Immunohistochemical Staining for Cyclin D1 and Ki-67 Aids in the Stratification of Atypical Ductal Hyperplasia Diagnosed on Breast Core Biopsy

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Abstract

A diagnosis of atypical ductal hyperplasia (ADH) after breast core biopsy usually is followed by an excisional biopsy to exclude the presence of a more significant lesion. To determine whether the immunohistochemical expression of cyclin D1 (CyD1) and Ki-67 can aid in case stratification for the likelihood of finding ductal carcinoma in situ (DCIS) on subsequent excision, we immunohistochemically stained 21 consecutive ADH cases diagnosed by core biopsy, and proliferation indices (PIs) were calculated for each case. Fluorescence in situ hybridization to detect CCND1 amplification was performed in 10 cases. In 5 cases, DCIS (with or without invasive carcinoma) was identified in the subsequent excision. The mean \( P_{\text{CyD1}} \) and \( P_{\text{Ki-67}} \) for these cases were significantly higher than in the remainder (\( P = .03 \) and \( P = .05 \), respectively). The sensitivities of \( P_{\text{CyD1}} \) and \( P_{\text{Ki-67}} \) for the presence of DCIS on subsequent excision were 100%, and the specificities were 75% and 69%, respectively. The specificity of the 2 markers combined was 88%. The number of cells with CCND1 amplification was higher in cases with DCIS or ADH on subsequent excision. Immunostaining for CyD1 and Ki-67 might help stratify cases of ADH on core biopsy and identify patients unlikely to have DCIS found on excision.

Image-guided core needle biopsy of the breast is very accurate in the diagnosis of benign breast disease and invasive carcinoma (IC).1-6 However, a significant number of cases diagnosed as atypical ductal hyperplasia (ADH) after core biopsy show ductal carcinoma in situ (DCIS) or IC on subsequent excision.1,5,7-18 This has led to the recommendation that a diagnosis of ADH by core biopsy should be followed by surgical excision. Better categorization of ADH diagnosed by such biopsies would be useful to identify cases more likely to show DCIS on excision and, ideally, to obviate the need for subsequent surgery for the remaining cases.

Overexpression of the cell cycle–associated protein cyclin D1 (CyD1) messenger RNA has been identified more frequently in cases of DCIS than in nonmalignant cases,19 and amplification of its encoding gene, \( \text{CCND1} \), has been identified in DCIS20,21 at a frequency higher than in ADH.21 By using different percentage cutoffs for CyD1 immunohistochemical positivity, most authors have found that it is expressed in DCIS and ADH,20-26 which might indicate that immunohistochemical analysis for CyD1 is not useful for distinguishing between the two.27 The percentage of positive cells however, was higher in DCIS than ADH in the studies specifically addressing this issue,23,25,26 suggesting that the proportion of CyD1+ cells in the lesions in question might, in fact, aid in the distinction between DCIS and ADH. The proliferation marker Ki-67 has also been found to be expressed in a higher percentage of cells in DCIS lesions compared with ADH,26,28,29 suggesting that it is another potential marker that could be used as an aid in distinguishing the two.

The expression of CyD1 and Ki-67 has not been studied in breast core needle biopsy specimens. We undertook this study to determine whether immunohistochemical analysis for...
these 2 proteins in breast needle core biopsy specimens might be useful to better stratify cases of ADH by predicting cases more likely to be associated with DCIS and/or IC on subsequent excisional biopsies. We also looked at whether CCND1 amplification, as determined by fluorescence in situ hybridization (FISH), can be detected in such cases and whether it correlates with immunohistochemical expression of CyD1.

Materials and Methods

Selection and Review of Cases

The study was approved by the St Luke’s-Roosevelt Hospital Center Institutional Review Board (New York, NY). A search of computerized files from the surgical pathology department of St Luke’s-Roosevelt Hospital Center during a 3-year period (January 1998-December 2001) revealed 1,037 breast Mammotome (Ethicon Endo-Surgery, Cincinnati, OH) biopsies, 68 (6.56%) of which were diagnosed as having ADH as the most significant lesion. Of these, there were 21 cases in which the diagnosis of ADH was confirmed by 2 pathologists (O.H. and H.M.) using published criteria30-32 and in which the diagnosis of ADH was considered positive for Ki-67, whereas only strong nuclear staining was considered positive for Ki-67.

