Surface Expression of Minichromosome Maintenance Proteins Provides a Novel Method for Detecting Patients at Risk for Developing Adenocarcinoma in Barrett's Esophagus

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ABSTRACT

Purpose: **The purpose is to determine whether a novel cell cycle marker, minichromosome maintenance protein 2 (Mcm2), predicted esophageal adenocarcinoma (AC) risk in Barrett's esophagus (BE) and whether this could be used in combination with a surface sampling method.**

Experimental Design: **Archival specimens [30 normal squamous esophagus (NE), 20 gastric antrum (GA), 13 duodenum (D2), 62 BE ± dysplasia, and 16 (AC)] were stained for Mcm2. Sequential biopsies from nine patients who developed AC during surveillance were compared with 18 matched controls who did not progress. Prospective endoscopic cytological brushings (61 NE, 90 BE ± dysplasia, and 11 AC) were scored as Mcm2 positive or negative.**

Results: **Mcm2 was not expressed on the luminal surface of NE, GA, and D2. In BE, the percentage of surface cells expressing Mcm2 correlated highly with the degree of dysplasia ($P < 0.0001$). In patients who developed AC, biopsies before dysplasia had higher Mcm2 expression than the matched control patients (mean, 28.4 and 3.4% positive cells, respectively, $P < 0.0001$). In the prospective cohort, the histopathological diagnosis of dysplasia or AC and the Mcm2-positive brushings were concordant in 91% of the patients ($P < 0.005$), and the results correlated with the frequency of cases with surface expression of Mcm2 in the retrospective study ($P < 0.0001$).**

Conclusions: **Surface expression of Mcm2 can be used to detect dysplasia and AC, as well as patients with BE at risk for subsequent development of dysplasia and AC. A brushing technique combined with Mcm2 staining has the potential to be exploited in surveillance and screening protocols.**

INTRODUCTION

The incidence of esophageal AC[2] has increased by 350% in the Western world over the last 30 years (1). Most patients present with dysphagia, and despite advances in multimodal treatment, the 5-year survival rates of symptomatic esophageal carcinomas remain <10% (2, 3). Because the major determinant of outcome is the stage of the cancer at presentation (4), early detection of esophageal cancer is essential to significantly improve survival rates. The identification of a multistage process of cancer development, akin to that described in colon carcinogenesis, provides the rationale for endoscopic surveillance in patients with diagnosed BE (5–8). Several studies have shown a significant improvement in the 5-year survival of patients with surveillance-detected AC (9, 10).

The clinical effectiveness of surveillance for patients with BE is currently hampered by the inadequate surveillance methods used. These are prone to sampling bias and the highly subjective histopathological diagnosis of dysplasia, with high inter- and intraobserver variability (11). Furthermore, we know that only a minority of patients with BE are diagnosed (12). As a result, there has been a suggestion that screening endoscopy should be performed in people with heartburn who are most at risk for developing BE and AC (13). However, the current dependence on endoscopic techniques means that screening is unlikely to be cost effective or feasible at the current time.

In an attempt to improve current surveillance methods, investigators have tried to identify biomarkers to stratify BE patients according to their cancer risk (7, 14). No single oncogene or tumor suppressor gene has been shown to be sensitive enough. Therefore, an alternative approach would be to use markers for the final common pathway after genetic instability, which is the loss of proliferative control. Until recently, assessment of cell proliferation on fixed tissue has relied on markers with disadvantages such as Ki67 and the proliferating cell nuclear antigen (15, 16). Now with an increased understanding of the proteins controlling the cell cycle, alternative markers are becoming available (17). The MCM protein complex binds to the origins of replication to ensure a single round of DNA replication. MCMs are expressed in all cycling cells throughout the cell cycle and are degraded when mammalian cells exit the

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[2] The abbreviations used are: AC, adenocarcinoma; BE, Barrett's esophagus; MCM, minichromosome maintenance; LGD, low-grade dysplasia; HGD, high-grade dysplasia; NE, normal squamous esophagus; GA, gastric antrum; D2, duodenum; GEJ, gastroesophageal junction; TBS-Tween, Tris-buffered saline-Tween (0.025%;v:v).

cell cycle into quiescent, differentiated, and senescent states (18, 19). As a result of the sensitivity of antibodies to MCMs as markers of cell cycle state and because of the aberrant entry into the cell cycle in dysplasia and malignancy, MCM proteins have been proposed as candidate markers for cancer screening, surveillance, and as prognostic markers (20–24). Williams *et al.* (23) demonstrated that Mcm2 and Mcm5 surface positivity correlated with the severity of dysplasia. These observations have led to the rationale for using surface sampling techniques in combination with MCM staining to detect dysplasia and malignancy (24). This approach has yielded promising results for cervical and colon cancer screening (22, 23).

