Prediction of Progression in Barrett's Esophagus Using a Tissue Systems Pathology Test: A Pooled Analysis of International Multicenter Studies

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BACKGROUND & AIMS: **Prediction of progression risk in Barrett's esophagus (BE) may enable personalized management. We aimed to assess the adjunct value of a tissue systems pathology test (TissueCypher) performed on paraffin-embedded biopsy tissue, when added to expert pathology review in predicting incident progression, pooling individual patient-level data from multiple international studies**

METHODS: **Demographics, clinical features, the TissueCypher risk class/score, and progression status were analyzed. Conditional logistical regression analysis was used to develop multivariable models predicting incident progression with and without the TissueCypher risk class (low, intermediate, high). Concordance (c-) statistics were calculated and compared with likelihood ratio tests to assess predictive ability of models. A risk prediction calculator integrating clinical variables and TissueCypher risk class was also developed.**

RESULTS: **Data from 552 patients with baseline no (n = 472), indefinite (n = 32), or low-grade dysplasia (n = 48) (comprising 152 incident progressors and 400 non-progressors) were analyzed. A high-risk test class independently predicted increased risk of progression to high-grade dysplasia/adenocarcinoma (odds ratio, 6.0; 95% confidence interval, 2.9–12.0), along with expert confirmed low-grade dysplasia (odds ratio, 2.9; 95% confidence interval, 1.2–7.2). Model prediction of progression with the TissueCypher risk class incorporated was significantly superior than without, in the whole cohort (c-statistic 0.75 vs 0.68; $P < .0001$) and the nondysplastic BE subset (c-statistic 0.72 vs 0.63; $P < .0001$). Sensitivity and specificity of the high risk TissueCypher class were 38% and 94%, respectively.**

CONCLUSIONS: **An objective tissue systems pathology test high-risk class is a strong independent predictor of incident progression in patients with BE, substantially improving progression risk prediction over clinical variables alone. Although test specificity was high, sensitivity was modest.**

Keywords: Biomarker; Esophageal Cancer; Screening; Surveillance.

Surveillance is recommended in Barrett's esophagus (BE) to detect dysplasia/esophageal adenocarcinoma (EAC).[1] However, progression rates are low,[2] progression rates are variable, and compliance with surveillance is suboptimal. Consequently, endoscopic surveillance is only modestly effective.[3] Although progression risk is determined by several factors,[4] surveillance recommendations are almost solely based on histology.[5,6]

Prediction of progression, although attractive (given the potential to tailor management), remains challenging. Clinical progression prediction models have been developed, but these include low-grade dysplasia (LGD) as a variable and hence their performance in nondysplastic BE (NDBE) is unclear.[7] Even in confirmed LGD, guidelines endorse surveillance as an alternative strategy to

Abbreviations used in this paper: **AMC, Academic Medical Center (Amsterdam); BE, Barrett's esophagus; c-statistic, concordance statistic; EAC, esophageal adenocarcinoma; EET, endoscopic eradication therapy; FFPE, formalin-fixed paraffin-embedded; HGD, high-grade dysplasia; IND, indefinite for dysplasia; LGD, low-grade dysplasia; NDBE, nondysplastic Barrett's esophagus; OR, odds ratio.**

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ablation, creating the need for risk stratification in this group to determine treatment strategies.

Several biomarkers to predict progression have been studied. However, few have advanced to phase 3 or 4 studies given challenges, including difficulty in assembling large cohorts and the need for long follow-up, resulting in substantial funding requirements and challenges in assay commercialization.[8] Consequently, biomarkers are not currently recommended for clinical use by most society guidelines.

