Improved Progression Prediction in Barrett's Esophagus With Low-grade Dysplasia Using Specific Histologic Criteria

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Abstract: Risk stratification of patients with Barrett's esophagus (BE) is based on diagnosis of low-grade dysplasia (LGD). LGD has a poor interobserver agreement and a limited value for prediction of progression to high-grade dysplasia or esophageal adenocarcinoma. Specific reproducible histologic criteria may improve the predictive value of LGD. Four gastrointestinal pathologists examined 12 histologic criteria associated with LGD in 84 BE patients with LGD (15 progressors and 69 nonprogressors). The criteria with at least a moderate (kappa, 0.4 to 0.6) interobserver agreement were validated in an independent cohort of 98 BE patients with LGD (30 progressors and 68 nonprogressors). Hazard ratios (HR) were calculated by Cox proportional hazard regression analysis using time-dependent covariates correcting for multiple endoscopies during follow-up. Agreement was moderate or good for 4 criteria, that is, loss of maturation, mucin depletion, nuclear enlargement, and increase of mitosis. Combination of the criteria differentiated high-risk and low-risk group amongst patients with LGD diagnosis ($P < 0.001$). When ≥ 2 criteria were present, a significantly higher progression rate to high-grade dysplasia or esophageal adenocarcinoma was observed (discovery set: HR, 5.47; 95% confidence interval [CI], 1.81-17; $P = 0.002$; validation set: HR, 3.52; 95% CI, 1.56-7.97; $P = 0.003$). Implementation of p53 immunohistochemistry and histologic criteria optimized the prediction of progression (area under the curve, 0.768; 95% CI, 0.656-0.881). We identified and validated a clinically applicable panel of 4 histologic criteria, segregating BE patients with LGD diagnosis into defined prognostic groups. This histologic panel can be used to improve clinical decision making, although additional studies are warranted.

Key Words: Barrett's esophagus, low-grade dysplasia, interobserver agreement, progression rate, histology

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The major risk factor for esophageal adenocarcinoma (EAC) is Barrett's esophagus (BE), a condition in which squamous epithelium of the distal esophagus is replaced by columnar epithelium with gastric and colonic differentiation. The EAC pathogenesis is suggested to be a gradual process with intermediate stages of low-grade dysplasia (LGD) and high-grade dysplasia (HGD).[1,2] The overall incidence of progression from BE to HGD or EAC is low (0.13% to 0.15%/y), as demonstrated by multiple BE cohort studies from different countries.[3,4] As a result, the rationale for BE surveillance as well as optimal approach for BE patients remains debated.[5] Endoscopic surveillance programs offer the opportunity for early detection and treatment of relevant neoplastic lesions in order to prevent development of advanced cancers.[3,4] Diagnosis of LGD in biopsies taken during Barrett surveillance is an important prognostic indicator for progression and the reason to intensify surveillance interval.[1,2,6,7] Alternatively, radiofrequency ablation might be indicated.[8] The current guidelines recommend endoscopic eradication therapy in patients with confirmed and persistent LGD with the goal of achieving complete eradication of intestinal metaplasia.[8,9]

In patients with LGD, major differences in rates of progression to HGD/EAC are reported in previous studies, varying from <1% to up to 13.4% per patient-year.[5,10–14] The differences in progression rate might reflect difficulties in discriminating true neoplasia from BE with reactive changes. Recent studies indicate that the predictive value of LGD diagnosis increases after expert review confirmation.[12,14,15] On the basis of this observation, LGD should be confirmed by a second pathologist with experience in gastrointestinal pathology, and especially in BE pathology.[1,2,9] However, overall interobserver variation for the diagnosis of LGD remains significant even among expert pathologists, with kappa values reported to be poor in most studies.[16–18] Adoption of standards for LGD diagnosis would increase

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agreement, but the descriptive histologic criteria for LGD are not sufficiently harmonized yet.[18,19] Therefore, the aim of the present study was to challenge the histologic criteria for LGD for their reproducibility and capacity to predict progression. We propose that a defined histologic criteria panel could improve prediction of progression in BE patients with LGD and thereby improve risk stratification in BE patients.

METHODS

Setting and Patients' Population

The study aimed to improve predictive value of LGD. Therefore, we examined the reproducibility of selected histologic criteria and tested their power to predict progression in patients with a BE, which was defined by development of HGD or EAC. Two independent cohorts of BE patients were identified retrospectively. The characteristics of both study populations are shown in Figure 1A.

The discovery set consisted of patients under endoscopic surveillance for BE at Erasmus Medical Center (EMC) (Rotterdam, the Netherlands), with at least 1 pathologic record of LGD during follow-up (LGD diagnosis was made between 2003 and 2014). Patients with LGD or HGD in their medical history had at least 1 year of follow-up before being eligible for inclusion in this study.