If cell cycle markers could be combined with a simple surface sampling technique, then this may provide a significant advance for identifying individuals at risk for the development of esophageal AC. This study had three main aims. Firstly, to determine the magnitude and pattern of expression of Mcm2 (24), as a prototype MCM protein, in histological samples from patients undergoing upper gastrointestinal endoscopy. Secondly, to determine whether aberrant surface expression of Mcm2 is predictive of cancer development using a retrospective case-control study. Thirdly, to determine whether detection of Mcm2 could be combined with a brushing technique and liquid-based cytology as a novel strategy for predicting those BE patients at risk for the development of AC.

PATIENTS AND METHODS

Patient and Tissue Collection. Approval was obtained from the Local Research Ethics Committee for the retrospective and prospective parts of this study.

Retrospective Cross-Sectional Study. Archival blocks were obtained from patients who had attended Addenbrooke's Hospital (Cambridge, United Kingdom). We studied 37 BE negative for dysplasia, 28 for LGD, 12 for HGD, and 17 with AC. Sections of normal tissue from non-BE and BE patients' NE ($n = 30$), the second part of the D2 ($n = 13$), and GA ($n = 20$) were used as control.

Retrospective Case-Control Study. Serial biopsies from nine patients whose AC or HGD was detected through a surveillance program (followed up for a median of 6 years, range from 3 to 13 years) were compared with those from 18 controls, matched for age and length of follow-up (with a median of 5 years, range from 3 to 7 years) who did not progress. The cancer patients had at least two endoscopies before the development of HGD, and they all progressed through the no dysplasia-LGD-HGD sequence. The control cases had no more than a diagnosis of focal LGD in one of their surveillance endoscopies. Hence, control patients with multifocal LGD at any time point of their surveillance program were excluded from the study.

Prospective Study. A total of 162 patients attending Addenbrooke's Hospital for an endoscopy for either heartburn symptoms, routine BE surveillance, or for assessment of dysplasia/AC as part of a tertiary referral service was recruited for esophageal brushings before biopsy (61 NE, 90 BE ± dysplasia, and 11 AC).

All patients with BE, in either the retrospective or prospective parts of the study, had an endoscopically visible columnar-lined segment and a histopathological diagnosis of specialized intestinal metaplasia. Esophagitis, occurring either in isolation or in conjunction with BE, was graded according to the Los Angeles classification with histopathological confirmation according to the established criteria (25). All patients had diagnostic biopsies taken 2 cm above the GEJ and in addition patients with BE had surveillance biopsies taken (each quadrant every 2 cm; Ref. 26).

Cytology Brushings. For all patients recruited prospectively, brush cytology was performed 2 cm above the GEJ using a disposable brush (Boston Scientific, Watertown, MA) under direct endoscopic vision. The brush was then put into PreservCyt solution (Cytyc Corporation, Boxborough MA), and from this, a monolayer of cells was obtained using a ThinPrep 2000 (Cytyc Corporation). One slide was stained conventionally with a Papanicolaou stain and analyzed for cytological abnormalities by an expert cytopathologist (M. O.). The second slide was stained for Mcm2. Only a subset of the samples (61 NE, 21 BE, 37 LGDs, 3 HGD, and 7 AC) was analyzed for routine cytology as it quickly became apparent that the results obtained showed variable correlation with the histopathological diagnosis (see "Results").

Immunostaining. Tissue sections were deparaffinized in xylene and rehydrated through alcohol solutions, water, and finally with TBS-Tween. Washing using TBS-Tween was performed between each step. An antigen-retrieval step was performed by pressure cooking samples for 3 min in 0.01 M Tris-sodium citrate buffer at pH 6.0. Staining was performed using the Dako autostainer (DakoCytomation Ltd., Ely, United Kingdom) and the staining kit Dako ChemMate for increased reproducibility. Blocking of nonspecific binding was performed using 10% normal goat serum (v:v) and 10% BSA (w:v) for 30 min at room temperature. The samples were incubated with monoclonal Mcm2 antibody (21, 22) at 1/10 or MIB-1 (anti-Ki67 antibody; DakoCytomation Ltd.) at 1/100 dilution in antibody diluent (DakoCytomation Ltd.) for 1 h at room temperature. Endogenous peroxidase activity was blocked with peroxidase-blocking solution (DakoCytomation Ltd.) 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 Ltd.) for 10 min. Sections were counterstained with hematoxylin. A negative control was performed by omission of the primary antibody.

For the cytological samples, the fixative was removed by a wash in 100% methanol for 5 min, and the cells were hydrated in TBS for 5 min and then washed in TBS-Tween. The cells were permeabilized in 4 mM sodium deoxycholate in TBS. The staining procedure was the same as for paraffin sections except that the primary antibody was applied for 2 h. A negative control was done by omission of the primary antibody using a monolayer slide made from a BE AC cell line SEG-1 (gift from Dr. David Beer, University of Michigan Medical School).

