosis of endometriosis by H&E results. The adjunctive use of CD10 IHC changed the histologic diagnosis in three women with minimal disease, whose H&E specimens were considered positive only in conjunction with CD10 IHC results. This represents 25% of the 12 women with initially negative results (Fig. 1). Using CD10+H&E adjunctively improved the sensitivity by 13.6%.

## TABLE 1

Results of H&E staining with and without CD10 immunohistochemistry in 108 specimens suspected to be endometriosis.

| | CD10 with H&E | | |
|---|---|---|---|
| H&E | Positive | Negative | Total |
| Positive | 38 | 0 | 38 |
| Negative | 11 | 59[a] | 70 |
| Total | 49 | 59 | 108 |

*Note:* McNemar's test *P* value = <.001.

[a] The five suggestive specimens by CD10 immunohistochemistry are included as negative.

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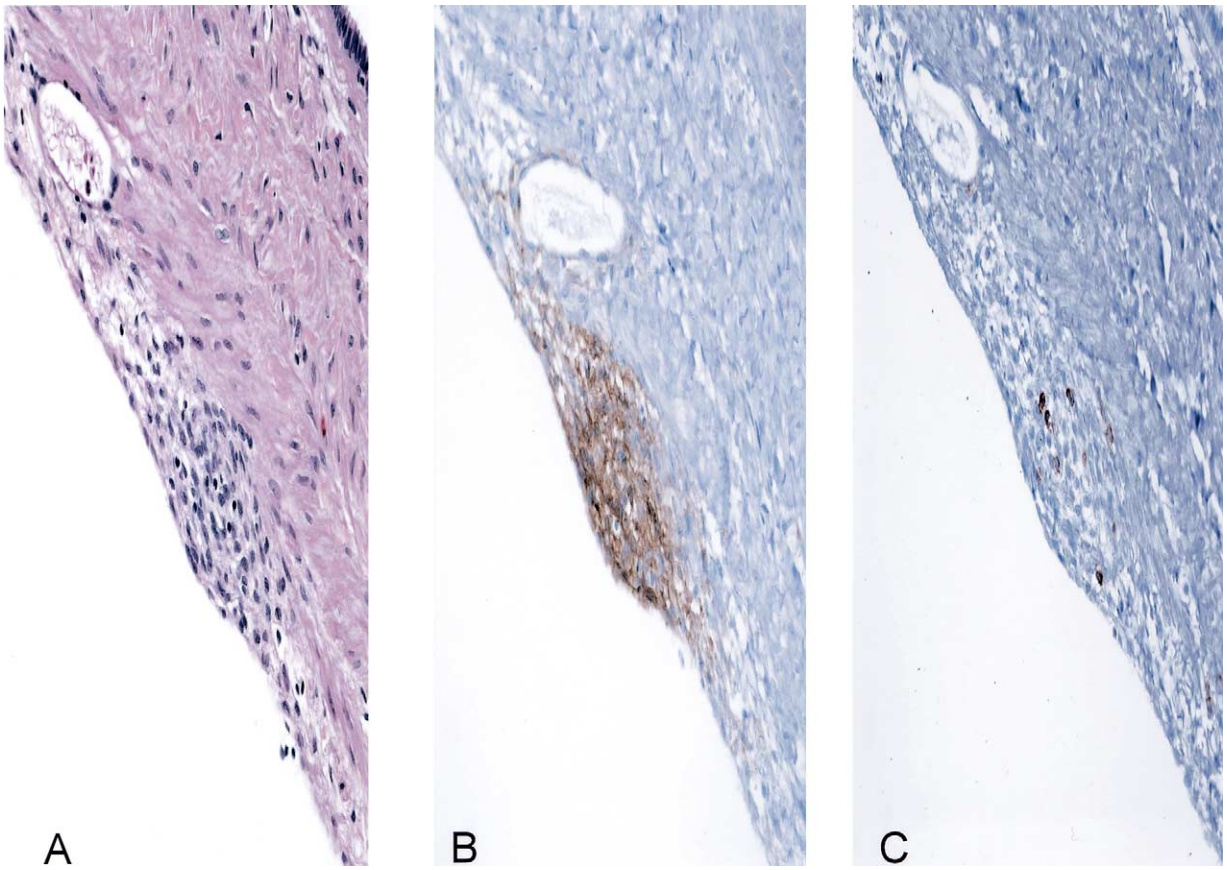
FIGURE 3

Light microscopy (magnification ×20) of a surgically identified endometriosis lesion. (**A**) H&E staining does not clearly identify endometriosis. (**B**) CD10 immunohistochemistry shows intense staining of cells that could be either stroma or inflammatory cells. (**C**) Leucocyte common antigen immunohistochemistry stains the inflammatory cells but does not stain the stroma cells, indicating an inflammatory infiltration in the endometriosis lesion.