The TissueCypher assay (Castle Biosciences, Inc, Pittsburgh, PA) uses a multiplexed fluorescence imaging platform that analyzes multiple biomarkers and tissue morphology to predict the risk of progression to high-grade dysplasia (HGD) and/or EAC. The assay is performed on formalin-fixed paraffin-embedded (FFPE) tissue obtained via endoscopic biopsies. Biomarkers included in the assay measure loss of tumor suppressor genes (*p53, p16*), alterations in lipid metabolism (AMACR), amplification of oncogenes (*HER-2*), markers of immune infiltration (CD68, COX2), and angiogenesis (HIF1 alpha, CD45RO). In addition morphometric features (nuclear size, shape, and amount of DNA) are also extracted, and make up 3 of the 15 features that a proprietary algorithm integrates to produce the risk score, which classifies patients into high, intermediate, and low risk of progression over 5 years.[9]

The assay has been studied in 5 cohorts, with 4 studying its ability to predict incident progression (HGD/EAC detected $\geq$12 months after BE diagnosis) and 1 studying its ability to detect missed prevalent ($\leq$12 months of BE diagnosis) HGD/EAC.[10] The initial study developed algorithm cutoffs,[11] and the subsequent 4 studies have validated these cutoffs in independent sample sets.[12-14] Given sample size limitations of individual studies, stratified analyses in NDBE separately has been challenging.

We pooled patient-level data from previous studies with the primary aims of: (1) assessing the incremental utility of the TissueCypher test in predicting progression in BE (NDBE, indefinite for dysplasia [IND], or LGD) patients with over clinical variables alone; and (2) assessing the incremental utility of the test in predicting progression in those with baseline NDBE over clinical variables. Secondary aims included: (1) assessing the incremental utility of the TissueCypher test in predicting both prevalent and incident HGD/EAC over 5 years; and (2) developing a BE progression risk score combining demographic, clinical, and TissueCypher test scores.

What You Need to Know

Background

Prediction of progression in Barrett's esophagus (BE) is challenging due to low rates of progression. Dysplasia, which is challenging to identify and diagnose histologically, is the only variable on which management is currently based.

Findings

In a pooled analysis of patient-level data from 4 multicenter studies, a tissue systems pathology test was able to significantly increase accuracy of progression prediction compared with clinical variables alone. A high-risk test class has high specificity (94%) but modest sensitivity (36%). A risk-prediction algorithm integrating clinical and biomarker test scores was also developed.

Implications for patient care

An automated and quantitative tissue systems pathology test may have a role in predicting progression in patients with BE without dysplasia or low-grade dysplasia, allowing personalization of management recommendations. This has the potential of making BE surveillance more efficient. While specificity is high, sensitivity of the assay should be improved.

Methods

Patient Accrual

Patient-level data from 4 studies predicting incident progression were pooled for the primary analysis (Supplementary Figure 1). Progression was defined in all 4 studies as the detection of HGD/EAC $\geq$1 year after a BE diagnosis. Deidentified data from these studies were shared with the authors with the permission of all investigators. Ethics/Institutional Review Board approvals were obtained for the individual studies, and this analysis was deemed exempt from additional approval. Although funding for statistical analysis was provided by Cernostics, the company or its employees did not participate in data analysis or writing of the manuscript. Histology was read by pathologists with expertise in gastrointestinal pathology at each expert center in all studies.

Study Details

Case-control Study 1.[11] This was a nested case-control study including samples from 4 institutions (University of Pittsburgh, University of Pennsylvania, Geisinger Medical Center, and the Academic Medical Center [AMC] in Amsterdam). The study included 79 progressors and 287 nonprogressors. Training and validation sets were used to establish and validate cutoffs. A 3-tier (high-, intermediate-, low-risk) classifier was validated. The training set cases and controls used to train the algorithm were excluded from the primary analysis for this manuscript.

Case-control study 2.[14] This validation study included samples from 2 institutions (University of Pittsburgh, Cleveland Clinic), with 58 progressors and 210 nonprogressors. Using cutoffs locked from study 1, a high-risk TissueCypher score predicted a 4.7-fold increased risk for progressing to HGD/EAC compared with those with a low-risk score.