Scoring Immunostaining. 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. Biopsies without surface epithelium were excluded from the analysis. All of the surface cells per biopsy were counted up to a maximum of 600 to determine

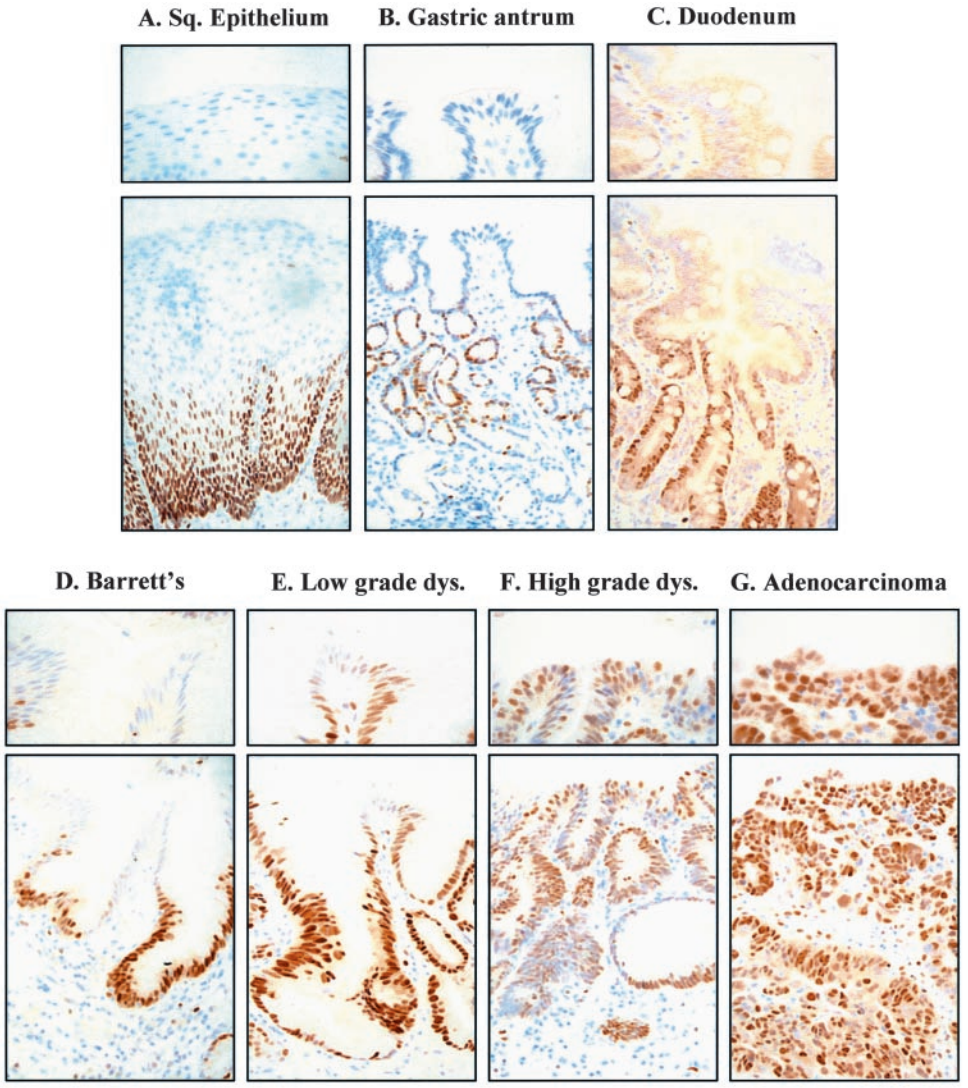

Fig. 1 Mcm2 surface expression in upper gastrointestinal tissues. Control tissues (*A–C*) and the Barrett's metaplasia-dysplasia-carcinoma sequence (*D–G*). Positive Mcm2 protein expression is demonstrated by *brown nuclear staining* with a *blue hematoxylin counterstain*. The *bottom panel* for each representative section is a low magnification view, including the usual proliferative basal (*A*) and glandular compartments (*B–G*; ×100), and the *top panel* demonstrates a high magnification view of the epithelial surface from the corresponding section (×400).

the frequency of Mcm2 expression. 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 for Mcm2 staining. Any positive cell was considered as abnormal (see "Results," Figs. 1 and 2), therefore, the brushing was positive if only one positive cell was present.

Statistical Analysis. The frequency of Mcm2 staining are expressed as a mean ± SE. The Jonckheere-Terpstra test (27) and the χ^2 test for trend were used to assess the correlation between Mcm2 expression and the degree of dysplasia. The Jonckheere-Terpstra test is a nonparametric statistic test, testing for a shift in ordered distributions (Mcm2 surface expression) when stratified by ordered categories (NE, BE, LGD, HGD, and AC). The Mann-Whitney test was used to identify specific differences between groups. In all cases, $P < 0.05$ was required for significance (28, 29).