One investigator (O.H.), blinded to subsequent follow-up pathology, calculated the proliferation index (PI) for each immunostain (PICyD1 and PIKi-67) by manually measuring the percentage of positive nuclei in at least 500 cells in the same duct space(s) involved by ADH (by direct comparison with the H&E-stained sections). The PICyD1 and PIKi-67 of the cases of ADH with a subsequent excisional biopsy that showed DCIS (with or without IC) then were compared with the remainder that did not have such a finding. Cutoff points were set to calculate the sensitivity and specificity of these indices for predicting the diagnosis in the subsequent excisional material.

FISH for CCND1 Amplification

A commercially available dual-color probe (Vysis, Downers Grove, IL) was used to determine the presence or absence of cells with CCND1 amplification in a subset of cases. The purchased probe was validated by mapping it to metaphase spreads. We cut 4-µm-thick sections from 10 needle core biopsies (using the same blocks selected for immunohistochemical analysis); the sections were placed on positively charged slides, deparaffinized, and pretreated according to the manufacturer pretreatment kit protocol. The slides and probes were denaturated at 75°C for 5 minutes, hybridized overnight at 37°C, washed according to the product insert, and then counterstained with DAPI II and coverslipped. By using the latter stain, the duct space(s) involved by ADH were identified (by comparison with the H&E- and the immunohistochemically stained sections).

At least 300 cells in these duct space(s) were scored by counting the ratio of orange signals (11q13, CyD1 region) to green signals (CEP11, 11p11.11-q11 centromere control). Cells that were unclear or too overlapped, poorly fixed, or otherwise difficult to discern were not scored. An orange/green signal ratio of 2 or more was considered to indicate CCND1 amplification. The amplification rate was calculated as the percentage of cells that showed gene amplification. The FISH studies were performed in the Department of Pathology, Washington University Medical Center, St Louis, MO, and the results were correlated with CyD1 immunohistochemical analysis and findings of subsequent excisional biopsies.

Statistical Tests

The Student t test was used to compare continuous variables and the χ² test to compare categorical variables. The
Pearson correlation coefficient was used to correlate between continuous variables. A $P$ value of .05 was considered statistically significant. Statistical analysis was performed using SPSS 11.0 for Windows software (SPSS, Chicago, IL).

**Results**

The clinical, radiologic, and pathologic features of the 21 cases of ADH diagnosed on needle core biopsy and their follow-up diagnoses on excision are given in Table 1. The women were 41 to 73 years old (mean, 60.7 years). Of the cases, 9 originated in the right breast and 12 in the left breast. All 21 biopsies were performed for mammographically detected microcalcifications using a stereotactic 11-gauge vacuum-assisted Mammotome.

Of the 21 patients, 5 had lesions categorized as BI-RADS category 3 (probably benign), 11 had lesions categorized as BI-RADS category 4 (suspicious), and 5 had lesions of an unknown BI-RADS category. A mean of 9 core samples (range, 5-16) was obtained from each lesion. Histopathologic patterns of ADH recognized on needle biopsy included a predominantly cribriform pattern (9 cases), micropapillary (5 cases), columnar cell change with atypia/columnar cell hyperplasia with atypia (3 cases), or a mixed pattern (3 cases). Follow-up mammography showed residual microcalcifications after biopsy in 4 cases.

There were 5 cases of ADH (24%) that had DCIS (Image 11) (including 2 cases with associated IC) on subsequent excision, 2 cases of lobular carcinoma in situ, 7 cases of ADH (Image 21 and Image 31), and 7 cases with only usual ductal hyperplasia (UH) (Image 41) in subsequent excisional biopsy material. None of the needle core biopsies with microcalcifications diagnosed as BI-RADS category 3 showed DCIS on excision. There were no statistically significant differences in the architectural pattern of ADH or the number of duct spaces involved between cases with a subsequent diagnosis of DCIS and those without such a diagnosis on excision ($P = .356$ and $P = .553$, respectively). Four cases of ADH had residual microcalcifications after needle biopsy, 1 with a subsequent diagnosis of DCIS on excision. The presence or absence of residual microcalcifications was not significantly different between cases with a subsequent diagnosis of DCIS and those without ($P = .397$).