Case-control Study 3.[13] This study was nested in the prospective REBUS cohort (patients with NDBE and LGD followed prospectively with endoscopy) from the AMC, Netherlands. Samples from 38 progressors and 38 nonprogressors were included. A high risk TissueCypher score was associated with a prevalence adjusted annual progression rate of 6.9% in patients with NDBE. Sensitivity increased from 31% to 50% to 69% by analyzing biopsies from multiple levels of a single endoscopy and from multiple endoscopies before progression, with specificity maintained at 95%.

Case-control Study 4.[12] This study analyzed samples from the screening cohort of the SURF LGD trial conducted in 9 European centers. One hundred fifty-five patient samples were reviewed by 3 expert pathologists and classified into LGD, IND, and NDBE. Sensitivity of the assay in detecting progressors was 68% compared with 76% (for 3 pathologists), specificity was 79% vs 64% to 77% for 3 pathologists. The assay also detected 56% of progressors in patients downstaged to NDBE.

Case-control Study 5.[10] This study was only included in the secondary analysis combining both incident and prevalent HGD/EAC prediction. Prevalent HGD/EAC was defined as that detected $\leq$1 year of BE diagnosis, with initial histology of NDBE or LGD. This was a case-control study including 4 institutions: Geisinger Health, University of Pittsburgh, University of Pennsylvania, and AMC Netherlands. The assay was performed in the baseline biopsies of 30 patients who had HGD/EAC diagnosed $\leq$1 year from BE diagnosis and from 145 patients without prevalent or incident HGD/EAC. The area under the receiver operating characteristic curve of the TissueCypher test to distinguish those with prevalent HGD/EAC from those without was 0.89.

TissueCypher Method

The assay uses a multiplexed fluorescence imaging platform that automatically extracts quantitative data on multiple tissue biomarkers and nuclear morphology (Supplementary Figure 2).[9] A multivariable classifier integrates quantitative image analysis data to provide a risk score from 0 to 10, which is stratified into low-, intermediate-, or high-risk classes. The assay is run and interpreted in a Clinical Laboratory Improvement Amendments-certified laboratory, on FFPE tissue blocks from the baseline surveillance endoscopy.

We utilized the highest TissueCypher assay class/score (if performed on multiple biopsy levels or on biopsies from more than 1 endoscopy) in all patients.

Patient Data

Individual-level patient demographics (age, sex), BE segment length, hiatal hernia presence, initial and expert reviewed pathology diagnoses, TissueCypher risk score, and class (high, intermediate, low) were analyzed.

Statistical Analysis

Multivariable conditional logistic regression models adjusting for covariates were used to assess the association of TissueCypher with incident progression. All models were stratified by matched group to account for the case-control study design. Adjustment variables were variables that could also be potential confounders for the TissueCypher results. Models with and without the TissueCypher risk classes/score were considered.

Missing data were assumed to be missing at random. Multiple imputation with 25 imputations was used, with regression models run on each imputed dataset and results pooled. Models with and without imputed data were analyzed to assess the impact of imputed data on model accuracy.

Results were summarized using 2 metrics:

1. First, concordance statistics (c-statistics) were calculated to evaluate the predictive ability of the models, with higher concordance suggesting greater ability to discriminate incident progression from nonprogression. A c-statistic of 0.5 indicates a model no better than random prediction, whereas c-statistic of 1.0 reflects perfect model discrimination.
2. Second, likelihood ratio tests compared c-statistics for models with and without inclusion of TissueCypher results. Results describing the association between model variables and incident progression were summarized as odds ratios (ORs), with 95% confidence intervals and *P*-values.

As a secondary analysis, the ability of the TissueCypher assay in predicting prevalent HGD/EAC (diagnosed within 12 months of BE diagnosis) and incident HGD/EAC was assessed combining the 5 case control studies (outlined earlier).

Using the case-control data, a preliminary predictive model for 5-year progression was also reported, with predictions using age, sex, expert BE pathology, hiatal hernia presence, BE segment length, and TissueCypher risk class. A conditional logistic regression model was fitted, excluding matched groups with case event time greater than 5 years. A 5-year progression intercept was pre-specified as a mean of 5% for a hypothetical subject with the mean of all covariates. A P-value $< .05$ was considered statistically significant, and no adjustment is made for multiple comparisons across several statistical models. Statistical analyses were done using R version 3.6.

