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Spatiotemporal Study of a Risk-Stratification Epigenetic-Based Biomarker Assay in Patients With Barrett Esophagus

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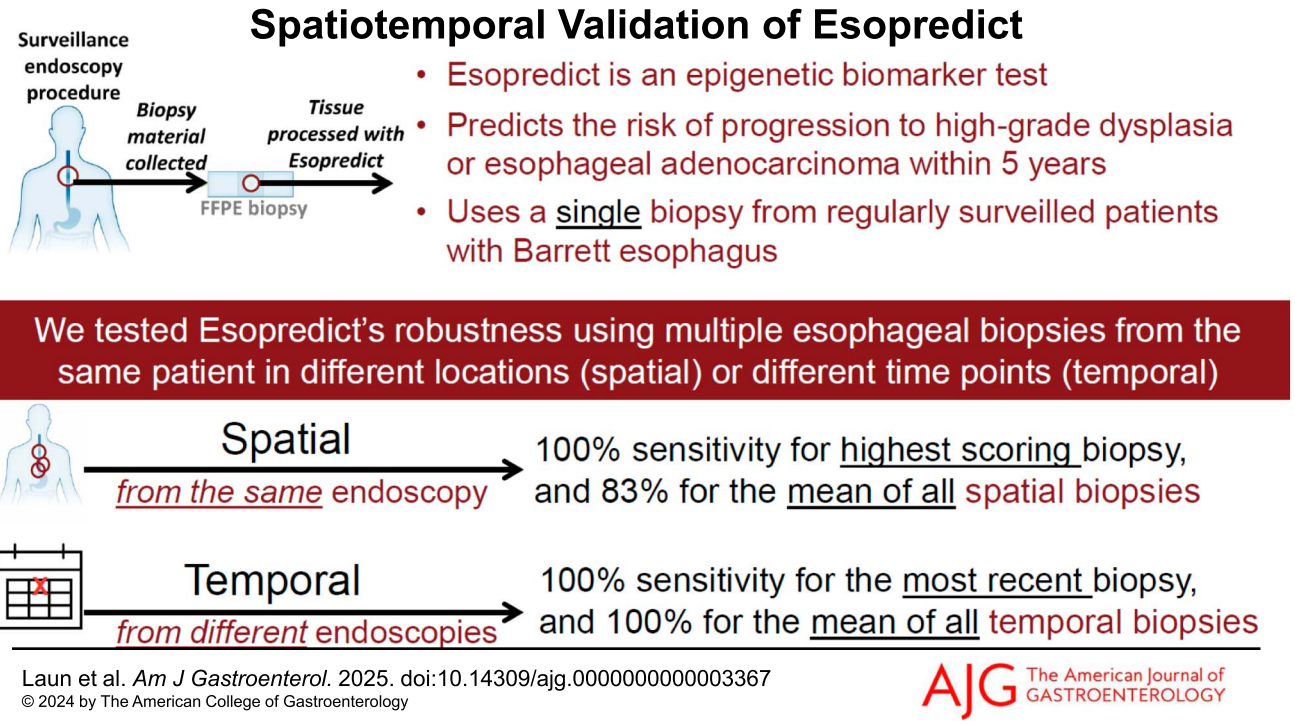
INTRODUCTION: **Barrett esophagus (BE) is the strongest known risk factor for developing esophageal adenocarcinoma (EAC), the second-most lethal cancer in the United States. Esopredict is a novel validated methylation-based biomarker assay that provides precise quantification of neoplastic progression risk in BE patients. Inherit challenges, including tissue heterogeneity, sampling error, interobserver variability, and inconsistent adherence to surveillance biopsy guidelines, may affect the predictive value results of Esopredict obtained at different anatomic locations or different sampling time points.**



Laun et al. *Am J Gastroenterol.* 2025. doi:10.14309/ajg.0000000000003367

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Received August 16, 2024; accepted January 24, 2025; published online February 12, 2025

ESOPHAGUS

METHODS: **To investigate the spatiotemporal performance of Esopredict across multiple spatiotemporal sampling points, we profiled 220 biopsies obtained from 58 BE patients, including 11 patients with overlapping spatial and temporal biopsies. We focused on spatial profiling (i.e., multiple biopsies obtained at several anatomic locations during a single endoscopy) and temporal profiling (i.e., biopsies obtained from multiple endoscopies performed at different time points). Each patient had an initial histologic diagnosis of nondysplastic Barrett esophagus, indefinite for dysplasia, or low-grade dysplasia. Final follow-up (endpoint) biopsies showed either high-grade dysplasia or EAC (progressors), or nondysplastic Barrett esophagus, indefinite for dysplasia, or low-grade dysplasia (nonprogressors). Biopsies were analyzed with Esopredict to compute a progression risk score, which quantified the likelihood of future progression to high-grade dysplasia or EAC within 5 years.**

RESULTS: **In 52 spatially profiled patients, Esopredict demonstrated a sensitivity of 81% (17/21 progressor patients), based on the highest-scoring biopsy from each patient; sensitivity increased to 100% (12/12) when end point biopsies occurred within 5 years of the index (initial) biopsy. In 28 temporally profiled patients, sensitivity was 100% (8/8 patients), based on the biopsy performed at the time point closest to the end point biopsy.**

DISCUSSION: **Esopredict showed high predictive performance in multiple spatiotemporal samples in BE patients. These data further support the use of Esopredict as a robust test to distinguish high-risk BE patients, who may benefit from endoscopic eradication therapy or increased surveillance frequency, from low-risk patients, who may be candidates for less frequent surveillance and noninterventional observation.**