All of the needle biopsy specimens were positive with antibodies against CyD1 and Ki-67. The mean $PI_{CyD1}$ in the cases that had DCIS on subsequent excision was 45.6% (range, 32%-72%) compared with 23.1% (range, 9%-75%) in the cases without such pathology on reexision ($P = .03$); corresponding $PI_{Ki-67}$ values were 3.4% (range, 2.2%-5.7%) and 1.7% (range, 0.2%-3.5%; $P = .05$). All cases with subsequent DCIS on reexision had $PI_{CyD1}$ and $PI_{Ki-67}$ of 32% or more and 2.2% or more, respectively. At a $PI_{CyD1}$ cutoff of 25%, the sensitivity for the presence of DCIS on subsequent excision was 100% and the specificity was 75%. With a cutoff of 2%, the

**Table 1**

Clinical, Radiologic, and Pathologic Features of 21 Cases of ADH Diagnosed on Needle Core Biopsy and Their Follow-up Diagnoses on Excision

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Lesion Location</th>
<th>BI-RADS Category</th>
<th>Architectural Pattern</th>
<th>No. of Involved Duct Spaces</th>
<th>$PI_{CyD1}$ (%)</th>
<th>$CCND1$ Amplification Rate (%)</th>
<th>$PI_{Ki-67}$ (%)</th>
<th>Follow-up Diagnosis</th>
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<tbody>
<tr>
<td>1</td>
<td>60/L, 12:00</td>
<td>4</td>
<td>Columnar</td>
<td>2</td>
<td>370</td>
<td>2.7</td>
<td>2.2</td>
<td>DCIS</td>
</tr>
<tr>
<td>2</td>
<td>58/L</td>
<td>4</td>
<td>Cribriform</td>
<td>3</td>
<td>72.0</td>
<td>6.7</td>
<td>5.7</td>
<td>DCIS</td>
</tr>
<tr>
<td>3</td>
<td>72/R</td>
<td>Unknown</td>
<td>Columnar</td>
<td>1</td>
<td>32.0</td>
<td>ND</td>
<td>2.8</td>
<td>DCIS</td>
</tr>
<tr>
<td>4</td>
<td>56/R</td>
<td>4</td>
<td>Mixed</td>
<td>2</td>
<td>34.0</td>
<td>ND</td>
<td>3.8</td>
<td>DCIS, IDC, LCIS</td>
</tr>
<tr>
<td>5</td>
<td>73/L, superior</td>
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<td>Cribriform</td>
<td>2</td>
<td>53.0</td>
<td>ND</td>
<td>2.4</td>
<td>DCIS, ILC</td>
</tr>
<tr>
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<td>Cribriform</td>
<td>2</td>
<td>10.0</td>
<td>ND</td>
<td>0.2</td>
<td>LCIS</td>
</tr>
<tr>
<td>7</td>
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<td>Unknown</td>
<td>Columnar</td>
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<td>20.0</td>
<td>ND</td>
<td>0.6</td>
<td>LCIS</td>
</tr>
<tr>
<td>8</td>
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<td>3</td>
<td>Micropapillary</td>
<td>2</td>
<td>29.0</td>
<td>ND</td>
<td>1.2</td>
<td>ADH</td>
</tr>
<tr>
<td>9</td>
<td>62/R</td>
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<td>2</td>
<td>75.0</td>
<td>ND</td>
<td>3.5</td>
<td>ADH</td>
</tr>
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<td>ND</td>
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<td>ADH</td>
</tr>
<tr>
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<td>0.7</td>
<td>ADH</td>
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</tr>
<tr>
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<td>ADH</td>
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<td>1.9</td>
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<tr>
<td>16</td>
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<td>Cribriform</td>
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<td>0.0</td>
<td>2.7</td>
<td>UH</td>
</tr>
<tr>
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<td>11.0</td>
<td>ND</td>
<td>0.8</td>
<td>UH</td>
</tr>
<tr>
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<td>Cribriform</td>
<td>2</td>
<td>13.0</td>
<td>ND</td>
<td>2.0</td>
<td>UH</td>
</tr>
<tr>
<td>19</td>
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<td>0.3</td>
<td>0.9</td>
<td>UH</td>
</tr>
<tr>
<td>20</td>
<td>63/R, LOQ</td>
<td>4</td>
<td>Micropapillary</td>
<td>1</td>
<td>13.0</td>
<td>0.0</td>
<td>0.9</td>
<td>UH</td>
</tr>
<tr>
<td>21</td>
<td>59/L, UOQ</td>
<td>4</td>
<td>Mixed</td>
<td>1</td>
<td>17.0</td>
<td>0.3</td>
<td>1.7</td>
<td>UH</td>
</tr>
</tbody>
</table>