The validation set consisted of patients with BE included in the ProBar study,[20] with LGD diagnosis made on follow-up. The study protocol has been described before.[20–22] In short, the ProBar study is a prospective study comprised of > 700 patients with known or newly diagnosed BE. The endoscopic diagnosis of BE was histologically confirmed by the presence of intestinal metaplasia. Patients with HGD or EAC on index endoscopy or a history of HGD or EAC were excluded from the ProBar study and were not encountered for the validation cohort. The ProBar patients were followed-up until they developed HGD or EAC, at which point they were treated and excluded from further follow-up. Of this cohort, all patients with LGD and progression to HGD or EAC during follow-up were selected and matched with patients with LGD during follow-up, but without progression to HGD or EAC in a ratio of 1:2.

All biopsies of the patients from EMC and the ProBar cohort were independently reviewed by 2 expert pathologists who confirmed the presence of LGD diagnosis before evaluating the criteria. If these pathologists were discordant on the grade of dysplasia, a third expert pathologist reviewed the case. Only biopsies with a consensus diagnosis of LGD were included in this study. The presence of HGD or EAC in progressors was also reviewed and confirmed by 4 expert pathologists (M.D., K.B., F.J.C.t.K., and F.J.W.t.K.), all actively participating in national BE studies, having extensive experience in the assessment of BE pathology.[14,21,23] Data analysis was performed based on histologic diagnosis on follow-up.

Endoscopic Follow-up

Clinical follow-up of all included patients was performed according to the guidelines of the American College of Gastroenterology, with a standardized endoscopy

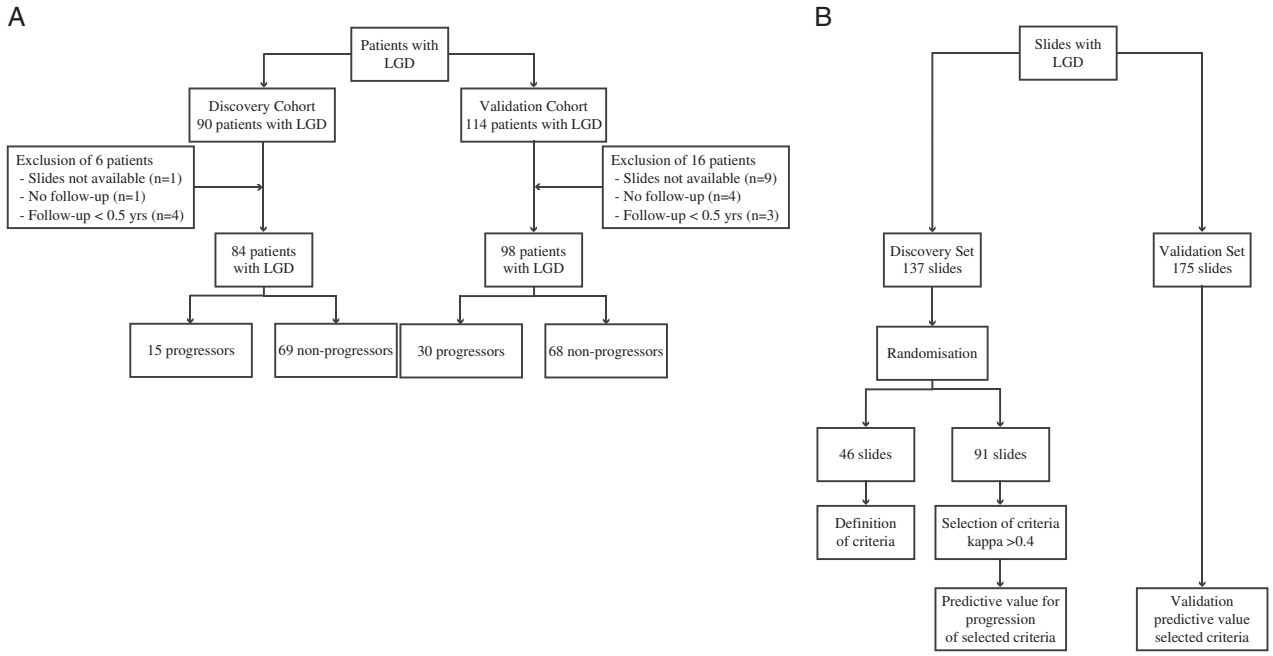

FIGURE 1. Flow chart of patients in this study (A) and study design (B). All slides of the discovery cohort were randomized and were assigned in a consecutive number from 1 to 137. The first 46 slides were used as a learning set to define the criteria, and the rest of the slides were used to calculate the interobserver correlation and the correlation to time to neoplastic progression. Progressors were defined as patients who developed HGD or EAC at follow-up. Nonprogressors were defined as patients without neoplastic progression during follow-up.

protocol, performed by experienced gastroenterologists.[24] Upper endoscopy biopsies were taken according to the Seattle protocol.[25] Duration of follow-up was calculated for each patient from the date of LGD endoscopy to the most recent endoscopic procedure with biopsies or the date of endoscopy in which HGD or EAC was diagnosed.

