Cyclin A Immunocytology as a Risk Stratification Tool for Barrett’s Esophagus Surveillance
Pierre Lao-Sirieix,1 Laurence Lovat,2 and Rebecca C. Fitzgerald1

Abstract

Purpose: Endoscopic surveillance of Barrett’s esophagus (BE) by histopathologic biopsy assessment is suboptimal. A proliferation marker, minichromosome maintenance protein 2, has potential as a biomarker but lacks specificity. We hypothesized that cyclin A, which detects a proportion of proliferating cells, would be more specific. Because cytologic sampling has clinical advantages, we also evaluated the efficacy of cyclin A in endoscopic brushing samples.

Experimental Design: A cross-sectional cyclin A immunostaining study was done in 77 patients attending for BE surveillance and 17 patients undergoing evaluation of esophageal adenocarcinoma. The control tissues were as follows: 30 squamous esophagus, 20 gastric antrum, and 13 duodenum. A nested case-control study was done within the same surveillance cohort (16 progressors compared with 32 matched controls) to determine the relative risk for progression. Immunocytology was done for endoscopic brushings collected prospectively from 75 BE dysplasia and 33 esophageal adenocarcinomas.

Results: Surface expression of cyclin A in BE samples correlated with the degree of dysplasia (P = 0.016). In the case-control cohort, patients with biopsies expressing cyclin A at the surface were more likely to progress to adenocarcinoma than those who did not (odds ratio, 7.5; 95% confidence interval, 1.8-30.7). The sensitivity and specificity of cyclin A expression in brushings for the detection of high-grade dysplasia and cancer patients were 97.8% and 58.7%, respectively. The associated negative predictive value was 97.4%.

Conclusions: Cyclin A immunopositivity correlates with cancer risk. Application of this marker to endoscopic brushings could be used as a first step to identify BE patients with the highest risk of progression.

The incidence of esophageal adenocarcinoma has risen 6-fold in the Western world over the last 30 years (1). Most patients with adenocarcinoma are diagnosed when their disease is already locally advanced (at least T3N1), and, as a result, the 5-year survival is only 5% to 13% (2–4). The existence of a preinvasive stage called Barrett’s esophagus (BE) has prompted the widespread implementation of endoscopic surveillance programs (5–7). The aim of surveillance is to identify BE patients with high-grade dysplasia and early carcinoma (T2N0 or less) who are still at a curable stage (8, 9).

Surveillance is subject to controversy because of the low conversion rate from BE to adenocarcinoma (0.5-2% per year; refs. 10, 11) and because the current methodology is cumbersome, costly, and not without risks (12). Clinical decision-making hinges on the histopathologic assessment of multiple biopsies. However, it is prone to sampling bias because the BE segment is heterogeneous and composed of different clones with variable malignant potential (13, 14). Furthermore, there is a high degree of intraobserver and interobserver variability in the grading of dysplasia even among expert upper-gastrointestinal pathologists (15–17) and it is not always possible to accurately predict the likelihood of cancer progression in patients with a diagnosis of low-grade or high-grade dysplasia (18–23).

The benefits of surveillance could be substantially improved if a methodology could be devised that is simpler, quicker to do, with reduced clinical risks and less subjectivity compared with the current methods. We hypothesized that brush cytology specimens in association with a highly specific and sensitive immunomarker would satisfy these requirements. A cytology brush can be deployed via the working channel of the endoscope and a large area of the BE segment can be assayed in a single sample. The small bleeding and perforation risks associated with multiple biopsies are negated and the time and subjectivity of the analysis could be substantially reduced if a binary scoring system, allowing for automation, could be used for specimen evaluation.