ADH, atypical ductal hyperplasia; BI-RADS, Breast Imaging Reporting and Data System (see text for explanation of categories); CyD1, cyclin D1; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; LCIS, lobular carcinoma in situ; LOQ, lower outer quadrant; ND, not done; PI, proliferation index; UH, usual ductal hyperplasia; UOQ, upper outer quadrant.
Image II (Case 2) Atypical ductal hyperplasia on core biopsy (A, H&E, ×200; B, H&E, ×400) with a cyclin D1 (CyD1) proliferation index (PI)_{CyD1} of 72 (C, CyD1 immunostain, ×400) and a PI_{Ki-67} of 5.7 (D, Ki-67 immunostain, ×400), for which the excisional biopsy showed ductal carcinoma in situ of high nuclear grade (E, H&E, ×200; F, H&E, ×400).
Image 2 (Case 8) Atypical ductal hyperplasia on core biopsy (A, H&E, ×200; B, H&E, ×400) with a cyclin D1 (CyD1) proliferation index (PI)\_CyD1 of 29 (C, CyD1 immunostain, ×400) and a PI\_Ki-67 of 1.2 (D, Ki-67 immunostain, ×400), for which the excisional biopsy also showed atypical ductal hyperplasia (E, H&E, ×200; F, H&E, ×400).
corresponding sensitivity and specificity for PI_{Ki-67} were 100% and 69%, respectively. Using the indices in combination increased the specificity to 88% Figure 1 and Table 2.

From 1 to 20 CCND1-amplified cells Image 5 per duct space were identified in 6 of 10 needle core biopsy specimens analyzed, with no amplification detected in the remaining 4 cases (Table 1). There was a strong positive correlation between the percentage of amplified cells in each case and the PI_{CyD1} (correlation coefficient, 0.9; \( P = .0001 \)). Of the 5 needle core biopsy specimens with findings of DCIS or ADH on subsequent excision, 4 showed CCND1 amplification in 2.7% to 6.7% of cells, whereas only 1 amplified cell (0.3%) was seen in each of the 2 (of 5) biopsy specimens with UH found on reexcision.

**Discussion**

Our data indicate that CyD1 and Ki-67 immunostaining of ADH in needle core biopsy specimens can help stratify cases as to the likelihood of showing DCIS in subsequent excision. Although the study is small, the findings suggest that patients with a diagnosis of ADH based on needle core biopsy with a low percentage of cells staining for either of these proteins in foci of ADH are unlikely to have DCIS on excision and might be spared unnecessary surgery.

A diagnosis of ADH based on image-guided needle core biopsy of the breast usually leads to surgical excision of the mammographic abnormality. This is because numerous studies\(^1\)\(^-\)\(^18\) have shown that subsequent excision
reveals DCIS and/or IC in a high percentage of cases diagnosed as ADH.

One of the main reasons for underdiagnosis of carcinoma is related to sampling, an inherent problem of the core biopsy technique. Because of the relatively small sample, there is always a possibility of some “underestimation” of the lesion compared with the final diagnosis by excision. Such underestimation might be due to incomplete removal of microcalcifications, the heterogeneity of DCIS, or both. This is well illustrated by the lower discordance rates associated with the use of larger caliber needles; these range from 33% to 87% for automated, 14-gauge, spring-loaded biopsy guns and 10% to 38% for the larger 11-gauge, vacuum-assisted devices such as the Mammotome.