RESULTS

Mcm2 Surface Staining Correlates with the Grade of Dysplasia. In normal control tissues (NE, GA, and D2), Mcm2 was not expressed at the luminal surface (Figs. 1, *A–C*, and 2). In NE, expression was confined to the parabasal and suprabasal compartments of the epithelium (Fig. 1*A*). In GA and D2, expression was restricted to the glands and lower crypts (Fig. 1, *B* and *C*). In BE samples, the proliferative compartment gradually extended toward the upper crypts and the surface coincident with the degree of dysplasia (Mcm2 expression in BE < LGD < HGD < AC, $P < 0.0001$; Figs. 1, *D–G*, and 2). For comparison, in BE the mean surface expression of Mcm2 and Ki67 was respectively 6.8 and 4.9% of epithelial cells in nondysplastic samples, 32.9 and 20.3% in LGD, 49.7 and 34.2% in HGD, and 66.5 and 59.9% in the AC. Hence, as expected, the Mcm2 antibody was more sensitive than Ki67, although this was difference was not statistically significant.

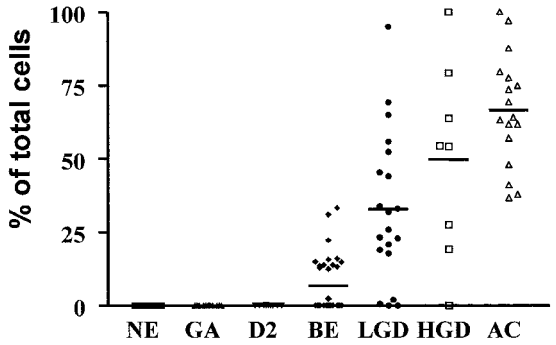

Fig. 2 Mcm2 surface expression. Mcm2-positive cells are presented as a percentage of the total surface epithelial cells. The horizontal line indicates the mean value for each patient group [NE ($n = 30$), GA ($n = 12$), D2 ($n = 9$), BE ($n = 34$), LGD ($n = 20$), HGD ($n = 8$), and AC ($n = 16$)]. There is a correlation between the degree of dysplasia and the level of Mcm2 expression ($P < 0.0001$).

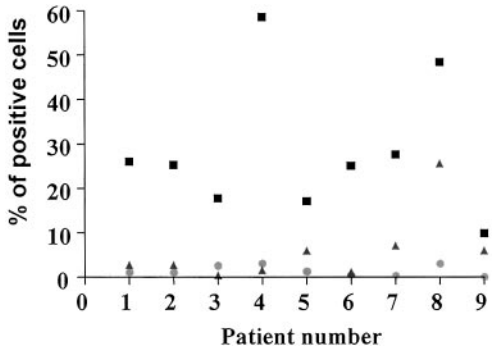

Fig. 3 Mcm2 surface expression in non dysplastic BE samples for the case-control patients. The mean Mcm2 surface expression of the non-dysplastic biopsies of patients destined to develop cancer (■, value is the mean of one to three biopsies depending on the number of years of follow-up) is compared with the two matched controls for each case (△ and ○, mean of three to seven biopsies depending on the number of years of follow-up). The Mcm2 surface expression in the cancer patients before dysplasia is significantly higher than that of control patients ($P > 0.0001$).

Interestingly, Mcm2 staining in patients with nondysplastic BE was dichotomous (unlike for Ki67, data not shown). Forty-four percent of BE samples were positive for Mcm2 surface expression with a mean of 15.5% positive cells/biopsy and 56% of patients were negative for surface Mcm2 expression (Fig. 2). Because normal epithelial tissues such as GA and D2 do not express surface Mcm2 and because Mcm2 surface expression correlates with dysplasia, we hypothesized that the population of nondysplastic BE patients with Mcm2 surface-positive staining might be more likely to progress down the dysplastic pathway than those who have no surface expression. This hypothesis formed the basis of the case-control study.

Mcm2 Surface Expression Allows Detection of Patients at Risk for Progression to AC. For each of the nine cases of AC, Mcm2 surface expression in biopsies before the diagnosis of dysplasia was higher than for the matched controls (Fig. 3). The mean expression of Mcm2 was 28.4% of total surface epithelial cells for BE biopsies in the group destined to develop AC and 3.4% for the control group ($P < 0.0001$; Fig. 3). The level of Mcm2 expression was maintained throughout the length of follow-up for the control group, but the expression levels in the AC group increased with time as the degree of dysplasia increased (P for degree of correlation with dysplasia in AC cases, $P < 0.05$ *versus* correlation between Mcm2 and time of biopsy, $P = 1$ for the controls; Fig. 4).

Mcm2 Staining of Esophageal Brushings. The ectopic surface expression of Mcm2 proteins in BE, which increased with dysplasia (Figs. 1, *D–G*, 2, and 3), suggested that a surface sampling method might enable noninvasive detection of dysplasia and malignancy and detection of BE cases destined to progress. To test whether Mcm2 staining of brushings gave a diagnostic advantage, a paired slide from each sample was sent for routine cytological assessment.