A matched case-control design with 152 matched sets, matched 1:2 to controls, with TissueCypher high/intermediate prevalence of 30% achieves 90% power to detect an OR of 2.19 calculated using conditional logistic regression with 2-sided alpha level 0.05. This assumes an R^2 statistic of 0.2 when TissueCypher is regressed on other covariates.

Results

Prediction of Incident Progression

Baseline characteristics of all included patients are displayed in Table 1. Most patients (85.5%) had NDBE at baseline, and 28% were progressors. Two-thirds of patients had long-segment BE. The median follow-up of non-progressors was 79.8 months (interquartile range, 59.9–111.8 months). Median time to progression was 38.1 months (interquartile range, 25.7–54.9 months). Seventy-nine percent of progression occurred within 5 years of the baseline endoscopy.

Of the 152 progressors (overall), 58 (38%) were in the high-risk class, 25 (16.4%) were in the intermediate-risk class, and the remaining 69 (45%) were in the low-risk class (Figure 1, *A, B*; Supplementary Table 1). The sensitivity of a high-risk class in predicting progression in the entire cohort was 0.38 with a specificity of 0.94. Sensitivity of a high-/intermediate-risk class in the entire cohort was 0.55, with a specificity of 0.82.

Clinical predictors of progression in all patients with BE are shown in Table 2. Increasing age, BE length, and confirmed LGD were independent predictors. The c-statistic of this clinical model was 0.68. In the model with the TissueCypher risk class included (Table 3), the c-statistic (accuracy of prediction) increased significantly (likelihood ratio test, P value $< .001$) to 0.75 (95% confidence interval, 0.66–0.83). TissueCypher high-risk class and confirmed LGD remained independent predictors of progression. The c-statistics of the models with the TissueCypher score as a continuous variable and with the TissueCypher risk class dichotomized into high + intermediate vs low or high vs intermedidate + low were similar (Supplementary Tables 2, 3, 3A).

We conducted an a priori stratified analysis in patients with NDBE. In the model without the TissueCypher class, increasing age and BE segment length were predictors of progression, with a model c-statistic of 0.63 (Table 4). With the TissueCypher risk class added to the model (Table 5), the c-statistic (accuracy of prediction) increased significantly to 0.72 (likelihood ratio test, P value $< .001$). A TissueCypher high-risk class was a strong (OR, 14.3) independent risk of progression. Analyses incorporating the TissueCypher score as a continuous variable and dichotomizing the risk class into high + intermediate vs low and high vs intermediate + low had similar results (Supplementary Tables 4, 5, and 5A). In the NDBE cohort, the sensitivity of a high-risk test class was 0.37 with a specificity of 0.96. Sensitivity of a high- + intermediate-risk vs low-risk class was 0.52, with a lower specificity of 0.85.

Models with and without imputed missing data revealed consistent results, as did models using BE

Table 1. Baseline Characteristics of All Patients With BE Included in the Primary Analysis

	Expert pathology diagnosis			
Variable	IND (n = 32)	LGD I(n = 48)	NDBE I(n = 472)	Total I(N = 552)
Age, *y*	60.8 (7.4)	63.9 (10.9)	61.3 (11.1)	61.5 (11.0)
Male sex	22 (68.8)	45 (93.8)	366 (77.5)	433 (78.4)
Long segment BE[a]	13 (43.3)	32 (69.6)	305 (67.5)	350 (66.3)
BE segment length, *cm*	4.2 (2.6)	5.2 (2.9)	4.8 (3.1)	4.8 (3.1)
Hiatal hernia present[a]	25 (86.2)	32 (84.2)	320 (85.1)	377 (85.1)
Progressors	9 (31.0)	31 (72.1)	112 (27.8)	152 (32.0)
Original pathology diagnosis				
IND	10 (31.3)	2 (4.2)	41 (8.7)	53 (9.6)
LGD	16 (50.0)	43 (89.6)	66 (14)	125 (22.6)
NDBE	6 (18.8)	3 (6.3)	365 (77.3)	374 (67.8)
TissueCypher risk score	4.9 (1.5)	6.0 (1.6)	4.4 (1.7)	4.6 (1.8)
TissueCypher risk class				
High	5 (15.6)	23 (47.9)	54 (11.4)	82 (14.9)
Intermediate	4 (12.5)	10 (20.8)	59 (12.5)	73 (13.2)
Low	23 (71.9)	15 (31.3)	359 (76.1)	397 (71.9)