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## DISCUSSION

This study demonstrates that the adjunctive use of CD10 IHC with H&E staining can increase the histologic detection of endometriosis. Several studies have indicated that CD10 is a sensitive marker of eutopic endometrial stroma and of endometrial stromal neoplasms (17). In a recent study of 25 biopsies, 22 were positive for endometriosis by H&E, and 22 demonstrated CD10 positivity. Only one of the three negative H&E specimens was positive by CD10 IHC (15). In another study of lesions in which endometriosis was suspected but stroma was not clearly demonstrated, 17 of 20 biopsies were considered diagnostic of endometriosis after CD10 staining (16). By contrast, of 70 negative lesions evaluated by CD10 IHC in this study, only 15% were found to have endometriosis. Taken together, these studies suggest that there is variability between pathologists in the rate of initial endometriosis diagnosis by H&E and that the addition of CD10 staining may be very useful when the diagnosis is suspected but not clear-cut.

Women falsely diagnosed with endometriosis may undergo treatments with their attendant risks and side effects, and those in whom the diagnosis is missed may not receive therapy. However, it is difficult to identify endometriosis with certainty in the absence of a diagnostic gold standard. The clinical diagnosis at surgery has important false-positive and false-negative rates; subtle, atypical, or deep lesions may be missed or lesions not confirmed by histology may be falsely classified. The operator-dependent false-negative rate (20) may improve with additional training and experience (21).

By contrast, histologic examination of suspected endometriosis has a very low false-positive rate but may erroneously consider a woman to be free of disease. Walter et al. (1) failed to confirm histologically the surgical diagnosis of

minimal endometriosis in 32% of 37 women. These may have been false-positive surgical diagnoses, or may represent a true failure of histologic detection. Some pathologists may diagnose endometriosis in specimens suggestive of endometriosis with only hemosiderin laden macrophages, endometrial glands, or endometrial stroma, whereas others may require both endometrial glands and stroma for diagnosis. Thus, another problem with the diagnosis of endometriosis is a lack of interobserver reproducibility if pathologists do not use the same diagnostic criteria.

The present study identified a small but important false-negative rate for histologic detection of endometriosis by H&E staining. The addition of CD10 IHC improved the rate of histologic detection from 35% to 45% of lesions examined, resulting in a new diagnosis of endometriosis in 3 of 12 women with negative results on H&E staining. All three had minimal endometriosis by the revised ASRM classification. We did not initially histologically confirm endometriosis in 43% (7 of 16) of women with minimal endometriosis, but CD10 decreased the rate of unconfirmed endometriosis to 25% (4 of 16), suggesting that use of CD10 IHC may be especially helpful in this setting.

There are several potential limitations to this study. It is possible that the histologic diagnosis was missed because of a sampling error in preparing the slides. We followed conventional procedures for processing tissues, and so did not section through and examine the entire specimen. Thus, if the slides used for CD10 staining contained more of a lesion, H&E staining might have been positive had that section been used for routine histopathology.

Another possible limitation is that CD10 IHC may not be specific enough for endometrial stroma, as it also identifies lymphocytes. As shown in our study, specimens with lymphoid infiltration will be positive for CD10 and might be falsely considered to indicate stroma of an endometriosis lesion. To avoid this error, one must identify adjacent glandular structures that confirm the diagnosis of endometriosis or identify the CD10-positive structures as lymphocytes. The LCA IHC can differentiate between stroma and inflammatory cells.

It is also possible that CD10 IHC will stain other nonendometriosis lesions, such as adenomyosis or mesenchymal tumors (11, 22). In general, these are not confused with endometriosis on H&E staining. Although dermal tissues of mesenchymal origin stain with CD10, we are not aware of reports demonstrating that fibroblasts are CD10 positive. Our data support this concept, as we did not find diffusely positive CD10 staining in fibrotic lesions.

Our study shows that the adjunctive use of CD10 IHC with H&E staining improves diagnostic sensitivity for endometriosis compared with H&E alone. Because CD10 IHC confirmed all positive diagnoses of endometriosis by H&E staining, we suggest that it be used only when H&E is negative in all specimens from a given woman so as to minimize expense. It may improve diagnostic accuracy for those with minimal endometriosis, which is essential for determining proper treatment.

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