Mcm2-positive nuclear staining was easily distinguished from the background staining of the negative cells (Fig. 5). Mcm2-positive staining of the brushings and the histopathological diagnosis of dysplasia or AC were concordant in 91% of the patients (Table 1). In keeping with the findings from the retrospective study, Mcm2 positivity in the brushings and the degree of dysplasia on biopsies correlated significantly ($P < 0.0001$). Furthermore, the frequency of BE patients that were positive for Mcm2 were similar in the retrospective histology and the prospective cytology study, suggesting that the brushings were indeed picking up the surface positive cells that were identified in the surface of the biopsy samples (Table 1). There was no correlation between the surface expression of Mcm2 and the degree of mucosal inflammation (data not shown, $P = 1$).

Only 43% of the patients with a histopathological diagnosis of BE had evidence of goblet cells by routine cytology (data not shown), and the concordance between histopathology and cytology for dysplasia was 48%, in keeping with other studies (30), compared with 91% for Mcm2 staining.

DISCUSSION

In this study, we have demonstrated that aberrant surface expression of Mcm2 increases as patients progress along the metaplasia-dysplasia-carcinoma sequence of esophageal AC. The case-control study suggests that patients with surface expression of Mcm2 in metaplastic BE may be more at risk for malignant progression. Because Mcm2 expression occurs on the surface of potentially premalignant tissues, but not on the surface of normal gastrointestinal tissues, a surface-sampling brush methodology may be applicable for detection of dysplasia and cases of BE destined to progress to AC. We have demonstrated comparable frequency of Mcm2 expression in endoscopic brushings *versus* multiple biopsies.

A recent publication demonstrated that Mcm2 and Mcm5 expression was increased in dysplastic BE (31). Interestingly, the pattern of Mcm2 and Mcm5 staining in histopathological sections of BE resembled that of gastric type mucosa with maximal expression in the crypts. However, it is not clear whether the presence of intestinal metaplasia was used in the study as part of the diagnostic criteria for BE. Here, we have restricted the definition of BE to the intestinal subtype containing goblet cells, which is also the type that confers the highest

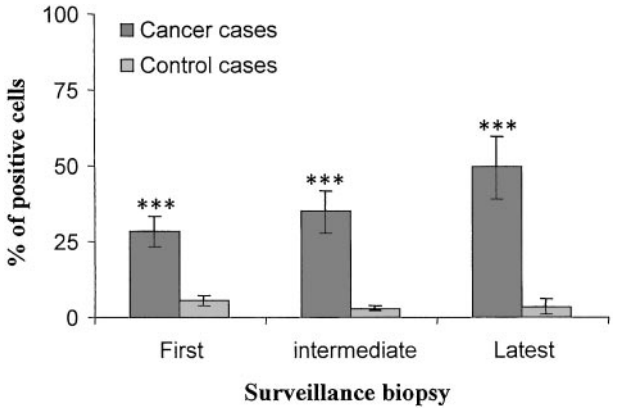

Fig. 4 Mcm2 surface expression over the follow-up period for the case-control study. The first surveillance biopsy refers to the biopsies taken at the first surveillance endoscopy after the diagnosis of Barrett's had been established and before the diagnosis of dysplasia. The intermediate biopsy refers to the biopsies taken at the time of the diagnosis of LGD in patients destined to develop AC (pooled if several biopsies show LGD) and the biopsy taken at the midpoint of the total surveillance interval for each control patient. The latest biopsy refers to the biopsy diagnosed with either HGD or AC for the cancer cases or the latest biopsy available for inclusion in the study for the control cases (*** represents $P < 0.0001$).

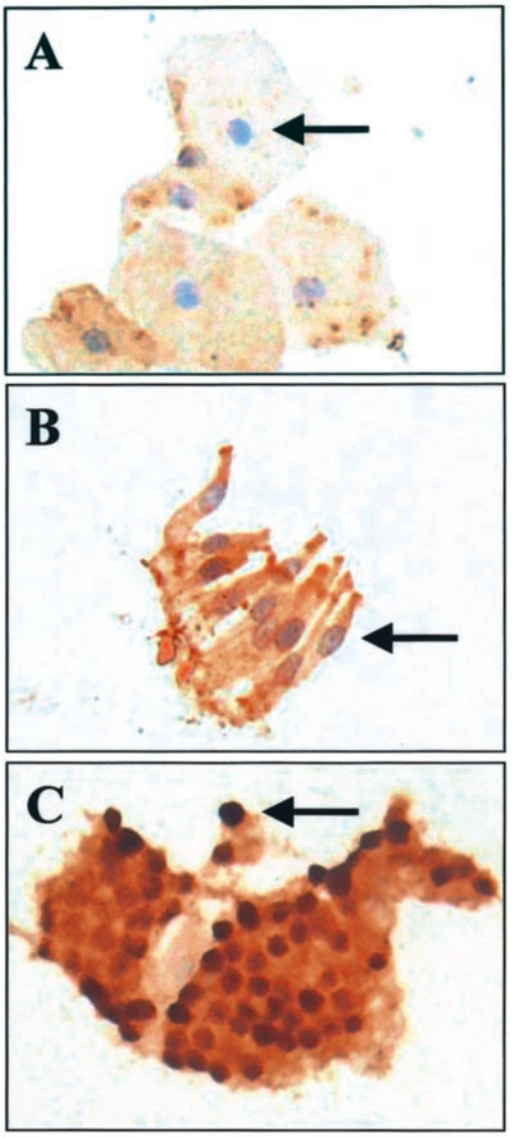

Fig. 5 Representative Mcm2 staining in esophageal brushings. Negative cells with *blue nuclei* in squamous esophagus cells (*A*) and BE cells (*B*) compared with the positive *brown nuclei* of the BE cells (*C*). The *black arrows* indicate the position of the nuclei (×400).

risk for malignancy (32), and the expression pattern of Mcm2 in BE resembles more that of D2 with a maximum expression in the lower crypts.