The National Cancer Institute Early Detection Research Network suggested that all studies of biomarkers should be carried out using five phases of evaluation (24). All the
candidate biomarkers for BE carcinogenesis that have progressed beyond phase 2 studies [i.e., studies assessing the values of a biomarker to predict the risk of progression to cancer: increased cyclin D1 expression (25); increased S-phase fraction (26): 17p and 9p loss of heterozygosity, tetraploidy, and aneuploidy (27); and increased surface expression of minichromosome maintenance protein 2 (Mcm2: ref. 28)] can be linked with cell proliferation. We have previously shown that Mcm2 could predict patients with an increased risk of progression to adenocarcinoma [odds ratios (OR), 136; 95% confidence interval (95% CI), 7.5-2464; P < 0.0001; ref. 28]. However, the large confidence interval and the low specificity (35%) for the detection of high-grade dysplasia and adenocarcinoma suggest that this marker is not reliable enough (28). We have previously analyzed a number of cell cycle markers in BE samples (29). Based on a qualitative assessment of the antibody staining, we hypothesized that surface expression of cyclin A (expressed in the S and G2 phases of the cell cycle) would be the most sensitive and specific marker of progression to adenocarcinoma. Cyclin A is expressed in the same epithelial compartments as Mcm2 and therefore should detect dysplastic cells at the epithelial surface. However, only a proportion of Mcm2-positive cells (30-40%) should theoretically express cyclin A (29).

The specific aims of this study were as follows: (a) to determine the degree of correlation between surface expression of cyclin A and the degree of dysplasia (Early Detection Research Network phase 1); (b) in the same population, to determine whether the levels of surface expression of cyclin A were predictive of the future risk of progression to high-grade dysplasia or adenocarcinoma in a nested case-control study (Early Detection Research Network phase 3); and (c) to determine whether immunocytologic assessment of cyclin A expression compared favorably with immunohistochemical data (Early Detection Research Network phase 2 for development of a clinically robust assay).

Materials and Methods

Patient and tissue collection

Approval was obtained from the Local Research Ethics Committees for this study. Patients attending for BE surveillance at Addenbrooke's Hospital, United Kingdom, between 2001 and 2006 were invited to take part in the research. Addenbrooke's Hospital is a large district general hospital serving a population of 1.6 million and 410 patients have undergone regular surveillance over this period. Eligibility criteria for this study were an endoscopically visible columnar-lined segment and a histopathologic diagnosis of intestinal metaplasia with goblet cells in patients >18 years of age who were able to provide fully informed consent. In addition, patients with an endoscopically normal esophagus attending endoscopy for a routine diagnostic procedure (e.g., assessment of anemia or diagnosis of coeliac disease) were recruited as controls for normal squamous esophagus, gastric mucosa, and duodenum.

All patients had diagnostic biopsies taken 2 cm above the gastroesophageal junction. In addition, patients with BE had surveillance biopsies taken (each quadrant every 2 cm; refs. 5, 6). The histopathologic diagnosis of dysplasia was made by two independent expert gastrointestinal pathologists using the Vienna classification (30) according to the standard clinical practice.

Methodologic approach

To adhere to the suggested five-phase approach designed by the Early Detection Research Network, this study was divided in three arms:

- A cross-sectional study (phase 1) to determine the usefulness of cyclin A as a biomarker by studying its expression in different stages of disease progression.
- A nested case-control study (phase 3) to evaluate the capacity of a biomarker to predict disease progression and to define criteria for a positive screening test.
- A brushing cytology study (phase 2) to assess the ability of the biomarker to distinguish subjects with cancer from patients without and to determine the sensitivity, specificity, as well as true- and false-positive rates, with a secondary aim to optimize a diagnostic assay.

Cross-sectional study. Paraffin-embedded tissues were selected from patients with different grades of dysplasia. The highest grade of dysplasia in any biopsy was used to categorize this patient: 37 patients with BE classified as negative for dysplasia, 28 with low-grade dysplasia, 12 with high-grade dysplasia, and 17 with adenocarcinoma. Sections of normal squamous esophageal tissue (n = 30), gastric antrum (n = 20), and the second part of the duodenum (n = 13) were used as controls. Controls originated from patients with (44%) and without BE (56%).