Better categorization of ADH diagnosed based on core needle biopsy would be useful to improve stratification of cases for the likelihood of finding DCIS on excision and might obviate the need for subsequent surgery. Previous studies addressing this issue showed that quantifying the degree of atypia or the extent of ADH in core biopsy samples may be of use in predicting the presence of DCIS in excision specimens.

Another factor possibly related to underdiagnosis of malignancy in patients with a core biopsy diagnosis of ADH is the often problematic distinction between ADH and low-grade DCIS. Although the histologic features of ADH are well characterized, they overlap with those of low-grade DCIS, often leading to significant interobserver variability in
The diagnosis of proliferative breast lesions.\textsuperscript{39-42} To overcome some of the limitations of morphologic examination, various attempts have been made to identify biologic or genetic markers that might be useful adjuncts to histopathologic examination in distinguishing between ADH and DCIS. Unfortunately, immunohistochemical expression of HER2/neu, p53, and bcl-2 proteins, as well as high-molecular-weight cytokeratins, seems not to be useful in this regard.\textsuperscript{27}

CyD1, a cell cycle regulatory protein involved in cellular growth, cellular differentiation, and organ development, is overexpressed in 30\% to 50\% of primary breast carcinomas.\textsuperscript{43} The findings of CyD1 messenger RNA overexpression in 76\% of cases of low-grade DCIS, compared with only 18\% of cases of ADH,\textsuperscript{19} and the immunohistochemical expression of CyD1 in 65\% of cases of low-grade DCIS, compared with 0\% of ADH,\textsuperscript{22} suggest that CyD1 is a potential marker that might be useful in distinguishing ADH from DCIS. Other authors,\textsuperscript{20,21,23-26} however, found a greater degree of overlap in the immunohistochemical expression of CyD1, with 36\% to 57\% of cases of low-grade DCIS and 8\% to 57\% of cases of ADH showing such expression, which might imply that CyD1 immunohistochemical analysis might be unable to discriminate between the two. A closer analysis of the published data shows that the percentage of CyD1\textsuperscript{+} cells in cases of low-grade DCIS is higher than that in cases of ADH in the 3 studies specifically addressing this issue.\textsuperscript{23,25,26} Considering the different methods and antibodies used in these studies, as well as the inevitable interobserver variability in the diagnosis of DCIS and ADH, the fact that such a trend remains evident would suggest that CyD1 expression indeed might prove helpful in the distinction between low-grade DCIS and ADH.

Ki-67 (MIB-1) is a nonspecific proliferation marker that frequently is used as an adjunct to histopathologic examination at various sites. Numerous studies have focused on its expression in breast cancer and its impact on prognosis,\textsuperscript{44} as well as its expression in DCIS.\textsuperscript{45} There are only a few studies in the English literature, however, that describe its expression in both low-grade DCIS and ADH.\textsuperscript{26,28,29} These all showed

\bold{Figure 1} Scatter chart showing the Ki-67 and cyclin D1 proliferation indices (PI\textsubscript{Ki-67} and PI\textsubscript{CyD1}) of cases of atypical ductal hyperplasia (ADH) on core biopsy stratified based on follow-up diagnosis. The vertical and horizontal lines represent PI\textsubscript{CyD1} and PI\textsubscript{Ki-67} cutoffs of 25\% and 2\%, respectively. DCIS, ductal carcinoma in situ; LCIS, lobular carcinoma in situ; UH, usual ductal hyperplasia.

