What makes an expert Barrett's pathologist?

Concordance and pathologist expertise within a digital review panel

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CHAPTER 3

BARRETT'S ESOPHAGUS PATIENTS WITH CONFIRMED AND PERSISTENT LOW-GRADE DYSPLASIA ARE AT INCREASED RISK OF NEOPLASTIC PROGRESSION

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ABSTRACT

Background & Aims

The diagnosis of low-grade dysplasia (LGD) in Barrett's esophagus (BE) is subjective and reported outcomes vary. Using data from a multicenter study of endoscopic therapy, the SURF Trial, we assessed potential predictors of progression to high-grade dysplasia or esophageal adenocarcinoma (HGD/EAC), in LGD.

Methods

We included 255 patients (78% men; mean age 63 years) in whom three expert pathologists reviewed the baseline as well as subsequent LGD specimens. All three pathologists separately evaluated each biopsy. Endpoint was development of HGD/EAC. Univariate logistic regression assessed the relationship between the endpoint and predictor variables, including number of pathologists confirming LGD, multifocality of LGD and persistence of LGD over time.

Results

Of 255 patients, 45 (18%) developed HGD/EAC during median 42 months follow-up (IQR 25-61) and a median of 4 endoscopies (IQR 3-6). The number of pathologists confirming LGD was strongly associated with progression, with a 47-fold increase when all three pathologists agreed on LGD (odds ratio (OR) 47.14; 95% CI, 13.10 to 169.70). When LGD was confirmed at baseline as well as on the subsequent endoscopy, the odds of neoplastic progression were highly increased (OR 9.28; 95% CI, 4.39 to 19.64). Multifocal LGD was not significantly associated with neoplastic progression.

Conclusions

The number of pathologists confirming LGD and persistence of LGD over time predict risk of neoplastic progression in BE patients with LGD. These simple, readily available variables can help stratify risk in BE with LGD and select patients for prophylactic ablation therapy.

INTRODUCTION

In Barrett's esophagus (BE), a precursor to esophageal adenocarcinoma (EAC), the squamous epithelial lining of the distal esophagus is replaced by columnar epithelium containing goblet cells. [1] If malignant progression in BE occurs, this is thought to develop through subsequent grades of dysplasia classified as non-dysplastic BE (NDBE), low-grade dysplasia (LGD) and high-grade dysplasia (HGD), eventually resulting in EAC. [1,2] Current international guidelines recommend expert histological confirmation of any dysplasia diagnosis in BE. Patients with HGD or EAC are recommended to undergo endoscopic or surgical therapy, depending on local expertise, patient fitness, and depth of invasion. [2–4] LGD in BE is an accepted risk factor for malignant progression, but due to difficulties in diagnosing LGD, the optimal management strategy for this patient group has been the subject of debate. This is mainly due to the highly variable neoplastic progression rates reported for LGD. In some studies, the neoplastic progression risk of LGD is not much higher than that of non-dysplastic BE, and has been reported as less than 1.5% per patient-year. [5–7] Several other studies have suggested a much higher progression rate of up to 13.4% per patient year. [8–11] These heterogeneous findings likely reflect varying degrees of misclassification between LGD and non-dysplastic BE (NDBE). Despite difficulties in making this diagnosis, if LGD is confirmed after expert review, current guidelines recommend offering these patients intensified endoscopic surveillance every 6-12 months, or considering them for radiofrequency ablation (RFA). [2,3,12]

A recent randomized controlled trial (the SUrveillance vs. RadioFrequency Ablation, or SURF Trial) compared endoscopic surveillance to RFA in BE patients with a confirmed diagnosis of LGD. [13] The SURF Trial demonstrated the superiority of RFA over surveillance, with an absolute risk difference of developing HGD/EAC of 25%, in favor of the patient group randomized to RFA. However, in 28% of patients in the surveillance arm of the SURF Trial, the LGD diagnosis was not reproduced on any of the four follow-up endoscopies, which likely reflects overstaging of initial biopsies. We can assume that a similar percentage of patients in the RFA arm thus underwent ablation for non-dysplastic BE. To date, however, the ability to accurately predict which patients with confirmed LGD will progress to more advanced disease is limited.