Study Design

Several histologic criteria for LGD are mentioned in the guidelines of the British Society of Gastroenterology[2]: loss of surface maturation, clonal step (sharp demarcation between nondysplastic epithelium and normal/reactive epithelium), loss of polarity, mucin depletion, stratification of nuclei, nuclear form, and nuclear features (enlargement, pleomorphism, hyperchromasia, prominent nucleolus), as well as increase in apoptosis and mitosis. To refine these histologic criteria, all 4 participating gastrointestinal pathologists discussed each of the individual criterion in a consensus meeting, and specific definitions for each of the criteria were documented. Therefore, 17 hemotoxylin and eosin slides of patients with LGD diagnosis and progression on follow-up and 29 slides of patients without progression were used from the discovery set. Thereafter, all refined criteria were applied by each of the 4 pathologists on the remaining slides of the discovery set (20 H&E slides of 11 progressors and 71 slides of 57 nonprogressors). The most reproducible histologic criteria defined by kappa value >0.4 were selected for further statistical analysis and correlation with clinical data.

Next, the criteria were validated in patients from the ProBar study, using 58 H&E slides of 30 patients showing progression and 117 slides of 68 patients without progression. The H&E slides were individually reviewed by 2 pathologists (F.J.C.t.K. and M.D.). If discordant on one of the selected criteria, a third pathologist (K.B.) reviewed the slide for all 4 histologic criteria.

All samples of patients in the discovery cohort and validation cohort were reviewed for the presence of histologic criteria for LGD. The pathologists involved were blinded to the diagnosis made by each other as well as the clinical and histologic follow-up results. The consensus was defined as such when ≥ 2 pathologists agreed on the presence or absence of each criterion. The flow diagram of the study design is shown in Figure 1B. In case of multiple biopsies with LGD during follow-up in 1 patient, the results from the index biopsy were used for the statistical analysis (see below).

Ethics

The study was approved by the Institutional Review Board of the EMC (code MEC-2016-042) and local medical ethical committees of all participating hospitals. On the basis of the opt-out registry, used in the EMC to document the objection of patients to use excess tissue materials for scientific research, none of the included patients had opposed.

Statistical Analysis

Median and interquartile ranges were calculated for continuous variables. Characteristics of progressors and nonprogressors were compared using the Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. Biopsies were analyzed for interobserver agreement on all individual histologic criteria, by using Fleiss kappa for the discovery set[26] and Cohen's kappa for the validation set. Strength of agreement was categorized as follows: 0.00 to 0.20, poor; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, good; and 0.81 to 1.00, very good.[27]

Cumulative risk for progression was calculated using Kaplan-Meier survival curves. The impact of pathologic criteria on time until progression was quantified using Cox regression with time-dependent covariates,[28] and frailty terms were included for the discovery set to account for patients with multiple progressions.[29] In the validation set, we performed Cox regression analysis with time-dependent covariates; no frailty terms were required, as each patient had at the most 1 progression. Multivariable Cox regression was corrected for patient age at endoscopy, length of the Barrett segment, and the presence of esophagitis. The predictive value of the combination of criteria was calculated after the optimal cutoff was determined using a receiver operating characteristic (ROC) curve and Youden's index.

Statistical calculations were performed using the statistical package for the social sciences (SPSS version 20.0; IBM Corp., Armonk, New York) and R version 3.2.1 (Vienna, Austria). Fleiss kappa was calculated using the irr package in R; Cox regression was performed using the survival package in R.

RESULTS

Patients and Characteristics

In total, 204 patients with BE were originally included in this study, 90 in the discovery, and 114 in the validation set (Fig. 1A). After exclusion for various reasons, 84 and 98 BE patients remained in the discovery and validation set, respectively. From 15 progressors in the discovery set, 11 had HGD in the past (treated by radiofrequency ablation and endomucosal resection), in contrast to none of the 30 progressors, in the validation set, who had no prior history of HGD or EAC.

Patient characteristics of the finally included cases in both data sets are given in Table 1. No statistical differences between both cohorts were found concerning sex, BE length, time of follow-up, or number of endoscopies performed. The patients of the discovery set were significantly younger, with a median age of, respectively, 67.7 years compared with 70.7 years in the validation set ($P=0.025$). The patient characteristics specified for progressors versus nonprogressors are given in supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/PAS/A614).