\bold{Table 3} Sensitivity, Specificity, and Negative and Positive Predictive Values of PI\textsubscript{CyD1}, PI\textsubscript{Ki-67}, and Both in Cases of ADH Diagnosed on Needle Core Biopsy for the Presence of DCIS in Follow-up Excision Specimens\textsuperscript{*}

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Negative Predictive Value (%)</th>
<th>Positive Predictive Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI\textsubscript{CyD1}</td>
<td>100 (46-100)</td>
<td>75 (47-92)</td>
<td>100 (70-100)</td>
<td>56 (22-85)</td>
</tr>
<tr>
<td>PI\textsubscript{Ki-67}</td>
<td>100 (46-100)</td>
<td>69 (41-88)</td>
<td>100 (68-100)</td>
<td>50 (20-80)</td>
</tr>
<tr>
<td>PI\textsubscript{CyD1} and PI\textsubscript{Ki-67}</td>
<td>100 (46-100)</td>
<td>88 (60-98)</td>
<td>100 (73-100)</td>
<td>71 (30-95)</td>
</tr>
</tbody>
</table>

ADH, atypical ductal hyperplasia; CyD1, cyclin D1; DCIS, ductal carcinoma in situ; PI, proliferation index.

\textsuperscript{*} The 95\% confidence intervals are given in parentheses.
that higher expression is seen in cases of low-grade DCIS than ADH, with mean proliferation indices of 9.6%26 and 6.4%28 for low-grade DCIS and 4.2%26 and 3.8%28 for ADH, indicating that Ki-67 immunostaining also might be a useful adjunct for distinguishing between low-grade DCIS and ADH.

In the present study, we assessed the immunohistochemical expression of CyD1 and Ki-67 in 21 cases of ADH in needle core biopsy material in which follow-up excisional material was available for review. These cases were selected from 68 consecutive cases of ADH, representing 6.56% of all breast needle biopsies. Despite the fact that we examined only 3 sections of each core needle biopsy specimen, a practice associated with a lower ADH detection rate,46 this figure is consistent with the incidence of ADH described in the literature, as was the 24% incidence of DCIS we found on follow-up surgical excision. These incidences suggest that the 21 cases of ADH we studied, although few, probably represent a good sample of all cases of ADH and can be used to calculate predictive values.

An important finding in this study was that in most cases, the microcalcifications were removed entirely during the biopsy procedure, which otherwise might be a confounding factor, the argument being that the DCIS detected on subsequent excision is due to incomplete sampling, as discussed earlier. The fact that the presence or absence of residual microcalcifications was not significantly different between cases with a subsequent diagnosis of DCIS and those without argues against such a possibility.

An unexpected finding was that only 1 of 5 cases that had DCIS on surgical excision originally had 3 or more duct spaces involved by ADH in the needle core specimen (Table 1). This contrasts with the findings of 2 studies in which 15 of 1526 and 3 of 3 cases37 with DCIS on surgical excision had 3 or more duct spaces involved by ADH in the needle core specimen. Although this difference is not statistically significant and can be explained by chance alone, it indicates that limited ADH in a needle biopsy specimen still might be associated with DCIS on excision.

The anti-CyD1 antibody stained a higher proportion of cases of ADH (all cases) and a larger proportion of cells compared with what has been described in the literature (Table 3). We believe that our prolonged (30 minutes) steam-induced method of antigen retrieval is the primary reason for this difference because it has been our experience that this method of heat-induced antigen retrieval produces much better staining results for CyD1, immunohistochemical demonstration of which is notoriously difficult.47 This seems to be less of a problem with the recently available rabbit monoclonal antibody, at least for the demonstration of CyD1 staining in the diagnosis of mantle cell lymphoma.47

Although the PIcyD1 of cases of ADH with a subsequent diagnosis of DCIS overlapped with cases without such a diagnosis, we were nevertheless able to set a PicyD1 cutoff that was 7% lower than the lowest index seen in cases with a subsequent diagnosis of DCIS to include all such cases (100% sensitivity) and only 4 cases without such a diagnosis (75% specificity). This contrasts with the 24% specificity obtained by relying on a light microscopic diagnosis of ADH alone. Using Pik67 in a similar manner resulted in a specificity of 69% for the diagnosis of DCIS on excision. Although this was less specific than PicyD1, the difference may be offset by the fact that immunohistochemical analysis for Ki-67 is more reliable and reproducible than that for CyD1. As often is the case when using diagnostic tests in combination, we found that using both PicyD1 and Pik67 together produced the highest specificity (88%). Although the positive predictive values of a PicyD1 more than 25% and/or a Pik67 more than 2% were too low (50%-71%) to be practically useful for management decisions, a negative predictive value of 100% associated with a PicyD1 less than 25% and/or a Pik67 less than 2% suggest that cases of ADH with low indices are unlikely to show DCIS on excision and patients might be spared additional surgery. The