In the SURF Trial, histological confirmation of LGD on a single time point by a single expert pathologist sufficed for inclusion. However, additional factors may help to

correctly identify BE patients with confirmed LGD who will progress to HGD/EAC, as well as those who will fail to demonstrate dysplasia during follow-up. LGD confirmation by multiple pathologists instead of a single pathologist diagnosis may carry a higher risk of progression. [14] Patients harboring multifocal dysplasia in their BE segment may also carry an increased risk of progression compared to patients with only focal dysplasia (spatial distribution of LGD).[15,16] Additionally, if LGD is confirmed on multiple endoscopies over time (temporal distribution of LGD), the risk of progressing to HGD/EAC may be increased as well. [13]

Among BE patients with LGD who were screened for the SURF trial, we aimed to investigate the risk of neoplastic progression associated with the number of expert pathologists confirming LGD, the spatial distribution and the temporal distribution of LGD.

METHODS

Source population

BE patients with a primary diagnosis of LGD were identified in nine Barrett's treatment centers in Europe and their referring institutions. The main inclusion criterion for the SURF Trial was a single endoscopy with biopsies demonstrating LGD, confirmed by a single expert pathologist, in the preceding 18 months. [13] The source population for the current study consisted of all patients who underwent the baseline pathology screening for eligibility for the SURF Trial. Patients were excluded from the original SURF study in case LGD was not confirmed, LGD occurred >18 months before screening, or in case of progression to HGD/EAC before randomization. These patients were eligible for inclusion in the current study. Informed consent for the current study was obtained (patients who declined enrollment in the randomized trial were able to consent to the current study). This study was reviewed by the institutional ethics committee.

Expert histological review

Patients who received RFA treatment were excluded since they were effectively ablated and, therefore, no relevant natural history outcomes were available. In all remaining patients, we aimed to retrieve the histopathological specimens with

intestinal metaplasia and a referral diagnosis of LGD, used for the SURF Trial pre-assessment, from the referring hospitals.

The central expert pathology panel consisted of 5 pathologists (FJWtK, CAS, SLM, MV, GJAO). The expert pathologists who participated in this study were considered as such by their (international) peers. They are dedicated to the field of Barrett's for a minimum of 5 years (range 5-30 years) and have a minimum caseload of 5-10 cases per week of which 75% is dysplastic. All pathologists have participated in the Dutch Barrett advisory committee for many years. [10,11,17] They also form the basis of the new national advisory platform for neoplasia of the esophagus, which will facilitate all LGD reviews in the Netherlands. All pathologists participated in multiple training programs for endoscopists and pathologists (www.best-academia.eu) and each has co-authored more than 10 peer reviewed publications in this field. For the purpose of the current study, three expert pathologists (FJWtK, CAS, SLM) independently reviewed all histological slides. For each endoscopy, these three pathologists separately evaluated each available level of biopsies. The presence and degree of dysplasia was separately recorded for each biopsy and classified according to the Vienna classification into NDBE, IND, LGD, HGD or EAC. [18] The BE segment was considered multifocal dysplastic if two or more biopsies showed LGD. These could be random as well as targeted biopsies obtained from a single or multiple locations. We excluded patients in whom one or more expert pathologists diagnosed HGD/EAC at baseline.

Outcomes, endoscopic and histological follow-up

To identify all follow-up endoscopic procedures with biopsies among non-SURF participants, we searched the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA database) for additional pathological samples from these patients. The PALGA database is a nationwide database which includes the reports from all pathology laboratories in the Netherlands since 1991. [19] All follow-up endoscopy and pathology reports for these patients until January 2014 were then retrieved from the respective hospitals. Patients who were assigned to the control group in the SURF trial underwent high-resolution endoscopy at 6 and 12 months after inclusion and annually thereafter.[13] Non-SURF participants underwent follow-up endoscopy in accordance with international guidelines (6-12 month interval in case of LGD, 3-5 year interval in case of NDBE). The Seattle biopsy protocol (4-quadrant biopsies from every 2 cm interval of BE epithelium) was adhered to in all patients.

In case HGD/EAC was detected, the original histology slides were retrieved and reviewed by one of the expert pathologists from the panel. Patient demographic information, endoscopic results (including procedure date, esophageal landmarks, presence of visible abnormalities) and histological outcome were entered in a dedicated database using a standardized data collection instrument.