Histologic Criteria for LGD and Prediction of Progression in the Discovery Set

Four pathologists scored all H&E slides from the discovery set patients using the 12 histologic criteria for

TABLE 1. Demographics of All Included BE Patients

	Discovery Set (N = 84)	Validation Set (N = 98)	*P*
Age at biopsy (y)			
Median (IQR)	67.7 (57.9-74.0)	70.7 (62.9-75.6)	0.025‡
Sex (n [%])			
Male	69 (82.1)	76 (77.6)	0.443†
Female	15 (17.9)	22 (22.4)	
Smoking (n [%])			
Yes	12 (14.3)	11 (11.2)	0.266†
No	57 (67.9)	86 (87.8)	
Not available	15 (17.9)	1 (1.0)	
Use of alcohol (n [%])			
Yes	52 (61.9)	72 (73.5)	0.783†
No	17 (20.2)	26 (26.5)	
Not available	15 (17.9)	0	
Esophagitis during follow-up (n [%])			
Yes	4 (4.8)	88 (89.8)	0.264*
No	80 (95.2)	10 (10.2)	
Length of BE (median [IQR])			
	5.0 (3.0-7.0)	5.0 (3.0-7.0)	0.994‡
Follow-up			
Median (IQR) (y)	7.5 (3.5-9.1)	5.3 (2.8-8.4)	0.191‡
Endoscopies			
Median number (IQR)	5.5 (4.0-6.75)	6.0 (4.0-7.0)	0.123‡
No. biopsies from individual patient			
Median number (IQR)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.967

*Fisher exact test.
†Pearson χ^2 test.
‡Mann-Whitney U test.
IQR indicates interquartile range.

LGD,[2] which had been discussed and specified by the involved pathologists during a prior consensus meeting (supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). Eight criteria showed a poor to fair interobserver agreement (kappa, −0.16 to 0.36) in the discovery set and were disregarded from further analysis (supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). The remaining 4 criteria, including loss of surface maturation (defined as no maturation of the epithelium seen on low power from the proliferation zone up to the surface), mucin depletion (defined as almost total to total disappearance of mucus from the surface columnar cells on high power), nuclear enlargement (defined as a nuclear size at least 2× as large as the nuclei of the normal not inflamed columnar epithelium), and increase of mitosis (defined as at least 1 mitosis at the epithelial surface or in the neck of the crypts; mitoses in the base of the crypt are disregarded), had a moderate agreement in the discovery set (kappa value, 0.55, 0.51, 0.41, and 0.48, respectively). The percentage of agreement for these criteria varied between 64.9% and 91.5% (supplemental Table 3, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). Histologic examples of the 4 criteria are given in Figure 2. In the multivariable Cox regression analyses, corrected for sex, age, length of BE, and esophagitis, all 4 parameters were significantly associated with neoplastic progression (Table 2; hazard ratio [HR], 5.93 (95% confidence interval—CI, 2.02-17); HR, 4.54 (95% CI, 1.55-13); HR, 4.23 (95% CI, 1.28-14); and HR, 7.27 (95% CI, 2.46-21) (see also supplemental Table 4, Supplemental Digital Content 1, http://links.lww.com/PAS/A614, for univariable analysis).

When combining these 4 criteria in a single panel, the most predictive cutoff for progression was calculated using an ROC curve and corresponding Youden index (supplemental Fig. 1, Supplemental Digital Content 2, http://links.lww.com/PAS/A615, and supplemental Table 5, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). This panel was considered to be positive if ≥ 2 criteria were present. Differences in progression time were found depending on the number of positive criteria: 9.0 years (95% CI, 8.2-9.8) for LGD with up to 1 criterion compared with 3.8 years (95% CI, 3.0-4.7) for LGD with ≥ 2 criteria. The corresponding Kaplan-Meier curve is depicted in Figure 3A. This shows a clear separation between patients with up to 1 criterion and those with >2 criteria, compared to the LGD diagnosis alone. During follow-up of a maximum of 10 years, 9.9% of the patients with up to 1 criterion showed progression, in comparison with 43.8% in biopsies with ≥ 2 criteria present (supplemental Table 6, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). In a multivariable Cox regression analysis, patients with 2 to 4 criteria in their first biopsy with LGD showed a significantly higher risk of progression to HGD and EAC, compared with patients with up to 1 criteria (HR, 5.47; 95% CI, 1.81-17; $P = 0.002$).