Nested case-control study. Sixteen patients from within the cross-sectional study were selected for a case-control study on the basis that they had developed incident adenocarcinoma (nine patients) or incident high-grade dysplasia (seven patients), which was detected through the surveillance program. Two controls were identified per patient matched, as far as possible, for age and length of follow-up, who did not progress. This small nested cohort was extremely well characterized and selected according to strict criteria. The cancer patients had at least two endoscopies before the development of high-grade dysplasia or cancer and they all progressed through the no dysplasia/low-grade dysplasia/high-grade dysplasia/cancer sequence. The control cases had at least three surveillance endoscopies with no more than a single diagnosis of low-grade dysplasia.

Biopsies were selected from three time points for each patient. The first time point was the first surveillance endoscopy after the diagnosis of BE had been established and before the diagnosis of dysplasia. The second time point was for biopsies taken at the midpoint of the total surveillance interval for each patient; this time point also corresponded to the development of low-grade dysplasia in all cases. The last time point was at the time of the high-grade dysplasia or adenocarcinoma diagnosis for the cases or the latest biopsy available for inclusion in the study for the controls. There, all the cases had an index biopsy with a diagnosis of nondysplastic BE and subsequently developed high-grade dysplasia or adenocarcinoma during the follow-up period.

Brushing cytology cohort. One hundred eight BE patients were recruited for esophageal brushings before biopsy (75 BE ± dysplasia and 33 esophageal adenocarcinomas). To increase the number of cases, high-grade dysplasia or BE-associated adenocarcinoma patients were also recruited from the Middlesex Hospital, which has a tertiary referral practice for the evaluation and treatment of patients with esophageal cancer. The cytology was done under direct endoscopic vision using a disposable brush (Boston Scientific, Watertown, MA). For patients with BE, brush cytology was done in each quadrant along the length of the segment starting at the most proximal extent of the gastric folds. In control patients with a normal squamous esophagus the brush was positioned 2 cm above the gastroesophageal junction, to avoid erroneous sampling of the gastric cardia, and brushed in each quadrant over a 3-cm length of esophageal mucosa. The brush was then put into PreservCyt solution (Cytec Corporation, Boxborough, MA) and from this a monolayer of cells was obtained using ThinPrep 2000 (Cytec).
Immunostaining

Biopsy sections were deparaffinized in xylene, rehydrated through alcohol solutions, water, and finally with TBS-Tween (0.025%, v/v). Washing with TBS-Tween was done between each step. An antigen retrieval step was done by pressure-cooking samples for 3 min in 0.01 mol/L Tris–sodium citrate buffer (pH 6.0). Staining was done using the Dako autostainer (DakoCytomation Ltd, Ely, United Kingdom) and the staining kit Dako ChemMate for increased reproducibility. Blocking of nonspecific binding was done using 10% normal goat serum (v/v) and 10% bovine serum albumin (w/v) for 30 min at room temperature. The samples were incubated with monoclonal anti–cyclin A antibody (Novocastra, Newcastle upon Tyne, United Kingdom) diluted in antibody diluent (DakoCytomation) for 1 h at room temperature. Endogenous peroxidase activity was blocked with peroxidase blocking solution (DakoCytomation) for 5 min. The secondary and the peroxidase-linked antibodies were each incubated for 30 min at room temperature followed by 3,3′-diaminobenzidine substrate (DakoCytomation) for 10 min. Sections were counterstained with hematoxylin. A negative control was done by omission of the primary antibody. The optimization of immunostaining using this antibody was done previously (29), and the specificity of the antibody was determined by a Western blot that confirmed a single band at the expected size of 58,000 Da (data not shown).

For the cytologic samples, the fixative was removed by washing in 100% methanol for 5 min, cells were hydrated in TBS for 5 min, and then washed in TBS-Tween. The cells were permeabilized in 4 mmol/L sodium deoxycholate in TBS for 7 min. The staining procedure was the same as for paraffin-embedded sections except that the primary antibody was applied for 2 h. The secondary and the peroxidase-linked antibodies were each incubated for 30 min at room temperature followed by 3,3′-diaminobenzidine substrate (DakoCytomation) for 10 min. Sections were counterstained with hematoxylin. A negative control was done by omission of the primary antibody using a monolayer slide made from a BE adenocarcinoma cell line SEG-1 (gift from Dr. David Beer, University of Michigan Medical School, Ann Arbor, MI).