Data analysis

The first LGD diagnosis that was reviewed by the expert pathology panel was considered the baseline endoscopy. The endpoint of the study was development of HGD/EAC diagnosed in clinical care by a community pathologist and confirmed by one of the expert pathologists from the panel, who was blinded for the previous histological outcomes. Duration of follow-up was calculated from the date of first LGD endoscopy with expert histology review to the date of the most recent endoscopic procedure with a histological diagnosis (non-progressors) or to the endoscopy date on which HGD/EAC was first detected (progressors). We examined effects of the number of expert pathologists confirming LGD, the number of biopsies with LGD (analysis per pathologist) and persistence of LGD over time (analysis per pathologist). We performed logistic regression to estimate the rate of progression to HGD/EAC during follow-up. We adjusted for gender and length of the BE segment. We used the Mantel-Haenszel method to estimate the common odds ratio across strata of individual pathologists. Kaplan–Meier survival analysis was performed to estimate the cumulative risk of progression to HGD/EAC. The predictive value of confirmed LGD was assessed for each individual pathologist. Subsequently, we examined the rate of progression in relation to confirmed LGD for each pair of pathologists, as well as for a concurrent diagnosis by all three pathologists. We examined the rate of progression to HGD/EAC in relation to the mean number of biopsies with confirmed LGD obtained from the baseline endoscopy. We also separately performed this analysis for each individual pathologist. The predictive value of persistent confirmed LGD was assessed for each individual pathologist. For each individual pathologist we selected patients in whom LGD was confirmed at baseline. For this analysis we only included those who underwent two or more follow-up endoscopies, and in whom the first follow-up endoscopy was performed within 18 months from baseline. That first follow-up endoscopy was then regarded as the baseline endoscopy, which converted the initial baseline endoscopy to a historic endoscopy. If the pathologist in question confirmed LGD on the new baseline endoscopy, LGD was considered persistent. Therefore, persistent LGD was

defined as confirmed LGD at baseline as well as on the previous endoscopy (performed less than 18 months before). Statistical calculations were performed using SPSS 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patients

Of 511 patients screened for the SURF trial, we excluded 71 patients who underwent RFA (**Figure 1**). The original specimens could not be retrieved in 28% of the source population (14 SURF Trial participants and 128 non-SURF participants), mainly patients enrolled in centers outside the Netherlands. Four patients did not consent to the study and 18 patients did not undergo endoscopic follow-up, resulting in an expert histology review population of 276 patients. Twenty-one patients were subsequently excluded since one or more expert pathologists diagnosed HGD/EAC at baseline, resulting in a study population of 255 patients (**Figure 1**). The mean age of included patients was 63.0 years (SD 10.2) and 78% were men. Further baseline characteristics are shown in **Table 1**. Of 255 included patients, a substantial minority (n = 113 [44%]) did not have LGD confirmed by any of the 3 expert pathologists. Median duration of follow-up was 42 months (IQR 25-61) and a median of 4 endoscopies (IQR 3-6) were performed.

Number of pathologists confirming LGD

During endoscopic follow-up, 45/255 (18%) patients developed neoplastic progression. The odds of neoplastic progression were 10-fold increased when a single pathologist confirmed LGD (OR 10.03; 95% CI, 6.40 to 15.72). For each of the three expert pathologists separately the odds of progression were 8 to 13-fold increased when LGD was confirmed compared with patients in whom LGD was downstaged (**Table 2**).

Figure 1: Flowchart of patients in this study

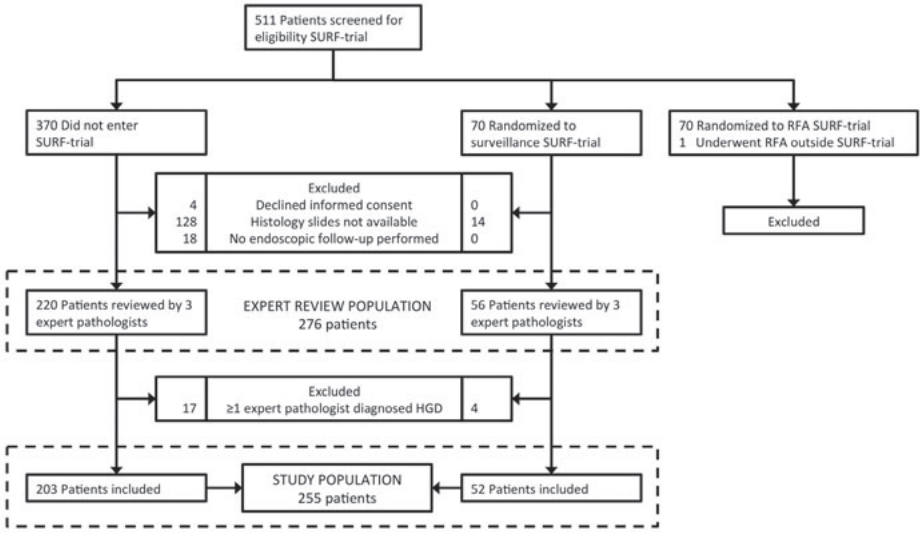


Table 1: Demographic and clinical characteristics.