Validation of the Histologic Criteria Panel and Individual Contribution of the Criteria for the Prediction of Progression

The interobserver agreement and predictive value of the criteria loss of surface maturation, mucin depletion, nuclear enlargement, and increase of mitosis were validated on the independent patient set. Two expert pathologists (M.D. and F.J.C.t.K) evaluated 175 H&E slides of 98 patients followed-up prospectively in the ProBar study. Thereby, a moderate or good interobserver agreement for all 4 criteria was found (kappa values: loss of maturation, 0.61; mucin depletion, 0.50; nuclear enlargement, 0.47; increase of mitosis, 0.46; combination of the criteria, 0.61; see supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/PAS/A614).

The panel consisting of these 4 distinct histologic criteria segregated patients with LGD diagnosis into prognostic groups ($P < 0.001$) (see Fig. 3B for corresponding Kaplan-Meier curve). When correlating with follow-up by multivariable Cox regression analysis, these criteria were significantly associated with neoplastic progression (HR, 3.41 [95% CI, 1.52-7.67]; HR, 2.76 [95% CI, 1.28-5.96]; HR, 4.01 [95% CI, 1.84-8.73]; and HR, 2.91 [95% CI, 1.36-6.24]) (see Table 2, univariable analysis in supplemental Table 4, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). Patients with >2 criteria in their index LGD biopsy showed a significantly higher risk of progression to HGD or EAC compared with patients with up to one of the criteria