**Table 3**

<p>| Cases of Low-Grade DCIS and ADH in the Literature Immunohistochemically Stained With Anti-CyD1 Antibodies |
|--------------------------------------------------|--------------------------------------------------|------------------------------|-------------------------------|--------------------------------------------------|--------------------------------------------------|------------------------------|</p>
<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of Cases</th>
<th>Positive Cells (%)</th>
<th>Positive Cells (%)</th>
<th>No. of Cases</th>
<th>Positive Cells (%)</th>
<th>Positive Cells (%)</th>
<th>Antibody (Clone; Dilution; HIAR Method)</th>
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<td>&gt;5</td>
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<td>DCS-6; 1:10; microwave</td>
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<td>8</td>
<td>NCL; 1:40; microwave</td>
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ADH, atypical ductal hyperplasia; CyD1, cyclin D1; DCIS, ductal carcinoma in situ; HIAR, heat-induced antigen retrieval; NA, not available.

* Mean quartile score for number of CyD1+ cells was higher in cases of DCIS.
situation in which such additional testing would seem most helpful is cases of ADH that lack conventional histopathologic features that have been found to be more likely associated with DCIS on excision, namely significant atypia and/or ADH involving 3 or more duct spaces.36,37

The strong correlation we found between \( \text{PI}_{\text{CyD1}} \) and amplification of \( \text{CCND1} \) is similar to the results of Simpson et al.20 Also similar to the findings of Simpson et al,9,10 of other authors21 is that many more cells show immunohistochemical expression of CyD1 than those that display amplification of its gene. This probably indicates that there are mechanisms, other than amplification, that would explain CyD1 overexpression.20,21 An interesting finding was that amplification rarely was identified in ADH cases with a subsequent diagnosis of UH, and higher levels of amplification were limited to cases with a subsequent diagnosis of ADH or DCIS.

There are several weaknesses in this study that need to be addressed. We have already alluded to the problems associated with CyD1 immunohistochemical analysis that might be resolved by using the newly available rabbit monoclonal antibody. This would probably entail a comparison between the \( \text{PI}_{\text{CyD1}} \) obtained by using the 2 antibodies. The fact that the method of antigen retrieval we used was different from that described previously also should be considered before attempting to reproduce these findings. One must also consider that there was a wide range of \( \text{PI}_{\text{CyD1}} \) and \( \text{PI}_{\text{Ki-67}} \) among the different cases, and although we were able to set discriminatory cut-off values for these indices, the thresholds set by these values were quite tight. We were limited by the number of cases in which follow-up excisional material was available for review; inclusion of more ADH cases would have added more strength to the study. In addition, long-term clinical follow-up might have been further instructive, as it would be interesting to know whether there is a difference in the subsequent development of DCIS between cases of ADH with low vs high PIs that were negative on the initial excision. Finally, although frequently performed in other settings and not a weakness of the study per se, manual counting of PIs might be too cumbersome to be practically performed in every case. This could easily be overcome by using an image analyzer if deemed necessary.

This study suggests that immunohistochemical staining of cases of ADH in needle core biopsy specimens with antibodies directed against CyD1 and Ki-67 can be used as an adjunct to histopathologic examination for predicting the likelihood of finding DCIS in subsequent excisional biopsy material. Despite its limitations, the study specifically suggests that cases with low \( \text{PI}_{\text{CyD1}} \) and/or \( \text{PI}_{\text{Ki-67}} \) are unlikely to have DCIS in subsequent excision specimens, and patients might be spared additional surgery, provided that there are no residual microcalcifications and that other histologic features known to be associated with DCIS are lacking. Additional larger studies with newer antibodies or other markers might further refine our ability to distinguish between ADH and DCIS and help predict the findings of excisional breast biopsies based on findings in needle biopsy specimens.