Characteristic	All patients (N=255)
Age, *y,* mean ± SD*	63.0 ±10.2
Male, n (%)	199 (78%)
Time since Barrett's diagnosis, *y,* median (IQR†)	3.4 (0-8)
Length of Barrett's segment, *cm*, median (IQR)	4 (3-7)
Circumferential Barrett's extent, *cm*, median (IQR)	2 (1-5)
No. of pathologists confirming LGD, n (%)	
0	113 (44%)
1	60 (24%)
2	34 (13%)
3	48 (19%)

*SD, standard deviation; †IQR, interquartile range.

Table 2: Univariate analysis of the number of pathologists confirming low-grade dysplasia (LGD) as a predictor of neoplastic progression among 255 patients screened for the Surveillance vs Radiofrequency Ablation Trial

Variable	Events, n (%)	OR (95% CI)	Adjusted OR (95% CI)*
Perspective of a single expert pathologists' review			
Pathologist 1			
LGD downstaged	7/155 (5%)	1 (referent)	1 (referent)
LGD confirmed	38/100 (38%)	12.96 (5.49 - 30.59)	11.29 (4.75 - 26.85)
Pathologist 2			
LGD downstaged	10/157 (6%)	1 (referent)	1 (referent)
LGD confirmed	35/98 (36%)	8.17 (3.81 - 17.50)	7.52 (3.48 - 16.26)
Pathologist 3			
LGD downstaged	13/181 (7%)	1 (referent)	1 (referent)
LGD confirmed	32/74 (43%)	9.85 (4.76 - 20.39)	8.82 (4.22 - 18.46)
Cumulative analysis of all 3 pathologists			
LGD downstaged	-	1 (referent)	-
LGD confirmed	-	10.03 (6.40 - 15.72)	-
Perspective of a two expert pathologists' review			
Pathologist 1 and 2			
0 confirmed, 2 downstaged	3/120 (3%)	1 (referent)	1 (referent)
1 confirmed, 1 downstaged	11/72 (15%)	7.03 (1.89 - 26.16)	6.72 (1.78 - 25.31)
2 confirmed, 0 downstaged	31/63 (49%)	37.78 (10.85 - 131.59)	31.41 (9.98 - 109.83)
Pathologist 1 and 3			
0 confirmed, 2 downstaged	7/141 (5%)	1 (referent)	1 (referent)
1 confirmed, 1 downstaged	6/54 (11%)	2.39 (0.77 - 7.48)	2.11 (0.67 - 6.63)
2 confirmed, 0 downstaged	32/60 (43%)	21.88 (8.77 - 54.55)	18.91 (7.49 - 47.74)
Pathologist 2 and 3			
0 confirmed, 2 downstaged	5/138 (4%)	1 (referent)	1 (referent)
1 confirmed, 1 downstaged	13/62 (21%)	7.06 (2.39 - 20.83)	7.00 (2.33 - 20.99)
2 confirmed, 0 downstaged	27/55 (49%)	25.65 (9.09 - 72.40)	21.98 (7.74 - 62.46)
Cumulative analysis of all 3 pathologist pairs			
0 confirmed, 2 downstaged	-	1 (referent)	-
1 confirmed, 1 downstaged	-	4.99 (2.60 - 9.60)	-