KEYWORDS: epigenetics; prognostic assay; biopsy assay; esophageal adenocarcinoma; personomics; clinical decisions; predictive biomarkers

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/AJG/D576; http://links.lww.com/AJG/D577

Am J Gastroenterol 2025;120:1285–1295. https://doi.org/10.14309/ajg.0000000000003367

INTRODUCTION

Barrett esophagus (BE) is a premalignant condition caused by chronic gastroesophageal reflux disease. BE can evolve into esophageal adenocarcinoma (EAC), a highly lethal cancer, with a 5-year survival of 21% (1–6). EAC incidence in the United States has increased 7-fold over the past 4 decades (7,8). Therefore, BE patients undergo lifelong surveillance esophagogastroduodenoscopy with biopsies to monitor for precancerous dysplasia or EAC (9,10). However, histologic interpretation of endoscopic biopsies is subject to considerable interobserver variability and

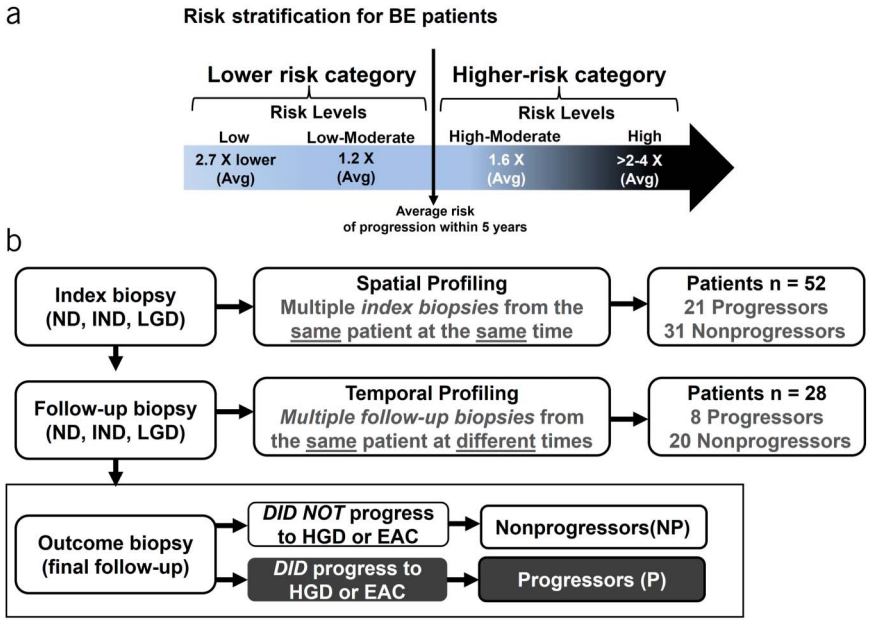

Figure 1. Risk stratification of Esopredict and consort diagram for spatiotemporal study. (**a**) Esopredict risk stratification based on previous publication (24). (**b**) Breakdown of spatial and temporal patients and the biopsies for each. A total of 58 patients and 220 biopsies were assayed with Esopredict, including 11 patients who had overlapping biopsies that met both criteria for spatial and temporal cohorts. Index biopsies are the earliest known biopsy, and outcome biopsies are the last known biopsy. Follow-up biopsies are in between index and outcome. BE, Barrett esophagus; EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia; IND, indefinite for dysplasia; LGD, low-grade dysplasia; ND, nondysplastic.

subjectivity; moreover, progression to high-grade dysplasia (HGD) or EAC can occur between screenings (11–15). Improved prediction tools to quantify the progression risk of BE patients may help identify patients who will benefit from intensified surveillance or endoscopic eradication therapy to prevent cancer progression. Endoscopic eradication therapy has high success rates, with 91% of treated patients achieving complete remission of dysplasia and 78% showing remission of underlying nondysplastic (ND) BE (NDBE), highlighting the potential impact of improved risk stratification in BE (14,16–20).

Esopredict is a molecular assay that predicts the risk of future neoplastic progression in BE patients, providing clinicians with personalized information for tailoring patient management (21–24). This epigenetic assay, which was recently studied in a retrospective multicenter cohort of 240 patients, stratifies patients into 2 primary categories: lower risk or higher risk, indicating either a below-average or above-average likelihood of progressing to HGD or EAC within 5 years (Figure 1a) (24). The Esopredict score (1–100) conveys a specific risk level of progression to HGD or EAC within 5 years. Risk categories (lower risk and higher risk) are further subdivided into low-risk levels (0–16), with a mean risk of 1.85% (nearly 3 times below the average risk of progression in BE); low-moderate risk (17–25) with a mean risk of 4.37% (close to the average risk based on prevalence); high-moderate risk (16–32) with a mean risk o 8.12%; and high-risk levels (33–100), with a mean risk of 21.5% (4 times above the average BE progression risk) (Figure 1a) (24).

This assay uses biopsy samples already obtained for pathological diagnosis during BE surveillance. The focal nature of dysplasia, which causes inherent sampling error; the subjectivity in histologic grading of dysplasia; and the inconsistency in adherence to endoscopic surveillance intervals and protocols may all adversely affect the current efficacy of surveillance esophagogastroduodenoscopy status (6,25–27). Notably, Esopredict detects specific hypermethylation events that silence the expression of important esophageal tumor suppressor genes or regulatory elements that may contribute to early esophageal carcinogenesis (19,28–34). These easily detectable molecular preneoplastic alterations may occur in adjacent non-neoplastic tissue or even precede the development of histologic dysplasia, a phenomenon known as field cancerization, or field effect, thus potentially enhancing the value of an epigenetic assay performed in multiple spatiotemporal samples (31,35–38).