Cytology alone is likely to be a poor screening or surveillance test for BE and associated dysplasia, as shown by our own results (Table 1), and as demonstrated in previous studies (33). The lack of cytological detection of intestinal metaplasia (28%) may be partly because of the variable distribution of goblet cells in BE. The assessment of LGD was difficult in some cases because of marked reactive changes attributable to inflammation. The air drying and spreading artifacts were partially overcome by using liquid-based cytology as opposed to conventional-based cytological techniques. Cytology was highly sensitive for the detection of HGD/AC, with a failure of detection only occurring because of inadequate material for assessment (2 of 7 AC cases missed).

However, analysis of Mcm2 expression in conjunction with cytology increases the diagnostic yield significantly. Given that the positive Mcm2 staining is localized to the nucleus, it is easy to distinguish true positive columnar cells from background staining in the liquid-based cytology monolayer (Fig. 5). The shape, size, and low background seen in squamous cells makes them easy to distinguish from columnar cells (Fig. 5). In our prospective study, any cells obtained from the brushings with nuclear staining were counted as positive. This makes scoring positive and negative brushings very simple and hence appealing for clinical practice. Furthermore, the exfoliative cytology technique used in this study enables a greater surface area of esophageal mucosa to be sampled compared with a conventional biopsy series. In the future, with the advent of newer cytological sampling devices, it should be possible to sample an even greater surface area of mucosa and to apply these techniques nonendoscopically (34). This has the potential to change radically the cost-effectiveness of esophageal screening and surveillance programs.

None of the biopsies from normal squamous esophagus had positive Mcm2 expression on the surface. In contrast, brushings from 13% of NE patients were Mcm2 positive. Half of these samples were positive squamous cells, and there was no correlation between the degree of inflammation and these results ($P = 1$). It is possible that the positive squamous cells originate from the basal layers and were brushed away from the epithelium as a result of an excessively harsh sampling technique. The other half of the positive brushings in NE patients represented positive glandular cells (two patients had diagnosed gastritis, one had intestinal metaplasia, and the last had a normal endos-

Table 1 A, routine cytology and Mcm2-stained brushings in the diagnosis of BE and associated dysplasia; and B, Mcm2 surface expression in the samples from the retrospective study

	A. Prospective study		B. Retrospective biopsy study
Histopathological diagnosis in biopsy samples	Cytological diagnosis of dysplasia	Mcm2-positive brushings	Mcm2 surface expression
NE	15% (9/61)	13% (8/61)	0% (0/30)
BE	28% (6/21)	42% (19/45)	44% (15/34)
LGD	43% (16/37)	88% (37/42)	85% (17/20)
HGD	100% (3/3)	100% (3/3)	88% (7/8)
AC	71% (5/7)	100% (11/11)	100% (16/16)

copy). Presumably, the positive glandular cells had originated from either the gastric cardia or from endoscopically undiagnosed ultrashort BE as 15% of biopsies from the GEJ of asymptomatic patients have been shown to have small foci of intestinal metaplasia (35).

It is also of note that 44% of nondysplastic BE patients had some degree of surface Mcm2 expression present in both the biopsy and the brushing samples. It is generally believed that there is a 0.5–1% risk/year of BE patients progressing to AC (36), and therefore, only a minority of the 44% of patients with Mcm2 surface expression are likely to progress. However, in the case-control study, we demonstrated that the patients who did progress to AC had significantly increased surface expression of Mcm2 at their initial endoscopy, before the development of dysplasia (mean, 28.4% positive cells *versus* 3.4% for controls, $P < 0.0001$; Fig. 3). It is interesting to note that of the 44% of nondysplastic BE patients with surface Mcm2 expression, the mean number of positive cells was 15.5%, and only three patients had >25% positive cells. Hence, whether a quantitative assessment of surface Mcm2 expression and the change in the level of Mcm2 expression over time is predictive of cancer development may be worthy of additional investigation. In the future, it is also possible that a combination of Mcm2 with other biomarkers may enable patients to be stratified additionally for risk of progression, although the histopathological diagnosis will remain the gold standard for dysplasia.