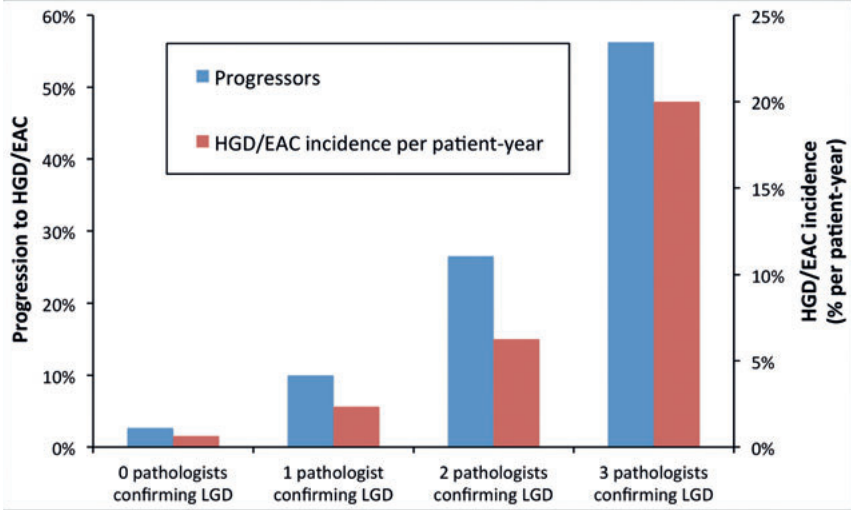
Table 2: Continued

Variable	Events, n (%)	OR (95% CI)	Adjusted OR (95% CI)*
2 confirmed, 0 downstaged	-	26.86 (14.74 - 48.94)	-
Perspective of a three expert pathologists' review			
0 confirmed, 3 downstaged	3/113 (3%)	1 (referent)	1 (referent)
1 confirmed, 2 downstaged	6/60 (10%)	4.07 (0.98 - 16.92)	3.82 (0.91 - 15.99)
2 confirmed, 1 downstaged	9/34 (27%)	13.20 (3.33 - 52.31)	11.97 (2.98 - 48.11)
3 confirmed, 0 downstaged	27/48 (56%)	47.14 (13.10 - 169.70)	38.79 (10.71 - 140.50)

*Adjusted for sex and Barrett's segment length

From the perspective of having two pathologists review the baseline LGD diagnosis, the odds of neoplastic progression were 27-fold increased (OR 26.86; 95% CI, 14.74 to 48.94) for patients in whom both pathologists confirmed LGD compared with patients in whom both pathologists downstaged LGD. The OR's ranged from 22 to 38 for the different pairs of pathologists. From the perspective of having three pathologists review the baseline LGD diagnosis, confirmation by all three expert pathologists was associated with an odds ratio of 47.14 (95% CI, 13.10 to 169.70). The odds ratios for the different analyses did not materially alter after adjusting for gender and length of the Barrett's segment (**Table 2**). The annual incidence rate of HGD/EAC gradually increased with an increasing number of pathologists confirming LGD. Patients who were downstaged by all three expert pathologists only had an annual HGD/EAC incidence rate of 0.6% per patient-year. The annual progression rate increased to 2.4% when a single pathologist confirmed LGD, 6.3% when two pathologists confirmed LGD and 20.0% when all three pathologists confirmed LGD (**Figure 2**). The cumulative incidence of neoplastic progression in relation to the number of pathologists confirming LGD is demonstrated in the Kaplan-Meier survival curve in **Figure 3**.

Figure 2: Proportion of patients progressing to and annual incidence rate of HGD or EAC in relation to the number of pathologists confirming LGD at baseline



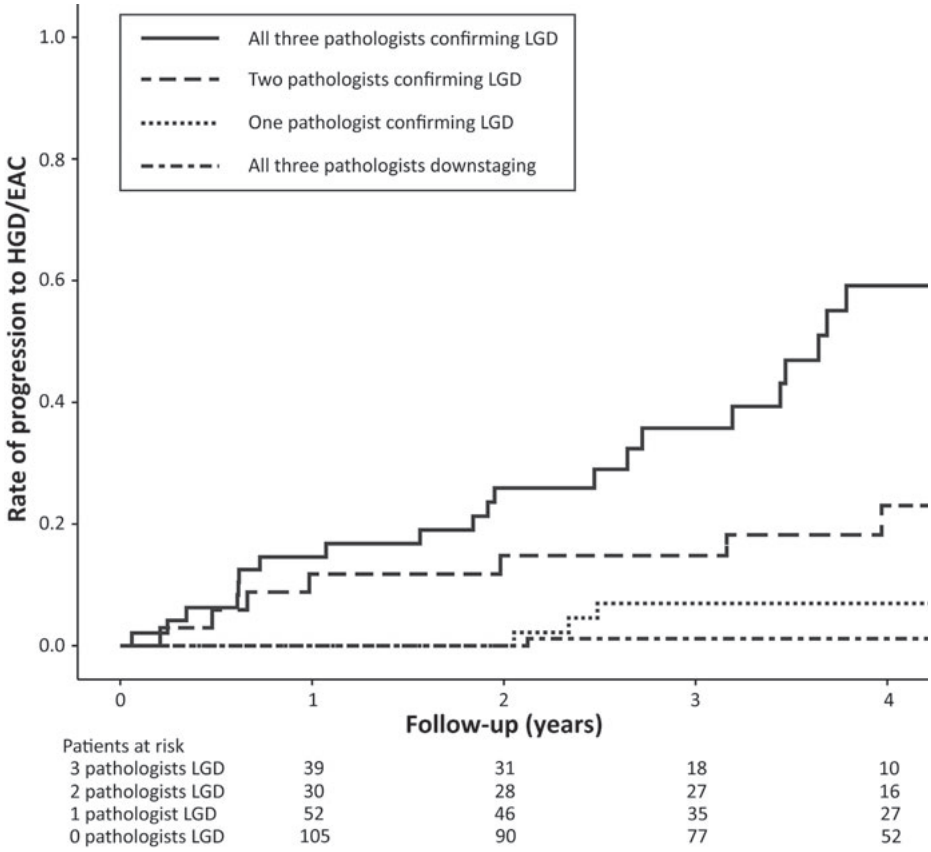
When LGD was confirmed in only a single biopsy, the odds of progression were 6-fold increased compared to nil biopsies with confirmed LGD (**Table 3**). The odds were 10-fold increased when two or more biopsies were diagnosed with LGD, yet compared to a single biopsy confirmation the increase in odds was not statistically significant. After adjusting for gender and length of the Barrett's segment these results did not change. The prognostic significance of multifocality was not statistically different for the three individual participating pathologists (**Supplementary Table 1**).