Note: Data are presented as number (%) or mean (standard deviation).
BE, Barrett's esophagus; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *NDBE*, nondysplastic Barrett's esophagus.
[a]Data on BE segment length class (long vs short) and hiatal hernia were missing in 4.3% and 8.3% of patients.

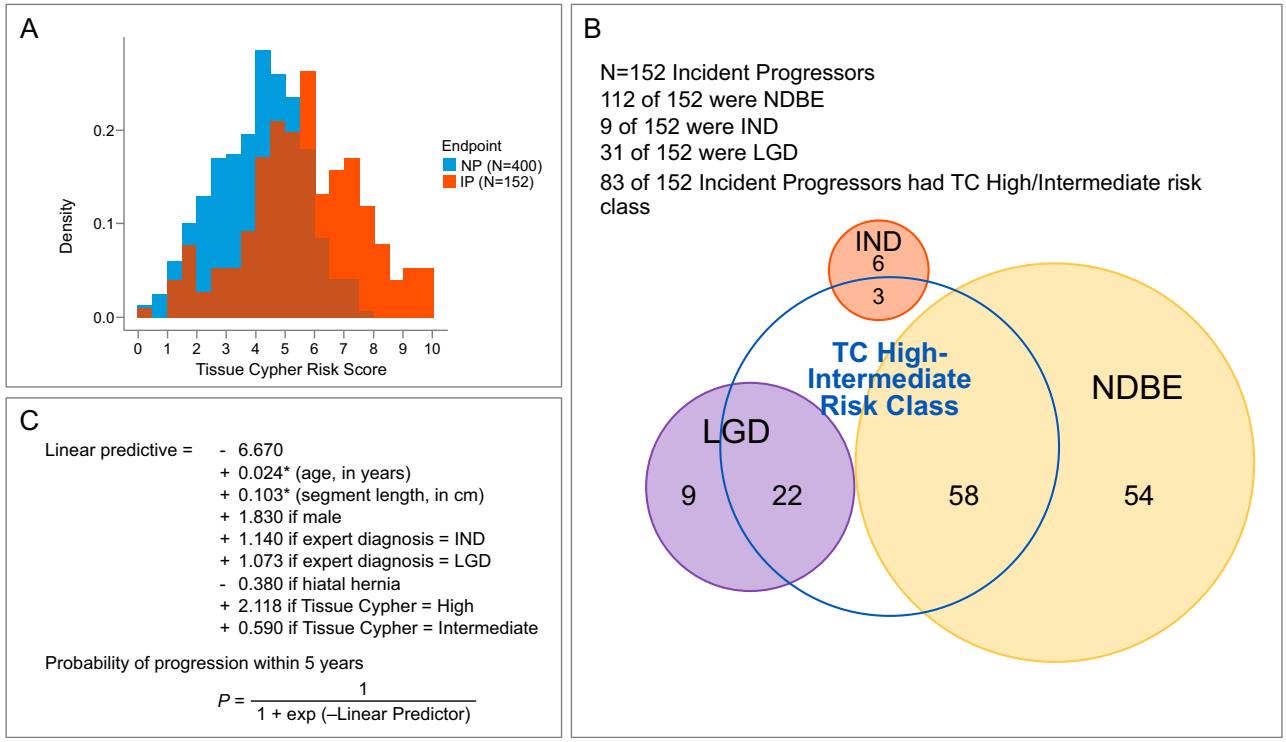

Figure 1. *A*, Overlapping histogram of TissueCypher (TC) score by case-control status. Cases (incident progressors, IP) are shown in *red*, whereas controls (nonprogressors, NP) are shown in *blue* highlight. *B*, Venn diagram of 152 IPs, showing expert pathologist classification and TC risk class. This shows that among cases (IPs), several NDBE and IND confirmed by expert pathologists were up-classified as TC high- or intermediate-risk. *C*, Model calculating predicted probability of progression within 5 years combining clinical factors and TC risk class.

length as continuous and dichotomized (long vs short segment) variables (Supplementary Tables 6, 7, and 8).

Prediction of Prevalent/Missed and Incident Progression to HGD/EAC

Patient-level data from all 5 studies were pooled for analysis of the secondary outcome: testing the predictive value of the TissueCypher test in predicting both prevalent and incident HGD/EAC. A total of 590 patients were analyzed, including 489 with NDBE, 68 with LGD, and 33 with IND, of whom 152 had incident HGD/EAC, 30 had prevalent HGD/EAC, and 400 were nonprogressors. Results were similar to the primary analysis, with the TissueCypher score remaining an independent predictor of both prevalent and incident HGD/EAC. The c-statistic of the prediction models increased significantly with the addition of the TissueCypher risk class or score. (Supplementary Tables 9–11).

Prediction of Progression Risk Combining Demographics, Clinical Variables, and TissueCypher Score