Scoring immunostaining

The scoring of immunostaining was done by an investigator (P.L.S.) who had no prior knowledge of the clinical diagnosis. In paraffin-embedded biopsies, the epithelial surface was defined as the superficial four layers of cells in the squamous esophagus and the most superficial layer of columnar cells for the glandular tissues, as described previously (28). All the surface cells per biopsy were counted up to a maximum of 600 to determine the frequency of cyclin A expression. When biopsies presented areas of intestinal metaplasia and dysplasia, both were counted to generate a single value. Only cells with diffuse nuclear staining were considered as positive. The immunopositive epithelial cells were expressed as a percentage of the total number of epithelial cells counted. The brushings were scored as either positive or negative. Any positive cell was considered as abnormal and therefore the brushing was positive if only one positive cell was present.

Statistical analysis

The frequency of cyclin A staining is expressed as a mean ± SE. The Spearman test was used to analyze the correlation between cyclin A expression and degree of dysplasia. The Mann-Whitney test was used to identify specific differences between groups. Multivariate analysis (binary logistic regression) was done using SPSS version 12.0 in the case-control study to determine which factors (age, gender, length of follow-up, and cyclin A expression at the luminal surface) were associated with progression to cancer. In all cases, \( P < 0.05 \) was required for significance (31).

Results

Patient demographics. The patient demographics are summarized in Table 1. Four hundred ten patients underwent surveillance at Addenbrooke’s Hospital from 2001 to 2006. Out of these patients, 267 consented to be in this study. During the study period, 16 patients were diagnosed with incident high-grade dysplasia or adenocarcinoma and were included in the case-control study. The characteristics of patients in each part of the study were not different from the characteristics of the overall clinical surveillance cohort.

Cross-sectional study. In normal gastrointestinal control tissues, cyclin A expression was restricted to the proliferative compartment, namely the basal cells and around the papillae of squamous epithelium, above the secretory glands in the gastric antrum, and at the bottom of the crypt in duodenum (Fig 1A). In nondysplastic BE samples, cyclin A expression was similarly localized to the proliferative compartment in 76% of samples (Fig 1B). However, with increasing grades of dysplasia, the expression of cyclin A shifted toward the upper crypts and the surface epithelium (Fig. 1A and B; \( P = 0.016 \) correlation between cyclin A and the degree of dysplasia). Indeed, in nondysplastic BE, only 24% of patients express cyclin A at the luminal surface compared with 59% of

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<tbody>
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<tr>
<td>Overall surveillance patients</td>
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<tr>
<td>BE study cohort</td>
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<tr>
<td>Cross-sectional study</td>
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<tr>
<td>BE patients</td>
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<tr>
<td>Control tissues</td>
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<tr>
<td>Case-control study</td>
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<tr>
<td>Cases</td>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>Brushing study</td>
</tr>
<tr>
<td>Nondysplastic BE</td>
</tr>
<tr>
<td>Dysplastic BE</td>
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<tr>
<td>Adenocarcinoma</td>
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</table>

NOTE: The demographics of the complete BE study cohort (n = 267) are compared with the patients undergoing surveillance as part of routine clinical care (n = 410). The breakdown is also given for the specific studies (cross-sectional, case-control, and brushing study). Abbreviation: N/A, not applicable.
low-grade dysplasia patients, 87% of high-grade dysplasia patients, and 100% of adenocarcinoma patients.

**Case-control study.** We then used a nested case-control study design to test the hypothesis that cyclin A expression at the luminal surface of BE specimens might predict the likelihood of progression to adenocarcinoma.