Table 3: Univariate analysis of the mean number of biopsies with confirmed low-grade dysplasia as a predictor of neoplastic progression among 255 patients screened for the Surveillance vs Radiofrequency Ablation Trial

				Adjusted
Mean no. of biopsies with LGD	**Events, n (%)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)***
0	13/173 (8%)	1 (referent)	Excluded	Excluded
1	13/39 (33%)	6.15 (2.57 - 14.74)	1 (referent)	1 (referent)
≥2	19/43 (44%)	9.74 (4.27 - 22.25)	1.58 (0.65 - 3.89)	1.17 (0.45 - 3.05)

*Adjusted for gender and Barrett's segment length

Figure 3: Kaplan-Meier survival curve demonstrating the cumulative risk of HGD or EAC in relation to the number of pathologists confirming LGD at baseline



LGD reproduced on subsequent endoscopies

For this analysis we selected 63, 72 and 54 patients for pathologist 1 through 3, respectively (see paragraph 'Data analysis'). Patients in whom LGD was confirmed on two subsequent endoscopies had a 9-fold increased odds of progression (OR 9.28; 95% CI, 4.39 to 19.64) compared with patients in whom LGD was not reproduced. The odds of neoplastic progression ranged from 4.96 to 18.00 for the individual expert pathologists (**Table 4**). The odds ratios were similar after adjustment was made for gender and length of the Barrett's segment.

Table 4: Univariate analysis of persistence of low-grade dysplasia (LGD) on subsequent endoscopies as a predictor of neoplastic progression among patients in whom LGD was confirmed at baseline by each individual pathologist

Variable	Events, n (%)	OR (95% CI)	Adjusted OR (95% CI)*
Pathologist 1			
LGD reproduced on first follow-up			
No	3/34 (9%)	1 (referent)	1 (referent)
Yes	16/29 (55%)	12.72 (3.16 - 51.21)	15.84 (3.55 - 70.73)
Pathologist 2			
LGD reproduced on first follow-up			
No	5/36 (14%)	1 (referent)	1 (referent)
Yes	16/36 (44%)	4.96 (1.57 - 15.68)	5.43 (1.63 - 18.11)
Pathologist 3			
LGD reproduced on first follow-up			
No	3/30 (10%)	1 (referent)	1 (referent)
Yes	16/24 (67%)	18.00 (4.16 - 77.81)	23.69 (4.51 - 124,38)
Cumulative analysis			
LGD reproduced on first follow-up			
No	-	1 (referent)	-
Yes	-	9.28 (4.39 - 19.64)	-

*Adjusted for gender and Barrett's segment length

Seven patients who had LGD confirmed at baseline but who did not have LGD confirmed on the first follow-up endoscopy progressed to HGD/EAC. In five (71%) of these patients, LGD was confirmed on a subsequent follow-up endoscopy prior to neoplastic progression. Two patients progressed without interval LGD. One patient underwent esophagectomy for poorly differentiated submucosal carcinoma, which was detected within one year of follow-up. No residual cancer or positive lymph nodes were detected and the patient remained disease-free 53 months post-treatment. The other patient progressed to focal HGD, was successfully treated with RFA and remained disease-free 29 months post-treatment.

DISCUSSION

This study included 255 BE patients originally diagnosed with LGD, generally by community-based pathologists, and who were screened for eligibility for the SURF trial. We demonstrated that the number of expert pathologists confirming LGD predicted increasing risk of neoplastic progression. The odds of progression were 10-fold increased when a single pathologist confirmed LGD. When two pathologists confirmed LGD the odds were 27-fold increased and when all three expert pathologists agreed on LGD the odds of neoplastic progression were 47-fold increased. Furthermore, we demonstrated that patients in whom LGD persisted on subsequent endoscopies had an increased risk of developing HGD/EAC. The odds of neoplastic progression were 9-fold increased for patients with confirmed LGD in whom LGD persisted on the first follow-up endoscopy compared with patients in whom LGD did not persist on the first follow-up endoscopy.