Accordingly, the aim of our study was to investigate Esopredict in 2 distinct cohorts: (i) a spatial profiling cohort, comprising 52 patients (130 corresponding samples) undergoing biopsies at multiple anatomical locations during a single endoscopic procedure, and (ii) a temporal profiling cohort, consisting of 28 patients (117 samples) obtained across multiple endoscopic surveillance sessions (Figure 1b). These included 11 patients who had multiple biopsies overlapping between spatiotemporal cohorts because they met the spatial criteria (n = 27) and the temporal criteria (n = 36).

Table 1. Baseline demographics and characteristics of patients

	Spatial profiling Patients (n = 52)		Temporal profiling Patients (n = 28)	
Age (yr)			by sample n = 94	
Mean (SD)	60.2 (11.7)		64.0 (10.9)	
Median [min, max]	62.0 [21.0, 79.0]		63.0 [38.0, 87.0]	
Sex (n)				
Female	5 (9.6%)		6 (21.4%)	
Male	47 (90.4%)		22 (78.6%)	
Index biopsy (yr)				
1995–2000	6 (11.5%)		4 (14.3%)	
2000–2010	17 (32.7%)		18 (64.3%)	
2010–2015	29 (55.8%)		6 (21.4%)	
Index biopsy, dysplasia (n)			by sample n = 94	
Nondysplastic	44 (84.6%)		47 (50%)	
Indefinite for dysplasia	2 (3.9%)		20 (21.4%)	
Low-grade dysplasia	6 (11.5%)		27 (27.7%)	
Progression status based on outcome biopsy (n)				
Nonprogressor	31 (59.6%)		20 (71.4%)	
Progressor	21 (40.4%)		8 (28.6%)	
Interval between index and outcome biopsies (yr)			by sample n = 94	
Mean (SD)	5.5 (2.9)		4.3 (3.2)	
Median [min, max]	5.7 [0.3, 13.5]		3.3 [0.6, 13.5]	
Average number of biopsies (n)				
Mean (SD)	2.5 (0.7)		3.3 (1.7)	
Median [min, max]	2.0 [2.0, 4.0]		3.0 [2.0, 7.0]	
Segment length (number, and mean, SD)				
Short (<3 cm) Long (>3 cm)	16 (30.8%) 29 (33.8%)	6.2 (4.2)	1 (3.6%) 11 (39.3%)	4.3 (2.7)
Unknown	7 (13.4%)	NA	16 (57.1%)	NA

NA, not applicable.

METHODS

Setting and study design

220 biopsies from 58 total patients with known BE diagnoses were retrospectively collected from 5 independent collaborating sites: Allegheny Health Network, Johns Hopkins University, Mayo Clinic, University of Maryland, and University of Connecticut, between May 2021 and June 2023. Of the 220 biopsies, 167 biopsies are newly tested for this study. 53 single biopsies from 53 patients were previously used in the clinical validation of Esopredict (24). A total of 11 patients had biopsies that met both the criterion for the spatial cohort (n = 27 biopsies) and the temporal cohort (n = 36 biopsies). Clinical metrics including age, sex, and diagnosis of dysplasia were recorded for each patient (Table 1). Quality control assessments revealed no significant differences between biopsies collected as far back as 32 years (1991) and those obtained more recently. Patient biopsies were received from our collaborating sites with defined criteria (see below in "Patient selection/biopsy procurement"). We reduced bias in the selection of patients and biopsies collected by having limited sample criteria and matching the exact criteria developed in our original clinical validation study. As a result, the specific methods of obtaining biopsies (e.g. the Seattle protocol) were unknown. The samples were processed randomly in batches; therefore, different sites and biopsy ages were processed in the same batch.

Patient selection/biopsy procurement

All patients required outcome data to be identified as either nonprogressors (having a >5-year outcome [i.e., follow-up]

biopsy not showing progression to HGD or EAC) or progressors (having an outcome biopsy showing progression to HGD or EAC within 5 years, Figure 1). Spatial profiling patients required minimum 2 biopsies obtained from the same endoscopy (i.e., multiple index biopsies). Temporal profiling patients required at least 2 biopsies from different endoscopies (i.e., 1 index biopsy and at least 1 follow-up biopsy). Consecutive sections were cut from formalin-fixed paraffin-embedded (FFPE) tissue blocks from 220 esophageal biopsies encompassing 58 patients with BE (Figure 1b). The pathologic diagnoses of the index biopsies (i.e., the assayed material) were ND BE, low-grade dysplasia (LGD), or indefinite for dysplasia (IND), and other relevant clinical metrics were recorded (Table 1). Index biopsies with histologic diagnoses by an expert pathologist were obtained from 1992 to 2018, with a mean year of 2007. Outcome biopsies (last follow-up biopsy on record), whose histologic diagnosis determined the patient outcome (nonprogressor or progressor), were from 1998 to 2022, with a mean year of 2012. Currently, there is no algorithm for predicting progression in patients who do not undergo Esopredict testing. Thus, because such a comparison is not possible, we did not include a group of patients who did not undergo Esopredict testing.