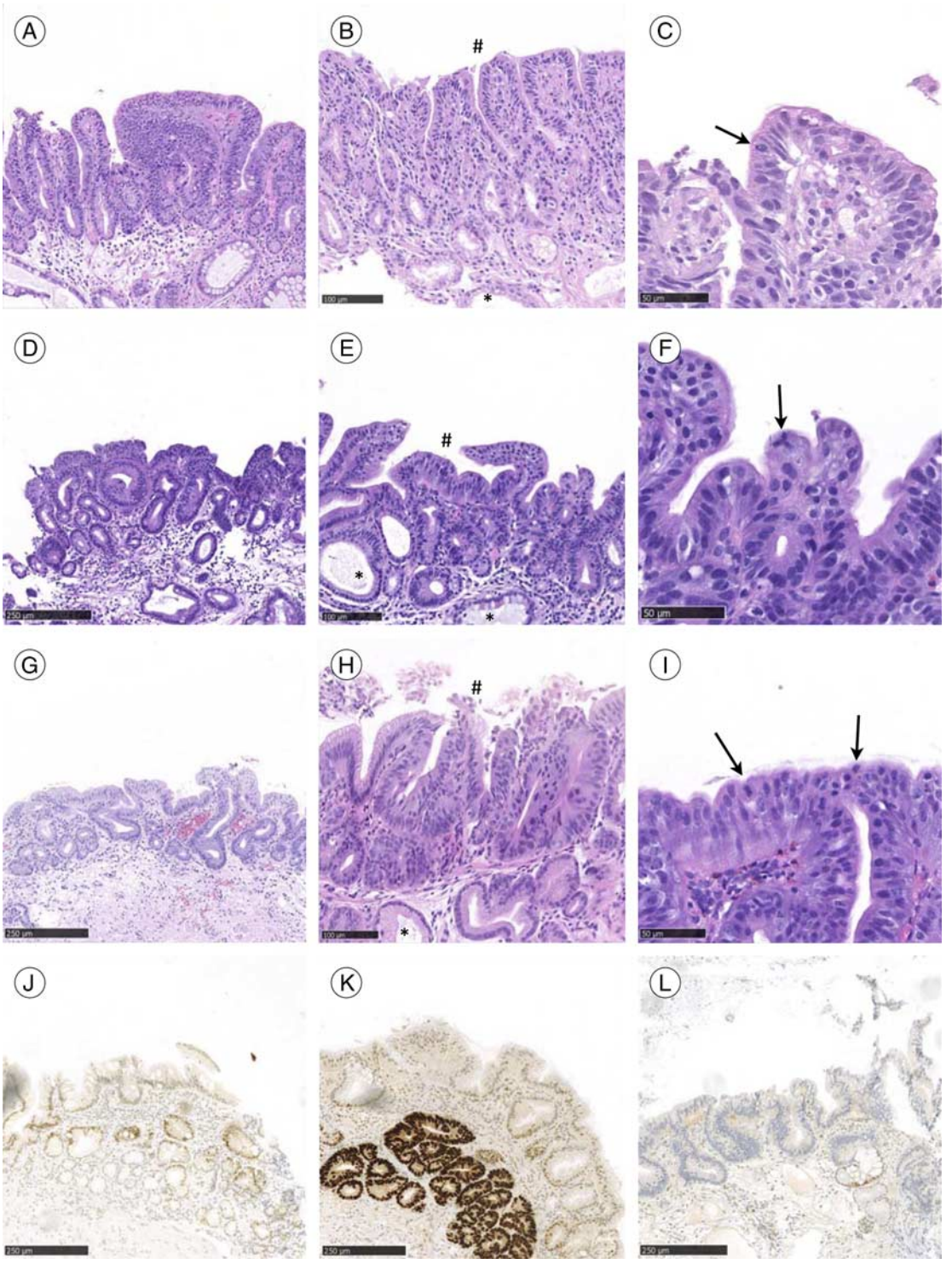

FIGURE 2. Examples of the histologic criteria and of the expression of p53. A, D, G, loss of surface maturation, that is, lack of normal epithelial maturation from the proliferation zone un to the surface. B, E, H, mucin depletion, that is, total or almost total disappearance of mucus from the surface columnar cells. Furthermore, nuclear enlargement can be appreciated if the dysplastic cells (indicated by #) are compared with the normal epithelium (indicated by *). C,F,I, increase in mitosis, indicated by arrows, present at the luminal side of the biopsy or in the neck of the crypt. J, K, L, example of p53 expression; J, normal expression of p53 with weak nuclear staining. K, overexpression of p53 with strong nuclear staining in crypts (compared with the adjacent normal expression in the epithelium). L, complete loss of p53 expression in epithelial cells.

TABLE 2. HR for Individual Histologic Criteria and Combination of These Criteria in a Multivariable Cox Regression Analysis for the Prediction of Progression to HGD or EAC

	HR in Multivariable Analysis					
	Discovery Set			Validation Set		
Histological Criteria	HR	95% CI	*P*	HR	95% CI	*P*
Loss of surface maturation	5.93	2.02-17	0.001	3.41	1.52-7.67	0.003
Mucin depletion	4.54	1.55-13	0.006	2.76	1.28-5.96	0.010
Nuclear enlargement	4.23	1.28-14	0.018	4.01	1.84-8.73	<0.001
Increase in mitosis	7.27	2.46-21	<0.001	2.91	1.36-6.24	0.006
Combination of criteria (ref. 0-1)						
2-4 criteria present	5.47	1.81-17	0.002	3.52	1.56-7.97	0.003

Adjusted for sex, age, length of BE, and esophagitis.
Ref. indicates reference.

(HR, 3.52; 95% CI, 1.56-7.97; $P=0.003$; Table 2). Data on progression incidence per patient-year, as well as 2-year and 5-year cumulative risk of progression, are given in supplemental Table 6 (Supplemental Digital Content 1, http://links.lww.com/PAS/A614).

We earlier investigated the prognostic value of p53 in the ProBar cohort and showed that the immunohistochemical pattern of p53 staining was related to progression (p53 expression was scored as normal expression, and aberrant expression was scored as being overexpression or loss of expression) (Fig. 2).[21] Therefore, we here correlated p53 with the distinct histologic criteria. Normal p53 staining and absence of the 4 histologic criteria were associated with lower progression rate (5.9% in the discovery and 18.9% in the validation set) compared with aberrant p53 staining and positive histologic criteria (42.9% and 68.0%, discovery and validation set, respectively; see supplemental Table 7, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). ROC using both histologic parameters and p53 were calculated, showing improved area under the curve, for combination of histologic criteria and p53 (see supplemental Fig. 2, Supplemental Digital Content 3, http://links.lww.com/PAS/A616).

DISCUSSION

During recent years, discussion has arisen about the value of histologic diagnosis of LGD as an instrument to determine surveillance interval in patients with BE. Many studies found only a weak correlation between LGD and the incidence of HGD/EAC with progression rate in patients with LGD as low as in all BE patients.[11,30] A major drawback is that the definition of LGD is inconsistent and includes a number of histologic features that are difficult to interpret. Lack of a precise definition of LGD causes differences in pathologic interpretation, resulting in high interobserver variability.[11,13,14,31,32] Furthermore, different