A model was developed to output predicted probability of progression within 5 years. It was a fitted subset to those case-control groups with case event time $\leq$5 years. Figure 1*C* demonstrates the prediction model. As an example, a 70-year-old male, with 5-cm segment length, no hiatal hernia, confirmed LGD, and TissueCypher high-risk class is predicted to have 63% probability of progression within 5 years. With the same patient characteristics but with a low-risk Tissue Cypher class, the probability of progression within 5 years would be reduced to 12% over 5 years. A 60-year-old male with a 5-cm segment length, no hiatal hernia, NDBE, and TissueCypher low-risk class is predicted to have 5% risk of progression; a female with the same characteristics is predicted to have $<$1% probability of progression.

Discussion

This analysis demonstrates that the addition of TissueCypher results significantly improved prediction of BE progression within 5 years, beyond that achieved by clinical factors alone. This also remained true in those with NDBE. Notably, in the multivariable models, both a high-risk TissueCypher class and expert-confirmed LGD were independent predictors of progression. Although the sensitivity of a high-risk class result was modest (37%–38%), the specificity was high (94%–96%). We also developed a BE progression risk calculator combining clinical variables with the Tissue Cypher risk

Table 2. Predictors of Progression in All Patients With BE Included in the Analysis Without TissueCypher Results

Variables	OR	95% CI		*P* value
Age, per year	1.08	1.01	1.16	.021
Male sex	3.55	0.84	14.99	.085
Expert diagnosis (IND vs NDBE)	2.25	0.86	5.84	.097
Expert diagnosis (LGD vs NDBE)	5.84	2.47	13.76	< .001
Presence of hiatal hernia	0.68	0.33	1.37	.27
BE segment length, per cm	1.15	1.01	1.30	.030

Note: C-statistic of model, 0.68 (95% CI, 0.59–0.76).
BE, Barrett's esophagus; *C-statistic*, concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *NDBE*, non-dysplastic Barrett's esophagus; *OR*, odds ratio.

class, setting the stage for its validation in subsequent prospective studies.