Previous studies have demonstrated that a confirmed LGD diagnosis by expert pathologists is a strong predictor of neoplastic progression. [8–11,14] Current international guidelines recommend review of all LGD in BE by at least one expert pathologist. [3,20] The SURF Trial provided evidence that patients with confirmed LGD should be considered for ablation therapy. However, this trial also demonstrated that accurate selection of these high-risk LGD patients proved difficult, since 28% of patients in the surveillance arm of the trial had no dysplasia detected during follow-up. [13] This finding was similar to what was reported in the AIM Dysplasia (Ablation of Intestinal Metaplasia Containing Dysplasia) trial (26%), and may have resulted from limited validity of the LGD diagnosis. [21] Considerable interobserver variation for diagnosing LGD, even among expert pathologists, limits the validity of this diagnosis. A smaller sized study demonstrated that a consensus LGD diagnosis by two or three expert pathologists significantly improved prediction of progression over a single pathologist diagnosis. [14] These data, in conjunction with the results presented in the current study, suggest that confirmation of LGD by more than one expert pathologist considerably increases the risk of neoplastic progression compared with LGD cases where expert pathologists do not agree.

A second important finding of the current study is the increased risk of neoplastic progression for patients in whom LGD persists on subsequent endoscopies. Most international guidelines recommend treatment of BE patients with LGD when the

diagnosis is confirmed and persists on multiple endoscopies. [3,12] The rationale for the latter part of that recommendation originates from several studies reporting that LGD may not be reproduced when endoscopy is repeated. [13,16,22] However, there is little evidence that the risk of progression to HGD/EAC is increased for patients with persistent LGD. The SURF Trial reported higher odds of neoplastic progression in patients who had multiple endoscopies with dysplasia prior to baseline. [13] A recent pathology registry study reported a hazard ratio of 3.5 for developing HGD/EAC when LGD persisted on two subsequent endoscopies. [23] Our current study demonstrated a 9-fold increased odds of neoplastic progression when an individual expert pathologist confirmed LGD at baseline as well as on the subsequent endoscopy performed within 18 months.

The extent of LGD within the BE segment has been thought to be a risk factor for neoplastic progression. [15,16] In the current study, finding LGD at multiple *locations* within the BE segment was not associated with an increased rate of neoplastic progression whereas confirming LGD at multiple *occasions* did show an increased odds ratio. Evaluating multiple biopsies from the same endoscopic procedure at the same time point is different from evaluating biopsies of distinct endoscopies, separated by a significant amount of time.

Why might multifocality not imply increased risk of progression, while both multiple confirmations by different pathologists, as well as multiple observations of LGD over time, increase this risk? While the current data cannot definitively address this issue, one possibility is that over-interpretation of reactive histological changes as dysplasia is more likely to carry over when the same observer is evaluating multiple biopsies of the same endoscopic procedure at the same time point than when these are assessed by independent observers (i.e. multiple expert pathologists) or at independent occasions (i.e. multiple endoscopies). If this is the case, then multiple reads of LGD by a single pathologist in a single biopsy run would be expected to have little or no impact on risk, as compared to multiple reads by different pathologists, or multiple, temporally separate reviews.

A confirmed histological diagnosis of LGD is both a predictor of neoplastic progression and a marker for prevalent HGD/EAC, as is demonstrated in **Figure 3**. A number of patients developed HGD/EAC in the first year of follow-up, however, the survival curve

also demonstrates a linear course of neoplastic progression in subsequent years of follow-up. In our opinion, all cases with confirmed LGD should therefore be referred to expert centers. In these patients adequate high-quality endoscopic inspection is imperative at baseline, with a low threshold for endoscopic resection.