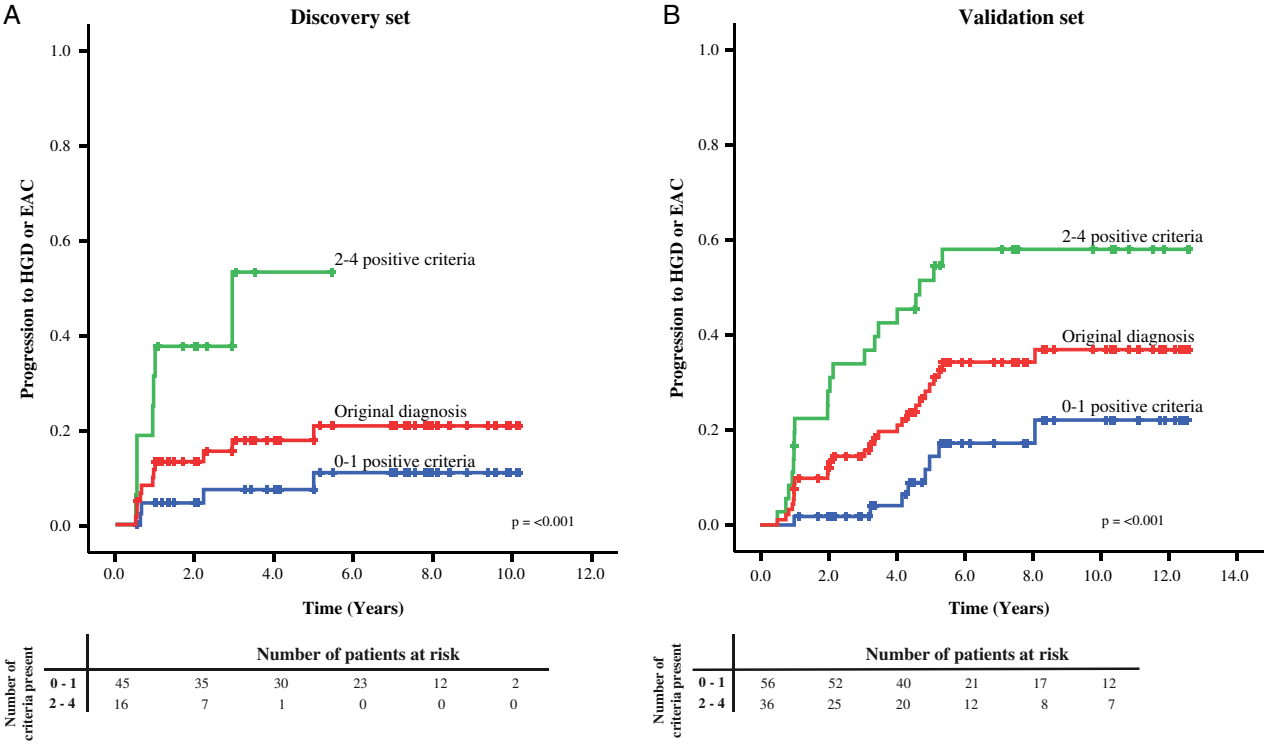

FIGURE 3. Kaplan-Meier plot, based on the first biopsy taken in the patient with LGD, showing the cumulative estimated risk of developing HGD or EAC in the discovery and validation set for the original LGD diagnosis compared with the combination of the criteria (loss of surface maturation, mucin depletion, nuclear enlargement, and increase in mitosis) (A, discovery set; B, validation set).

forms of LGD were described in the past, which contribute to the complexity of the decision making for pathologists.[33] A standardized application of well-defined histologic criteria would provide more objective methodology to analyze BE samples. Therefore, the present study was undertaken to determine whether specific histologic criteria can be identified that are interpreted reliably by pathologists and whether such criteria help to improve discrimination of patients with high versus low risk for developing neoplastic progression.

First, we challenged all 12 histologic criteria associated with LGD diagnosis for the interobserver agreement. As expected, even after refining of the criteria by the experts, the agreement between pathologists was low for most criteria. Only 4 of the 12 criteria, including loss of surface maturation, mucin depletion, nuclear enlargement, and increase of mitosis, showed a moderate or good agreement defined by kappa values >0.4. The complete agreement for the combination of the criteria was high in our study (75% to 85%; kappa value, 0.46; see supplemental Table 6, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). The high level of agreement was confirmed in the independent set of 98 patients and was higher than in most LGD studies, with kappa values being as low as 0.11 to 0.27, even among expert pathologists.[11,16,17,19] Only few earlier studies using a selected group of highly experienced European and US pathologists could demonstrate such an improved interobserver agreement for LGD diagnosis.[14,31]

Failure of maturation to the surface is suggested to be the most important characteristic of the dysplastic Barrett epithelium. Furthermore, truly dysplastic cells are likely to show significant nuclear abnormalities and mitotic activity.[34] Therefore, not surprisingly, increase in mitosis, nuclear enlargement, loss of surface maturation, and associated mucin depletion were predictive of progression to HGD/EAC in our patients (Table 2). When >1 criterion was present, high cumulative incidence of progression was detected (43.3% and 51.9% in the discovery and validation set, respectively), whereas in patients with up to 1 criterion, a low progression rate was found (8.9% and 14.3%, respectively). We did not further analyze other histologic and cytonuclear criteria, which might be useful for the diagnosis of LGD, including nuclear pleomorphism and clonal step (sharp demarcation between nondysplastic epithelium and normal/reactive epithelium). The interobserver agreement for these criteria was weak in our hands, and therefore their application for risk stratification is questionable.