A large proportion of patients with BE in our prospective study had LGD. This reflects the tertiary referral of patients with dysplasia in BE in our practice. In the future, there needs to be a prospective study of Mcm2 staining applied to a nonselected surveillance cohort with appropriate follow-up. In addition, with the advent of nonendoscopic cytological sampling devices, it would be valuable to apply our immunocytochemical technique to a cohort of heartburn patients who are subsequently endoscoped. For example, in parts of mainland China, nonendoscopic cytological sampling of the esophagus (balloon cytology) has been used for decades as a screening test for squamous esophageal cancer (37, 38). During the current study, five patients with squamous esophageal carcinoma attended for an endoscopy and had brushings taken. All five of these patients had positive Mcm2 surface expression, although they were excluded from subsequent analysis in view of the squamous cell histology.

In view of the alarming increase in the frequency of AC of the esophagus, inexpensive and effective tests for detection of patients at risk are badly needed. The detection of MCM proteins in samples from the esophageal surface may offer a diagnostic advance.

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REFERENCES

1. Devesa, S. S., Blot, W. J., and Fraumeni, J. F., Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer (Phila.), *83:* 2049–2053, 1998.

2. Lund, O., Kimose, H. H., Aagaard, M. T., Hasenkam, J. M., and Erlandsen, M. Risk stratification and long-term results after surgical treatment of carcinomas of the thoracic esophagus and cardia. A 25-year retrospective study. J. Thorac. Cardiovasc. Surg., *99:* 200–209, 1990.

3. Berrino, F. C. R., Esteve, J., Gatta, G., Hakulinen, T., Micheli, A., Sant, M., and Verdicchia, A. Survival of cancer patients in Europe: the EUROCARE-II study, IACR Sci. Publ. No. 151. Lyon, France: International Agency for Research on Cancer, 1999.

4. Streitz, J. M., Jr., Ellis, F. H., Jr., Gibb, S. P., Balogh, K., and Watkins, E., Jr. Adenocarcinoma in Barrett's esophagus. A clinicopathologic study of 65 cases. Ann. Surg., *213:* 122–125, 1991.

5. Hameeteman, W., Tytgat, G. N., Houthoff, H. J., and van den Tweel, J. G. Barrett's esophagus: development of dysplasia and adenocarcinoma. Gastroenterology, *96:* 1249–1256, 1989.

6. Jankowski, J. A., Harrison, R. F., Perry, I., Balkwill, F., and Tselepis, C. Barrett's metaplasia. Lancet, *356:* 2079–2085, 2000.

7. Fitzgerald, R. C., and Triadafilopoulos, G. Recent developments in the molecular characterization of Barrett's esophagus. Dig. Dis., *16:* 63–80, 1998.

8. Souza, R. F., Morales, C. P., and Spechler, S. J. Review article: a conceptual approach to understanding the molecular mechanisms of cancer development in Barrett's oesophagus. Aliment Pharmacol. Ther., *15:* 1087–1100, 2001.

9. Peters, J. H., Clark, G. W., Ireland, A. P., Chandrasoma, P., Smyrk, T. C., and DeMeester, T. R. Outcome of adenocarcinoma arising in Barrett's esophagus in endoscopically surveyed and nonsurveyed patients. J. Thorac. Cardiovasc. Surg., *108:* 813–821; discussion 821–812, 1994.

10. van Sandick, J. W., van Lanschot, J. J., Kuiken, B. W., Tytgat, G. N., Offerhaus, G. J., and Obertop, H. Impact of endoscopic biopsy surveillance of Barrett's oesophagus on pathological stage and clinical outcome of Barrett's carcinoma. Gut, *43:* 216–222, 1998.

11. Reid, B. J., Haggitt, R. C., Rubin, C. E., Roth, G., Surawicz, C. M., Van Belle, G., Lewin, K., Weinstein, W. M., Antonioli, D. A., Goldman, H., *et al.* Observer variation in the diagnosis of dysplasia in Barrett's esophagus. Hum. Pathol., *19:* 166–178, 1988.

12. Cameron, A. J., Zinsmeister, A. R., Ballard, D. J., and Carney, J. A. Prevalence of columnar-lined (Barrett's) esophagus. Comparison of population-based clinical and autopsy findings. Gastroenterology, *99:* 918–922, 1990.

13. Sampliner, R. E. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. Am. J. Gastroenterol., *97:* 1888–1895, 2002.

14. Montesano, R., and Hainaut, P. Molecular precursor lesions in oesophageal cancer. Cancer Surv., *32:* 53–68, 1998.

15. Pellish, L. J., Hermos, J. A., and Eastwood, G. L. Cell proliferation in three types of Barrett's epithelium. Gut, *21:* 26–31, 1980.

16. Gray, M. R., Hall, P. A., Nash, J., Ansari, B., Lane, D. P., and Kingsnorth, A. N. Epithelial proliferation in Barrett's esophagus by proliferating cell nuclear antigen immunolocalization. Gastroenterology, *103:* 1769–1776, 1992.

17. Blow, J. J., and Hodgson, B. Replication licensing: defining the proliferative state? Trends Cell Biol., *12:* 72–78, 2002.