As shown in Table 2, the male to female ratio, the length of follow-up, and the length of time to the second time point biopsy did not vary significantly between the cases and controls. Within the strict selection criteria, it was not possible to match completely for age and the male to female ratio was 7:1 in the cases and 2.6:1 in the controls (Table 1). Interestingly, the number of biopsies analyzed per time point was slightly greater for the controls, and, as the likelihood of detecting abnormal cyclin A expression increases with the number of biopsies taken, the findings were not biased in favor of the cases.

For each of the 16 cases, the expression of cyclin A at the surface in biopsies taken before the development of high-grade dysplasia or adenocarcinoma was increased compared with samples from matched controls taken at the equivalent time point (Fig. 2). We then quantified the risk of progression (OR) of patients expressing any discernible cyclin A at the surface at a given time point before the diagnosis of high-grade dysplasia or adenocarcinoma. When considering only the first time point, or in other words samples taken at the first surveillance endoscopy in which all patients had a diagnosis of nondysplastic BE, multivariate analysis showed that the risk of progression was increased 7.6-fold (95% CI, 1.6-37.0; Table 3) in patients who expressed any level of cyclin A at the surface compared with patients without surface expression ($P < 0.012$; Table 1). The age, gender, and the length of follow-up were not associated with an increased risk of progression.

### Table 2. Patient characteristics of the case-control cohort

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>16</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Age at recruitment, y</td>
<td>62.2 ± 1.9</td>
<td>56.7 ± 1.7</td>
<td>0.037</td>
</tr>
<tr>
<td>M:F ratio</td>
<td>7:1</td>
<td>2.6:1</td>
<td>0.233</td>
</tr>
<tr>
<td>Length of follow-up, y</td>
<td>6.5 ± 0.6</td>
<td>5.5 ± 0.3</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>(3-13)</td>
<td>(3-9)</td>
<td></td>
</tr>
<tr>
<td>Time at midpoint</td>
<td>3.2 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td>0.118</td>
</tr>
<tr>
<td>No. biopsies</td>
<td>3.31 ± 0.15</td>
<td>3.92 ± 0.20</td>
<td>0.026</td>
</tr>
</tbody>
</table>

![Fig. 1. Surface expression of cyclin A in gastrointestinal biopsy tissue. Normal squamous esophagus (NE), gastric antrum (GA), second part of duodenum (D2), BE, low-grade dysplasia (LGD), high-grade dysplasia (HGD), and adenocarcinoma (AC). A, representative immunohistochemical staining. B, cumulative data. Y axis, number of cells at the luminal surface expressing cyclin A as a percentage of the total number of surface cells. There was a statistically significant correlation between cyclin A and the degree of dysplasia, $P = 0.016$.](image-url)
However, not all cases expressed cyclin A at the surface at their initial endoscopy. If biopsies taken at any time point before the development of high-grade dysplasia or adenocarcinoma were considered, then 15 of 16 cases had surface expression of cyclin A and the risk of progression increased further (from OR of 7.6 to 13.2, \(P < 0.005\); for 95% CI, see Table 3). When cyclin A expression was considered as a function of the number of slides analyzed, the confidence interval decreased significantly, thus increasing the statistical significance of the findings (Table 3).

Because cyclin A was expressed at the epithelial surface of patients with a higher risk of progression to adenocarcinoma, these cells should be detectable by immunocytology. To test this, we did a prospective study of cyclin A expression in endoscopic esophageal brushings.

**Brushing study.** In cytologic brushings, normal squamous epithelial cells were easily distinguished from columnar cells (Fig. 3A) and cyclin A–positive nuclear staining was easily distinguishable from negative glandular cells within the same clump of cells (Fig. 3B).

The number of brushing samples that scored positive for cyclin A in each diagnostic category of dysplasia and cancer (low-grade dysplasia, high-grade dysplasia, and adenocarcinoma) was similar to the retrospective biopsy study. However, the detection rate of nondysplastic BE samples in the cytologic specimens was significantly higher than the surface expression observed in biopsies (35% versus 25%; Table 4), possibly because of the larger surface area sampled by the cytology brush.