Various predictive biomarkers have been studied previously in BE patients, including and especially p53. Normal expression of p53 has generally been accepted as a faint heterogenous staining to almost no nuclear staining, whereas overexpression has been defined as a homogenous strong nuclear staining in at least 1 crypt.[21] Loss of expression, defined as the complete absence of expression, has recently been recognized as a previously underestimated specific expression pattern associated with stop codon TP53 mutations.[35] The use of p53 has been shown not only to reduce interobserver variation but also to improve prediction of progression.[22,36–38] The results of the present study indicate independent additional value of p53 to the model using the specifically defined histologic features. This observation makes sense by biology, as these histologic criteria might result from chromosomal instability and multiplication of DNA elements, leading to decreased maturation and increased mitotic activity. In BE this is frequently preceded by altered p53 function, which causes a diminished feedback-loop upon DNA damage. However, BE is a heterogenous disease with higher rate of mutations than many common cancers, and various genes are involved in the development of dysplasia.[39]

The clinical management of BE patients with LGD diagnosis is still under debate. International guidelines suggest either endoscopic eradication treatment or active surveillance.[40–43] The decision for one of the options might be difficult, as the risks of endoscopic eradication therapy might outweigh its benefits, while surveillance might create significant burden to the patient and compliance problems.[41,44,45] The current recommendation is that the decision should be made on individual basis, and that endoscopic therapy is appropriate in patients at highest risk of progression.[9,41] As higher accuracy of risk prediction is improved by an expert review,[9,13,14,46–48] confirmation by at least 1 expert pathologist is indicated. However, it is not clear yet which of the histologic features drives the LGD diagnosis in the eyes of an expert.[19] This implies significant limitations for pathologists, clinicians, and patients. The problems in the interpretation come to light when observing the significant differences in progression rates reported in the literature.[11,13,14,31,32] This is also true for the geographical differences, as European pathologists might have higher interobserver agreement compared with US pathologists.[14,19,31] In general, if all pathologists would use the same histologic criteria according to standardized protocol, this could contribute to a more accurate decision-making in daily practice. Our study is intended to be the first step toward standardization of pathologic assessment of BE samples. Application of a simple histologic panel using the 4 aforementioned criteria is feasible not only for expert BE pathologists but also for pathologists with less experience in the field of BE after appropriate histologic training pertaining to the 4 specific criteria.

There are, however, sources of possible bias in our study population to be kept in mind. Because of the retrospective set-up of the study, not all clinical data were noted in a uniform manner, although long-term follow-up data for progression was known for each patient. As EMC is a referral center for complex endoscopic procedures, a high proportion of patients with prior HGD/EAC were found in the discovery set. Therefore, interpretation of progression rate might be limited for a more general hospital. However, this study was not intended as an incidence report but was designed to develop a new tool for improved prediction of progression in patients with LGD. Because the results derived from the discovery cohort might have been impacted by the fact that the majority of

the progressors in this group had recurrence of LGD and a history of HGD or EAC, an independent group of patients with LGD diagnosis derived from the ProBar cohort was studied.[20–22] ProBar patients were prospectively followed-up according to a stringent follow-up scheme and standardized endoscopy and biopsy protocol. The progression rate for the baseline LGD diagnosis in patients derived from this cohort is comparable to recent European BE studies, being 30%.[14,15,18] Furthermore, the follow-up period of some patients could be considered short, although the majority (75%) of patients without progression were followed-up for at least 4 years. The predictive value of the criteria, however, also remained significant in a more stringent analysis applying a 3-year follow-up (supplemental Table 8, Supplemental Digital Content 1, http://links.lww.com/PAS/A614). In summary, we have shown that specific histologic criteria including loss of maturation, mucin depletion, nuclear enlargement, and increase of mitosis stand out from other histologic criteria showing at least moderate interobserver agreement and may be valuable to improve prediction of neoplastic progression in patients with LGD diagnosis. This finding might have great impact on the current surveillance practice, as these specific criteria could be used by a broader pathology community. Until now, the majority of patients diagnosed with LGD according to current standards undergo intensified follow-up, which is unnecessary, as the diagnosis is false, and hence the risk of progression is low. In contrast, the presence of criteria proposed in the current study indeed indicates a high risk of progression, which has important management consequences, such as a therapeutic intervention to ablate the dysplastic mucosal surface or intensified follow-up. In the absence of these criteria, patients could be followed-up less rigorously. Future studies in a prospective setting are warranted to confirm our observations.

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