Sample processing/assay

Tissue processing, assay methods, and predictive probability calculations are presented in a previous study (24). All patients in this study had 1 outcome biopsy which was not assayed, used to determine progressor status, and was the last recorded biopsy for that patient. Each patient in the spatial profiling cohort underwent 1 endoscopy containing 2–4 biopsies assayed with Esopredict. Each patient in the temporal profiling cohort included multiple endoscopies across time, i.e., including 1 index biopsy (the earliest known biopsy), as well as 2–6 additional biopsies obtained at different endoscopic surveillance time points before the outcome endoscopy. 11 patients had overlap between cohorts because they featured biopsies that represented both spatial (multiple biopsies from a single endoscopy) and temporal (biopsies taken at multiple endoscopies) samples. These 11 patients comprised 5 progressors and 6 nonprogressors (Figure 1a). Following established protocols, the Esopredict assay was run on each biopsy sample at the Previse CLIA-certified laboratory in Baltimore, Maryland (CLIA #: 21D2256153). This assay returns a personalized risk score and probability of progression within 5 years. Figure 1a displays the average risk of progression of each risk category relative to the average prevalence of progression (24). The assay parameters and the predictive model were locked as discussed and validated in prior studies (21–24).

Designation	Esopredict
True-positive (TP)	Progressors in the higher risk (high moderate and high-risk levels)
False-positive (FP)	Nonprogressors in the higher risk (high moderate and high-risk levels)
True-negative (TN)	Nonprogressors in the lower risk (low and low moderate-risk levels)
False-negative (FN)	Progressors in the lower risk (low and low moderate-risk levels)

Statistical analyses

The algorithm and specific cutpoints result in distinct categories of risk and associated risk scores summarized in our published clinical validation study (24). To assess the variability of risk scores of the different biopsies of the same patient (spatial), we estimated the average positive agreement (APA) and the average negative agreement (ANA). APA and ANA measure agreement when there is no clear reference. A result is considered positive if the risk score falls within the higher risk range (high-moderate to high) and negative if the risk score falls within the lower risk range (low to low-moderate).

$$\text{APA} = \frac{\text{Number of times biopsies agree on a positive}}{\text{The total number of positives identified}}$$

$$\text{ANA} = \frac{\text{Number of times biopsies agree on a negative}}{\text{The total number of negatives identified}}$$

The sensitivity and specificity based on true-positive, false-positive, true-negative, and false-negative were calculated as follows:

$$\text{Sensitivity} = \frac{\text{TP}}{(\text{TP} + \text{FN})}$$

$$\text{Specificity} = \frac{\text{TN}}{(\text{TN} + \text{FP})}$$

Ethical statement

All histologic tissue sections from retrospective patients diagnosed with BE were used in this study. The tissue samples were prepared at the time of surgery and were not collected for this study. Archival tissue samples were completely anonymized and deidentified, and there was no possibility of linking them back to

Table 2. Assessment of variability in spatial profiling patients based on Two Raters Agreement (FDA-approved measures)

	Rep 1—Positive	Rep 2—Negative
A. Total spatial patients (n = 52, 130 biopsies)		
Rep 1—Positive	20	0
Rep 2—Negative	10	22
	APA = 80%	ANA = 81%
B. Total spatial nonprogressor patients (n = 31)		
Rep 1—Positive	10	0
Rep 2—Negative	6	15
		ANA = 83%
C. Total spatial progressor patients (n = 21) (0-12 years)		
Rep 1—Positive	10	0
Rep 2—Negative	4	7
		APA = 83%

A. APA and ANA of the total spatial profiling patients across replicate samples from the same patient. B. ANA of the total nonprogressor spatial profiling patients across replicate samples from the same patient. C. APA of the total progressor spatial profiling patients across replicate samples from the same patient.
ANA, average negative agreement; APA, average positive agreement.

Table 3. Sensitivity and specificity of Esopredict in spatial profiling samples

A. Spatial cohort (36 patients, 89 biopsies)			
Highest-scoring biopsy of each patient			
	Lower risk (n = 13)	Higher risk (n = 23)	Overall (n = 36)
Nonprogressors	13	11	24
Progressors	0	12	12
Sensitivity = 100%, specificity 54%			
Mean score of all the biopsies of each patient (2–5 biopsies each)			
	Lower risk (n = 20)	Higher risk (n = 16)	Overall (n = 36)
Nonprogressors	18	6	24
Progressors	2	10	12
Sensitivity = 83%, specificity 75%			
B. Progressors with higher risk scores in the spatial cohort (21 patients total)			
	All biopsy intervals 0–12 yr	**Within 0–5 yr**	**Within 5–12 yr**
Highest-scoring biopsy	17/21 (81%)	12/12 (100%)	5/9 (56%)
Mean score of all biopsies	14/21 (67%)	10/12 (83%)	4/9 (44%)

1. Accessing progressor status in 0–5 yr after index biopsies in the spatial cohort (n = 36, 84 total samples) showing the number of patients in lower risk (low and low-moderate) vs higher risk (high and high-moderate) in nonprogressors and progressors comparing the biopsy with the highest score (top) and the mean score (bottom) of all total biopsies of each patient. 2. Progressors only, n = 21 with 0–12-yr biopsy interval range. The number of progressor patients with higher risk scores compared with the overall number of patients, based on all time intervals, within 0–5 yr, and 5–12 yr.

patients. The specimens were prepared and provided by personnel without any role in this research study except for providing the tissue specimens. The study was approved by review by the Institutional Ethics Committee of Johns Hopkins University, Institutional Review Board NA_0036.