18. Tye, B. K. MCM proteins in DNA replication. Annu. Rev. Biochem., *68:* 649–686, 1999.

19. Todorov, I. T., Werness, B. A., Wang, H. Q., Buddharaju, L. N., Todorova, P. D., Slocum, H. K., Brooks, J. S., and Huberman, J. A. HsMCM2/BM28: a novel proliferation marker for human tumors and normal tissues. Lab. Investig., *78:* 73–78, 1998.

20. Hunt, D. P., Freeman, A., Morris, L. S., Burnet, N. G., Bird, K., Davies, T. W., Laskey, R. A., and Coleman, N. Early recurrence of benign meningioma correlates with expression of mini-chromosome maintenance-2 protein. Br. J. Neurosurg., *16:* 10–15, 2002.

21. Rodins, K., Cheale, M., Coleman, N., and Fox, S. B. Minichromosome maintenance protein 2 expression in normal kidney and renal cell carcinomas: relationship to tumor dormancy and potential clinical utility. Clin. Cancer Res., *8:* 1075–1081, 2002.

22. Davies, R. J., Freeman, A., Morris, L. S., Bingham, S., Dilworth, S., Scott, I., Laskey, R. A., Miller, R., and Coleman, N. Analysis of minichromosome maintenance proteins as a novel method for detection of colorectal cancer in stool. Lancet, *359:* 1917–1919, 2002.

23. Williams, G. H., Romanowski, P., Morris, L., Madine, M., Mills, A. D., Stoeber, K., Marr, J., Laskey, R. A., and Coleman, N. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. Proc. Natl. Acad. Sci. USA, *95:* 14932–14937, 1998.

24. Freeman, A., Morris, L. S., Mills, A. D., Stoeber, K., Laskey, R. A., Williams, G. H., and Coleman, N. Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. Clin. Cancer Res., *5:* 2121–2132, 1999.

25. Riddell, R. H., Goldman, H., Ransohoff, D. F., Appelman, H. D., Fenoglio, C. M., Haggitt, R. C., Ahren, C., Correa, P., Hamilton, S. R., Morson, B. C., *et al*. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. Hum. Pathol., *14:* 931–968, 1983.

26. Sampliner, R. E. Practice guidelines on the diagnosis, surveillance, and therapy of Barrett's esophagus. The Practice Parameters Committee of the American College of Gastroenterology. Am. J. Gastroenterol., *93:* 1028–1032, 1998.

27. Hodges, J. L., and Lehmann, E. L. Nonparametric statistical methods. New York: John Wiley & Sons, 1963.

28. Landis, J. R., and Koch, G. G. The measurement of observer agreement for categorical data. Biometrics, *33:* 159–174, 1977.

29. Altman, D. Practical statistics for medical research. London: Chapman and Hall, 1991.

30. Spechler, S. J. Barrett's esophagus: should we brush off this ballooning problem? Gastroenterology, *112:* 2138–2142, 1997.

31. Going, J. J., Keith, W. N., Neilson, L., Stoeber, K., Stuart, R. C., and Williams, G. H. Aberrant expression of minichromosome maintenance proteins 2 and 5, and Ki-67 in dysplastic squamous oesophageal epithelium and Barrett's mucosa. Gut, *50:* 373–377, 2002.

32. Haggitt, R. C., and Dean, P. J. Adenocarcinoma in Barrett's epithelium. *In:* R. K. Goyal (ed.), Barrett's Esophagus: Pathophysiology, Diagnosis, and Management, pp. 153–166. New York: Elsevier, 1985.

33. Falk, G. W., Chittajallu, R., Goldblum, J. R., Biscotti, C. V., Geisinger, K. R., Petras, R. E., Birgisson, S., Rice, T. W., and Richter, J. E. Surveillance of patients with Barrett's esophagus for dysplasia and cancer with balloon cytology. Gastroenterology, *112:* 1787–1797, 1997.

34. Rader, A. E., Faigel, D. O., Ditomasso, J., Magaret, N., Burm, M., and Fennerty, M. B. Cytological screening for Barrett's esophagus using a prototype flexible mesh catheter. Dig. Dis. Sci., *46:* 2681–2686, 2001.

35. Spechler, S. J. Short and ultrashort Barrett's esophagus: what does it mean? Semin. Gastrointest. Dis., *8:* 59–67, 1997.

36. Shaheen, N. J., Crosby, M. A., Bozymski, E. M., and Sandler, R. S. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? Gastroenterology, *119:* 333–338, 2000.

37. Liu, S. F., Shen, Q., Dawsey, S. M., Wang, G. Q., Nieberg, R. K., Wang, Z. Y., Weiner, M., Zhou, B., Cao, J., Yu, Y., *et al*. Esophageal balloon cytology and subsequent risk of esophageal and gastric-cardia cancer in a high-risk Chinese population. Int. J. Cancer, *57:* 775–780, 1994.

38. Shu, Y. J. Cytopathology of the esophagus. An overview of esophageal cytopathology in China. Acta Cytol., *27:* 7–16, 1983.

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