The sensitivity and specificity of the immunocytologic test for the detection of adenocarcinoma and high-grade dysplasia were 97.8% and 58.7%, respectively, with a negative predictive value of 97.4%. In other words, if a brushing sample is negative for cyclin A, it is 97.4% likely that the patient does not have high-grade dysplasia or adenocarcinoma. This is especially important because these are the patients that should never be wrongly diagnosed. If, on the other hand, this test was used to differentiate between dysplastic and nondysplastic samples, the sensitivity and specificity are 88.3% and 64.2%, respectively, with a negative predictive value of 81.6% (Table 5).

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**Table 3.** Summary table for cyclin A expression in the case-control study

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. No. patients (first time point only)</td>
<td>7.6 (1.6-37.0)</td>
</tr>
<tr>
<td>B. No. patients (any time point before HGD/AC)</td>
<td>13.2 (1.5-112.3)</td>
</tr>
<tr>
<td>C. No. biopsies (any time before HGD/AC)</td>
<td>6.2 (2.3-17)</td>
</tr>
</tbody>
</table>

NOTE: Rows A and B correspond to the number of patients with cyclin A expression and row C corresponds to the number of biopsies expressing cyclin A for each patient.

Abbreviations: HGD, high-grade dysplasia; AC, adenocarcinoma.
reduction of the sample processing and analysis. However, such a protocol of fewer endoscopies would not be applicable to routine clinical practice. In this study, these patients are likely to have an increased risk of progression to adenocarcinoma compared with the negative patients.

An obvious limitation of the study is that only 3.31 ± 0.15 and 3.92 ± 0.2 biopsies for the cases and the control patients, respectively, were used to calculate the OR and 95% CI for expression of cyclin A as a marker of progression. The number of biopsy is low because in Addenbrooke's Hospital, surveillance was rarely done according to the quadrant biopsy protocol before 2001. In most cases, the first and second time points were taken before implementation of this strict protocol. The 95% CI for the OR obtained with cyclin A, although narrower than with Mcm2, is still a relatively wide. A study carried out on a larger cohort of patients would be required to fully assess the value of cyclin A as a marker of progression. The sensitivity, specificity, negative predictive value, and positive predictive value have to be taken into consideration carefully as they only apply to the cohort of patients presented in this study. These data warrant further testing in a larger patient population to determine the true sensitivity, specificity, and negative predictive values.

### Table 4. Percentage of cyclin A–stained brushings in the diagnosis of BE and associated dysplasia

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<tr>
<th>Cyclin A–positive cytology samples (%)</th>
<th>Biopsy samples with surface cyclin A expression (%)</th>
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<tr>
<td>BE</td>
<td>35 (17/48)</td>
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<tr>
<td>LGD</td>
<td>60 (9/15)</td>
</tr>
<tr>
<td>HGD</td>
<td>92 (11/12)</td>
</tr>
<tr>
<td>AC</td>
<td>100 (33/33)</td>
</tr>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>59</td>
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<td></td>
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NOTE: The values for the cyclin A–positive brushings represent the number of patients with any discernible cyclin A expression (scored negative or positive). The values for biopsy samples with cyclin A expression are from the retrospective cohort study for comparison and represent the percentage of patients with cyclin A expression.

Abbreviation: LGD, low-grade dysplasia.

### Table 5. Results of analysis of cyclin A–stained brushings as a diagnostic test for esophageal adenocarcinoma and dysplasia

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Efficiency of the test (%)</th>
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<tr>
<td>AC and HGD vs other (LGD, BE)</td>
<td>97.8</td>
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<td>88.3</td>
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NOTE: The efficiency of test is calculated as (true positive + true negative) / number of samples × 100.

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

Discussion

In this study, we have shown that expression of cyclin A at the luminal surface increased throughout the metaplasia-dysplasia-carcinoma sequence. The nested case-control study showed that surface expression of cyclin A in BE patients was associated with an increased risk of progression to adenocarcinoma. The cells expressing cyclin A were detected by immunostaining of cytologic samples with a very high negative predictive value (~97%) for cancer and high-grade dysplasia.