RESULTS

Baseline clinical characteristics

For each patient, documentation included dysplasia grade; i.e., NDBE (spatial cohort, n = 44; temporal cohort, patients: n = 21, samples: n = 47), LGD (spatial cohort, n = 6; temporal cohort, patients: n = 6, samples: n = 27), and IND (spatial cohort, n = 2; temporal cohort, patients: 1, samples: n = 20), including the 11 patients with overlapping spatial and temporal biopsies. Both cohorts predominantly consisted of male patients, which aligns with the higher incidence of this disease in men (90% in the spatial cohort, and 79% in the temporal cohort, Table 1). The median ages were 62 and 63 years in the spatial and temporal cohorts, respectively. Notably, in the spatial cohort, progressors were older (mean age = 64 years) compared with nonprogressors (58 years, $P = 0.04$), reflecting an age-related trend in disease progression. However, the temporal cohort showed no significant age difference between progressors and nonprogressors (mean age of both cohorts = 61 years).

Performance in the spatial profiling cohort

Within the spatial profiling cohort, patients had multiple index biopsies, between 2 and 4, during a single endoscopic procedure, which were analyzed using Esopredict (n = 130 total biopsies). In addition, patients had 1 outcome biopsy (not assayed) to determine progression status. To evaluate the variability across different anatomical locations from the same procedure, we used the FDA-approved Two Raters Agreement. For the entire spatial cohort (n = 52), the APA was 80%, and the ANA was 81% (Table 2A). Specifically, the ANA for the patients who did not progress to HGD or EAC within 5 years was 83%, and the APA for patients who progressed to HGD or EAC within 5 years was also 83% (Table 2B,c).

The performance of Esopredict within the spatial cohort was evaluated by comparing the number of accurate classifications of progressor patients based on their Esopredict scores comparing the lower-risk category vs the higher-risk category. For the spatial cohort, we limited the analysis to the time frame of 0–5 years, which is clinically validated and clinically relevant (24). Using this criterion, there were 36 patients and a total of 89 biopsies, with the highest-scoring biopsy, ranging from 2 to 4 different anatomical locations per patient. The sensitivity of Esopredict in these patients was 100%. Averaging the scores from all biopsies per patient (2–4 per patient, total of 89 biopsies), the sensitivity was 83% (Table 3A). Further analysis of progressor patients, segmented into biopsy intervals of 0–5 years (clinically validated) and 5–12 years (beyond clinically validated), revealed an increase in the sensitivity from 81% to 100% for the highest-scoring biopsy and from 67% to 83% for the mean score across all biopsies per patient (Table 3B). For spatial samples of patients that progressed to HGD or EAC within 5 years, we examined the Esopredict scores and sensitivity at different biopsies/levels, ranging between 2 and 4 per patient (Supplemental Figure 1, http://links.lww.com/AJG/D576).

Performance in the temporal profiling cohort

In the temporal profiling cohort, patients had 1 index biopsy, between 1 and 6 follow-up biopsies at different time intervals, which were analyzed with Esopredict. In addition, an outcome biopsy (most recent biopsy) was used to determine the progressor status of each patient (Figure 1). The performance within this cohort was evaluated by how many patients fell into the lower risk category vs the higher risk category. The sensitivity of Esopredict,

Table 4. Sensitivity and specificity of Esopredict in temporal profiling samples

Temporal cohort (28 patients total)			
Based on the MOST RECENT biopsy of each patient			
	Lower risk (n = 12)	Higher risk (n = 16)	Overall (n = 28)
Nonprogressors	11	9	20
Progressors	1	7	8
Sensitivity = 88%			
Based on the EARLIEST-KNOWN biopsy of each patient			
	Lower risk (n = 16)	Higher risk (n = 12)	Overall (n = 28)
Nonprogressors	13	7	20
Progressors	3	**5**	8
Sensitivity = 63%			
Based on the MEAN of all biopsies of each patient (range of 2–7)			
	Lower risk (n = 11)	Higher risk (n = 17)	Overall (n = 28)
Nonprogressors	11	9	20
Progressors	0	8	8
Sensitivity = 100%			
Based on the MEDIAN of all biopsies of each patient (range of 2–7)			
	Lower risk (n = 11)	Higher risk (n = 17)	Overall (n = 28)
Nonprogressors	11	9	20
Progressors	0	8	8
Sensitivity = 100%			

1. Accessing progressor status in 0–5 yr after index biopsy in the temporal cohort showing the number of patients in lower risk (low and low-moderate) vs higher-risk (high and high-moderate) in nonprogressors and progressors comparing the scores from the biopsy with the most recent (closest in time to outcome), the scores from the biopsy with the earliest known (furthest in time from outcome), and the mean score of all total biopsies of each patient. 2. Progressors only. The number of progressor patients with higher risk scores compared with the overall number of patients, based on all time intervals, within 0–5 yr, and 5–12 yr.