A strength of this study was the histological review of all biopsies by three expert pathologists, all of whom are expert based on their cumulative work in Barrett's dysplasia and participation in previous studies on BE diagnosis and treatment. [24–26] Furthermore, homogeneity between participating expert pathologists was considerable, judged by the comparable odds ratios as well as previously reported interobserver agreements. [10,11] A limitation of this study is heterogeneity in follow-up regimen and duration of included patients. Patients who participated in the SURF Trial underwent at least three years of high quality surveillance endoscopies at strictly regimented intervals, whereas patients who did not enter the SURF Trial underwent surveillance endoscopies in regular clinical practice where surveillance regimen and biopsy practice could not be mandated. However, differential misclassification of the outcome seems unlikely, since the percentage of progressors among SURF Trial participants (23%) was lower than among non-participants of the SURF Trial in whom at least one pathologist confirmed LGD at baseline (33%). Furthermore, none of the odds ratios described in the results section changed significantly on sensitivity analysis excluding all SURF trial participants (data not shown). The quality and homogeneity of the expert pathology panel is one of the strengths of the current study, but may also hamper generalizability of our findings. A different panel of pathologists might not produce equally convincing results. However, results from multiple previous studies regarding expert review of LGD and neoplastic progression risk support our contention of the value of confirmatory reads. [8–11,27] Other studies have reported conflicting results, which might be due to a relatively limited number of progression cases leading to imprecise estimates as well as histology review that was performed to a variable extent and with a different composition of pathologists in the expert panel. [7,28,29] Therefore, when the validity of the expert panel of pathologists is ensured, the current results will likely be reproducible. We may have introduced selection bias by excluding 28% of patients from our source population for whom we could not retrieve the original histology slides. This is due, in part, to the international multicenter setting of the SURF Trial, which introduced logistical and legal difficulties with retrieving histological material. However, obtaining repeat endoscopic and histologic sampling from patients

followed for a number of years is challenging. For instance, a similar US study reported that histological slides could not be retrieved in 58% of patients. [7]

Low-grade dysplasia currently represents one of the most challenging clinical dilemma within the management algorithm of BE patients. Expert histological review is instrumental in making management decisions in these patients, yet this is not widely available and quality of expert reviews is not uniform. To overcome these issues, much research effort is focused on identifying objective biomarker panels that can predict the natural course of disease. [30–34] However, to date, these biomarkers have generally not come into clinical practice. Our study suggests that simple clinical predictors of progression, which are either already available or attainable, might be used to improve risk stratification in the setting of LGD.

In conclusion, this study describes predictors of neoplastic progression in 255 BE patients with confirmed LGD who were screened for the SURF Trial. While confirmed LGD is increasingly accepted and advocated as a valid indication for RFA, this group of patients may still include those who actually have more innocent disease, who could safely be spared the cost and potential complications of endoscopic eradication therapy. The number of expert pathologists confirming LGD proved to be a strong predictor of developing HGD/EAC. Furthermore, persistence of LGD on subsequent endoscopies was associated with a considerably increased risk of neoplastic progression.

How might these findings be used in managing BE patients diagnosed with LGD? In order to confirm 'true' dysplastic changes, review of the histology slides by an expert pathologist is the first and imperative step. Where available, histological review by more than one expert pathologist can further improve risk stratification. Patients in whom multiple expert pathologists confirm LGD might immediately qualify for endoscopic ablative therapy. However, while in the Netherlands a system is in place to centralize histology reviews using electronically scanned slides, we realize that review by multiple expert pathologists might not be feasible in most clinical settings. Therefore, for patients in whom only a single expert pathologist confirms LGD as well as for patients in whom only a single expert review is available, a repeat endoscopy could further stratify risk. In this situation, if the repeat endoscopy confirmed LGD, ablative therapy could be performed.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1: Univariate analysis of the number of biopsies with confirmed low-grade dysplasia as a predictor of neoplastic progression among 255 patients screened for the Surveillance vs Radiofrequency Ablation Trial

Variable	Events, n (%)	OR (95% CI)	OR (95% CI)
Pathologist 1			
Number of biopsies with LGD			
0	7/155 (5%)	1 (referent)	Excluded
1	12/38 (32%)	9.8 (3.52 - 27.09)	1 (referent)
≥2	17/43 (40%)	13.8 (5.22 - 36.61)	1.42 (0.57 - 3.55)
Pathologist 2			
Number of biopsies with LGD			
0	10/157 (6%)	1 (referent)	Excluded
1	8/24 (33%)	7.4 (2.54 - 21.28)	1 (referent)
≥2	17/48 (35%)	8.1 (3.37 - 19.28)	1.10 (0.39 - 3.09)
Pathologist 3			
Number of biopsies with LGD			
0	13/181 (7%)	1 (referent)	Excluded
1	13/34 (38%)	8.0 (3.28 - 19.53)	1 (referent)
≥2	16/33 (49%)	12.2 (5.02 - 29.49)	1.52 (0.58 - 4.02)

NOTE. Excludes 19, 26 and 7 patients for pathologist 1 through 3, respectively, in whom the number of biopsies with LGD was not recorded