The recently published British Society of Gastroenterology guidelines for the diagnosis and management of BE suggest that when surveillance is done, the sampling should comprise quadranctic biopsies every 2 cm in line with the American Gastroenterology Association guidelines (5, 6). However, several studies have shown that the current endoscopic surveillance is not cost-effective (32), and a significant part of the cost results from the collection and specialist histopathologic review of multiple biopsy specimens. It has been suggested that a modified protocol involving fewer endoscopies or restricting surveillance to patients with dysplasia would be cost-effective (32). One possible way to reduce the cost would be to replace multiple biopsies with a cytologic specimen. This has the potential to enable sampling of a greater surface area and to reduce the associated costs of sample processing and analysis.

Cytology alone has been shown to be a poor diagnostic tool for dysplasia (33, 34). However, several groups have tested the possibility of assessing cytogenetic changes in cytologic samples obtained from endoscopic brushings using fluorescence in situ hybridization. Detection of loss of heterozygosity at 9p and 17p by fluorescence in situ hybridization has been evaluated in cytologic specimens and seems promising; however, because, in most cases, the loss of heterozygosity status of paired biopsies taken from the same site was not evaluated, it is difficult to ascertain the sensitivity and specificity of such techniques (35, 36). Furthermore, fluorescence in situ hybridization was shown to be unable to detect loss of heterozygosity without copy number change, and dual-probe fluorescence in situ hybridization might be required for patients with tetraploidy occurring before loss of heterozygosity (37).

We previously showed that Mcm2 was a marker of progression in a smaller case-control cohort (28); however, a cutoff point of 15% surface expression was necessary to obtain the optimum OR for Mcm2 expression (OR, 136; 95% CI, 7.5-2464; P < 0.0001). The 95% CI for these data was very wide, and a quantitative evaluation of immunohistochemical staining would not be applicable to routine clinical practice. In this study, any luminal expression of cyclin A was predictive of cancer progression with a much narrower confidence interval (95% CI, 1.8-30.7). This allowed the use of a simple binary scoring of esophageal brushing for expression of cyclin A that was highly sensitive (97.8%) for high-grade dysplasia and adenocarcinoma and associated with a very high negative predictive value (97.4%). The high negative predictive value suggests that virtually none of the very high-risk patients would be missed using this technique. A number of low-grade dysplasia and nondysplastic BE patients also scored positive, which decreased the specificity; however, as shown in the case-control study, these patients are likely to have an increased risk of progression to adenocarcinoma compared with the negative patients.

The number of biopsy is low because in Addenbrooke's Hospital, surveillance was rarely done according to the quadrant biopsy protocol before 2001. In most cases, the first and second time points were taken before implementation of this strict protocol. The 95% CI for the OR obtained with cyclin A, although narrower than with Mcm2, is still a relatively wide. A study carried out on a larger cohort of patients would be required to fully assess the value of cyclin A as a marker of progression. The sensitivity, specificity, negative predictive value, and positive predictive value have to be taken into consideration carefully as they only apply to the cohort of patients presented in this study. These data warrant further testing in a larger patient population to determine the true sensitivity, specificity, and negative predictive values.

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NOTE: The efficiency of test is calculated as (true positive + true negative) / number of samples × 100.

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.
One possible application of our data would be to do cyclin A immunostaining on a single cytologic specimen obtained at the index endoscopy. Patients with cyclin A positivity would undergo the quadrantic biopsy protocol with histopathologic assessment at their next endoscopy. Patients with a negative result could have a more prolonged surveillance interval with a repeat cyclin A assessment. This would decrease the number of patients requiring multiple biopsies and histopathologic analyses by ~60%.

Overall, in view of the association between cyclin A staining and cancer risk and the very high negative predictive value on cytologic specimens, our data provide preliminary data to suggest that immunocytoLOGY may provide an initial surveillance tool. This method could provide increased sensitivity and specificity with reduced procedural risk and significant cost savings to the health care system.

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**References**


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Cyclin A Immunocytology as a Risk Stratification Tool for Barrett's Esophagus Surveillance

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