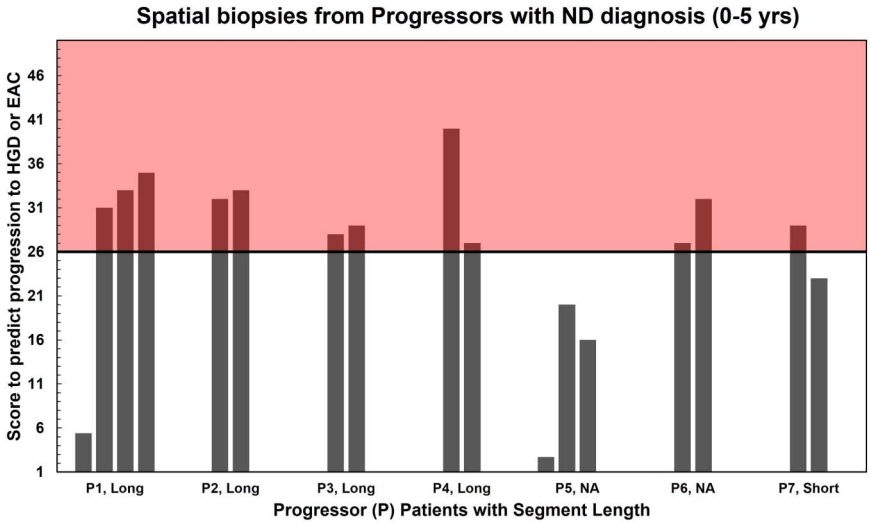

Figure 2. Esopredict risk scores of spatial profiling patients with index biopsies of NDBE. Patients with index (initial) biopsies that were ND, who then progressed to HGD or EAC within 5 years. The x-axis shows each patient (n = 7) had between 2 and 4 spatial biopsies with segment length noted when available (short < 3 cm, long > 3 cm). The y-axis shows the Esopredict score to predict progression within 5 years. The line at the risk score of 26 designates the lower risk (low and low-moderate) from the higher risk (high-moderate and high) scores (red background). BE, Barrett esophagus; EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia; ND, nondysplastic.

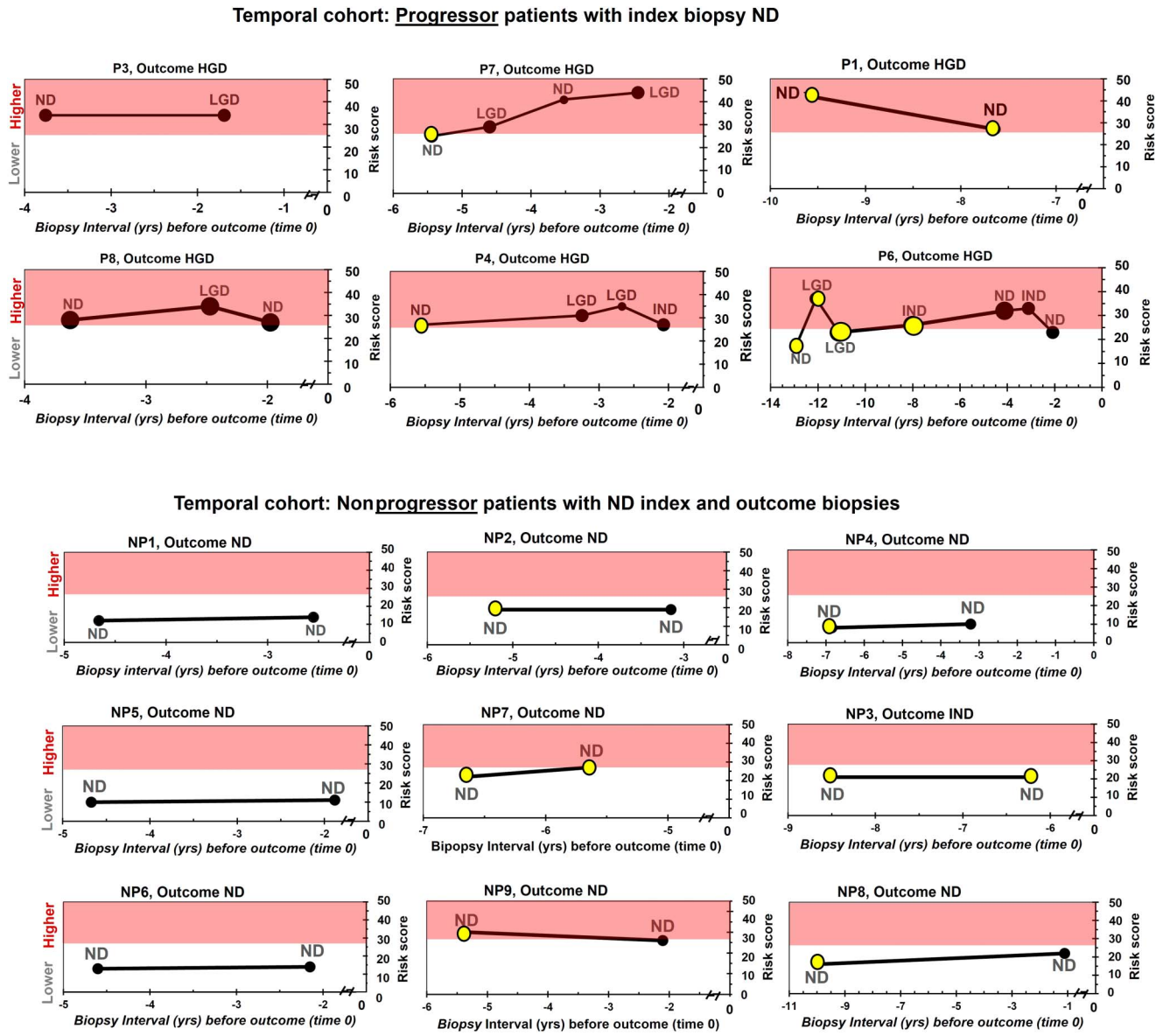

Figure 3. Esopredict risk score of temporal profiling patients with index biopsies of NDBE. (**a**) Patients with index (initial) biopsies that were ND who then progressed to HGD or EAC (progressors). Each x-axis shows each biopsy interval in years before the outcome biopsy (time 0). Each y-axis shows the risk score to predict progression within 5 years. The line at 26 designates the lower risk (low and low-moderate) from the higher risk (high-moderate and high) scores (red background). Note: Yellow highlighted data points represent Esopredict scores in biopsies outside the 5-year prediction. (**b**) Patients with index (initial) biopsies that were ND who then remained ND (nonprogressors). Each x-axis shows each biopsy interval in years before the outcome biopsy (time 0). Each y-axis shows the risk score to predict progression within 5 years. The line at 26 designates the lower risk (low and low-moderate) from the higher risk (high-moderate and high) scores (red background). Note: Yellow highlighted data points represent Esopredict scores in biopsies outside the 5-year prediction. HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; LGD, low-grade dysplasia; ND, nondysplastic; NP, nonprogressor patients.

using the most recent biopsy, closest in time from the outcome biopsy, was 88%. However, the sensitivity is reduced to 63% in the biopsies that were furthest in time from the outcome biopsy (up to 13 years). The sensitivity of Esopredict was 100% when averaging the scores of all the biopsies per patient (ranging from 2 to 7 biopsies) (Table 4A). For progressor patients stratified into biopsy intervals of 0–5 and 5–13 years, the sensitivity for the most recent biopsy improved from 88% to 100%. Similarly, for the furthest in time from the outcome biopsy, the sensitivity increased from 63% to 67%. The sensitivity remained at 100% for the mean score of all biopsies of each patient (Table 4B).

Performance in a subset of patients with index biopsies of ND

Given that 90% of all BE patients are diagnosed as ND, a focused subset analysis was conducted for both spatial and temporal cohorts targeting those patients who had index biopsies of NDBE. Within the spatial cohort, 7 patients who initially presented with NDBE progressed to HGD or EAC within 5 years. Figure 2 displays the number of spatial samples for each patient including the score of each biopsy. All 7 patients (100%) had at least 1 biopsy, and 5 of the 7 patients (71%) had more than 1 biopsy with a score falling into the higher risk category. Collectively, out of the 17 total samples from these 7 patients, 14 (82%) indicated scores in the higher risk category.

Within the temporal cohort, 6 patients had an NDBE index biopsy and progressed to HGD or EAC. All 6 patients (100%) had more than 1 or all biopsies in the higher risk category. This included patients with >10 years between the index and outcome biopsy (P1 and P6). Of the total 22 biopsy samples from these patients, 18 biopsies (82%) indicated higher risk scores (Figure 3a). Conversely, among the 9 nonprogressor patients who remained NDBE in outcome biopsies, 7 (78%) had biopsies scored in the lower risk category (Figure 3b). One of the nonprogressor patient 7 had 1 biopsy indicating lower risk and 1 biopsy indicating higher risk, and the other, nonprogressor patient 9, had both biopsies fall into higher risk levels. This included patients with over 10 years between index and outcome biopsies. Of the collective 18 samples from these 9 patients, 15 (83%) indicated lower risk scores.

Performance in a subset of overlapping spatial and temporal patients with index biopsies of NDBE

Eleven patients had biopsies that fulfilled the criteria for both spatial and temporal profiling cohorts (Figure 1). Each patient had at least 1 temporal biopsy that coincided with 2 or more spatial biopsies at that same time point. Among the 5 progressor patients, consistent risk categorization (either lower risk or higher risk) was maintained across all spatial biopsies corresponding to 6 different time points (Supplemental Figure 2, http://links.lww.com/AJG/D577). Conversely, for the 6 nonprogressor patients, category shifts between lower risk and higher risk were observed in 4 of 11 time points. However, 5 of 6 nonprogressor patients progressed from NDBE to LGD or IND.

DISCUSSION

Esopredict is a clinically available laboratory-developed prognostic test that risk stratifies patients with BE with a 5-year percent risk to support gastroenterologists' management of patient care (24). This study demonstrated high accuracy of Esopredict across biopsies from multiple locations of the esophagus from the same endoscopic procedure, with APA and ANA both 83% (Table 2). Sensitivity was high (12/12 patients, or 100%) in spatially profiled progressors based on the highest-scored biopsy, where 2–5 biopsies were obtained per patient. If the mean scores of all biopsies obtained per patient were used, sensitivity was still high (10/12 patients, or 83%), establishing robust assay performance across multiple locations. The high APA and sensitivity across multiple spatial locations in progressors suggest that an Esopredict score will be obtained, regardless of where biopsies are collected among different anatomical sites.

As anticipated, assay sensitivity was slightly higher in biopsies taken closer to the outcome time point. Using the most recent biopsy, of the 8 patients who later progressed to develop HGD or EAC, Esopredict placed 7 (88%) in the higher risk category with a mean interval between assayed biopsy and outcome of 2.5 years. Sensitivity across all patients was 63% (5/8 progressors), with a mean interval of 5.9 years to outcome biopsy. Of the 6 progressors with biopsy intervals limited to the clinically validated time of 0–5 years, sensitivity increased to 100% (6/6 patients). 24 The mean score, calculated as the average of all scores of each biopsy within the same patient, in this same subset had a sensitivity of 100% (8/8 progressors) in all biopsy intervals within 0–13 years. The consistently high sensitivity and reproducibility observed across these spatiotemporal samples also support the robustness of this assay, notwithstanding inherent challenges associated with biopsy sampling (39–42).

Field cancerization (or field effect) defines protumorigenic molecular alterations, including epigenetic changes, in tissues that seem histologically normal. These molecular lesions can develop before phenotypical alterations are detectable, often present in the margins and surrounding areas outside a region of visible disease (35,37,43–50). Indeed, this phenomenon has been observed during the progression of esophageal cancers including EAC (50–57). BE tends to be multifocal, so detecting molecular changes in adjacent non-neoplastic tissue or even preceding the development of histologic changes enhances the prognostic utility of this assay. Significantly, hypermethylation of the specific biomarkers measured by Esopredict (p16, RUNX3, HPP1, and FBN1) has been identified in field cancerization and aberrant hypermethylation during early disease progression (58–69). This finding supports a substantial advantage for a prognostic that quantitatively assesses epigenetic modification of biomarkers known to become altered before detectable clinical disease develops. Our approach addressed challenges posed by sampling error, timing error, and decreased adherence to recommended endoscopy intervals or spatial sampling (Seattle protocol) (27,39–42,70–73).

Some limitations of our study include a relatively limited sample size, as well as enrichment of progressor patients relative to the known overall population prevalence (31,74–78). Owing to the low prevalence of progression among BE patients, our case-control design was enriched for patients who progressed to HGD or EAC compared with the general BE population. 220 biopsy samples were assayed with Esopredict in this study, as there were multiple biopsies for each patient, and there were 58 patients in total. As shown in Figures 2 and 3, Esopredict is accurate in ND patients, however, given that 90% of the BE population under surveillance are ND, a study focused specifically on the NDBE population will be useful. A limitation is we did not have a central pathology review of our samples to determine the dysplasia diagnosis of each patient including biopsies tested with Esopredict and outcome biopsies not tested. We intentionally relied on pathological diagnosis from each clinical center (both index and outcome) similar to our initial clinical validation study because even though there is a higher likelihood of sampling error or interobserver variability, this study mimics the real-world use case of Esopredict, which is intended to supplement the pathological reports from different pathologists. Owing to the case-control design of this study and our previous clinical validation, we are unable to evaluate a time to progression. We observe consistency with Esopredict scores in samples of temporally profiled patients that are beyond the 5-year window of prediction of our validated assay (Figure 3 and Supplementary Figure 2, highlighted yellow on graphs, http://links.lww.com/AJG/D577). This strongly suggests a rationale for developing a new case-cohort study to validate the assay's predictive capability of estimating specific time to progression. This would greatly benefit the current assay so that risk percent over different periods such as 1, 2, 5, and 10 years could be useful to clinicians making surveillance interval decisions integrated with variable risk factors such as segment length, family history, and dysplasia diagnosis. Other tests evaluate the risk of progression of patients with BE, such as Progression in BE score and tissue systems pathology (TSP-9) test (TissueCypher). There are challenges with comparing the assays without testing the same patient population, as small sample sizes

and different patient populations would not lead to an accurate comparison. We previously evaluated a small subset of patients with enough clinical information to produce a Progression in Barrett's (PIB) score in our clinical validation, however found it insufficient to evaluate based on data size and that the percent risk of progression of risk categories between the 2 tests was not comparable (24).

A reliable prognostic tool such as Esopredict can enhance clinical practice by stratifying patients according to their progression risk relative to the average BE patient. Esopredict complements clinical prognostic factors, such as segment length, grade of dysplasia, and patient history, in refining decision-making for subsequent surveillance intervals and treatment. For example, patients categorized as lower risk can continue with standard surveillance schedules or consider reducing surveillance frequency in those with additional low-risk factors such as short-segment BE. Similarly, a patient with a moderate Esopredict risk score (e.g., 8% progression risk, which is slightly higher than average) can continue with surveillance as scheduled, with consideration given to increasing surveillance frequency or performing endoscopic treatment if additional clinical risk factors are present, such as LGD, IND, or long segment length. Thus, by aligning with the principles of personalized medicine, this prognostic tool is designed to tailor medical interventions or decisions in individual patients, thereby optimizing efficacy and minimizing unnecessary treatment or endoscopies. The observed performance of Esopredict across spatiotemporally profiled patients further supports its utility as a complementary tool for risk assessment in a population challenging to risk stratify based on the current standard of care, which relies heavily on histologic diagnosis.

CONFLICTS OF INTEREST

Guarantor of the article: Stephen J. Meltzer, MD.

Specific author contributions: S.E.L. designed and conducted the study; provided administrative, technical, and material support; acquired, analyzed, and interpreted the data; drafted, reviewed, and revised the manuscript, and approved the final draft submitted. L.K. and D.L. designed and conducted the study; provided administrative, technical, and material support; analyzed and interpreted the data; reviewed and revised the manuscript, and approved the final draft submitted. S.E.L., and J.B. analyzed and interpreted the data; drafted, reviewed, and revised the manuscript, and approved the final draft submitted. S.G. and A.K. reviewed and revised the manuscript and approved the final draft that was submitted. F.P., S.K. acquired and analyzed data; reviewed the manuscript, and approved the final draft submitted. K.M. and Y.C. provided material support, reviewed the manuscript, and approved the final draft submitted. C.L.L., B.D.G., A.H.Z., A.N.O., L.K. provided material support; T.M., S.J.M. provided guidance for the study's design; supervised the study; provided administrative, technical, and material support; interpreted the data; reviewed and revised the manuscript; and approved the final draft submitted.

Financial support: Support from the following grants: R44DK136424, R41CA261376, R01DK118250, R01CA287294.

Potential competing interests: S.E. Laun, D.G. Lunz, and L. Kann are paid employees, equity holders of Previse, and inventors of the patented technologies described. S.J. Meltzer is an equity holder of Previse and an inventor of the patented technologies described. Y. Chen is an inventor of the patented technologies described. K. Ma is an equity holder of Previse. S. Gilbert and F. Pierre are paid employees and equity holders of Previse. T. Maddala and J. Braun are paid consultants of Previse. A.K., C.L., B.G., A.Z., L.K. have no conflicts of interest to disclose in relation to this research.

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