A biomarker-based strategy to predict progression may have potential advantages in the elusive quest to predict BE progression. If measured in an objective (quantitative and automated) manner, biomarkers could overcome inherent subjectivity in histological assessment by pathologists. Additionally, biomarker level changes may precede histological changes predictive of progression. Several biomarkers have been explored for this purpose. These include genetic (mutations, copy number variations, aneuploidy) and epigenetic (methylated DNA) markers. Jin et al assayed a panel of methylated DNA markers on FFPE specimens from a multicenter retrospective cohort with a sensitivity of 44% and specificity of 90%.[15] A Dutch group assessed fluorescence in situ hybridization biomarkers, in a prospective cohort of 428 patients with 22 progressors, reporting a sensitivity of 86% and a specificity of 54%.[16] Other investigators have used polymerase chain reaction-based technology and next-generation sequencing to discover promising biomarkers.[8,17] Challenges with some technologies include the need for fresh frozen tissue or endoscopic brushings, lack of independent validation beyond the initial study describing the markers (falling in the Early Detection Research Network phase 2, retrospective phase 3 categories),[18] and challenges with scaling assay technology. The TissueCypher assay is performed on easily accessible FFPE biopsy tissue, utilizing automated, quantitative scoring of multiple biomarkers classes, performed in a Clinical Laboratory Improvement Amendments-certified laboratory and has been validated in multiple independent cohorts.

However, some limitations should also be acknowledged. Biopsies sample a small proportion of the total BE mucosa. Hence, an assay performed on a single biopsy is likely to be inadequately sensitive. Despite the use of the highest risk class from all analyzed biopsies (as some patients had biopsies from multiple levels and endoscopies analyzed), sensitivity of a high-risk test class was

Table 3. Predictors of Progression in All Patients With BE With TissueCypher Risk Class Incorporated

Variables	OR	95% CI		*P* value
Age, per year	1.06	0.99	1.14	.11
Male sex	2.95	0.64	13.69	.17
Expert diagnosis (IND vs NDBE)	2.13	0.76	5.99	.15
Expert diagnosis (LGD vs NDBE)	2.92	1.18	7.24	.021
Presence of hiatal hernia	0.71	0.33	1.52	.38
BE segment length, per cm	1.13	0.99	1.30	.082
TissueCypher risk class (high vs low)	6.00	2.99	12.01	< .001
TissueCypher risk class (intermediate vs low)	1.58	0.80	3.12	.19

Note: C-statistic of model, 0.75 (95% CI, 0.66–0.83).
BE, Barrett's esophagus; *C-statistic*, concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *NDBE*, non-dysplastic Barrett's esophagus; *OR*, odds ratio.

Table 4. Predictors of Progression in Patients With BE Without Dysplasia Without TissueCypher Results

Variables	OR	95% CI		*P* value
Age, per year	1.08	1.01	1.16	.037
Male sex	2.39	0.52	11.00	.26
Presence of hiatal hernia	0.70	0.29	1.57	.36
BE segment length, per cm	1.17	1.03	1.34	.017

Note: C-statistic of model, 0.63 (95% CI, 0.53–0.74).
BE, Barrett's esophagus; *C-statistic*, concordance statistic; *CI*, confidence interval; *OR*, odds ratio.

Table 5. Predictors of Progression in Patients With BE Without Dysplasia With TissueCypher Results

Variables	OR	95% CI		*P* value
Age, per year	1.06	0.98	1.15	.15
Male sex	1.05	0.20	5.50	.95
Presence of hiatal hernia	0.57	0.21	1.54	.26
BE segment length, per cm	1.14	0.99	1.32	.071
TissueCypher risk class (high vs low)	14.23	5.16	39.19	< .001
TissueCypher risk class (intermediate vs low)	1.69	0.74	3.86	.21

Note: C-statistic of the model, 0.72 (95% CI, 0.61–0.83).
BE, Barrett's esophagus; *C-statistic*, concordance statistic; *CI*, confidence interval; *OR*, odds ratio.

modest (38%). Strategies to further improve sensitivity may include inclusion of additional markers, recalibrating the prediction algorithm, and dichotomizing results by combining high- and intermediate-risk classes (vs a low-risk class) (this approach increased sensitivity to 55% but with a lower specificity of 82% in the current analysis) or assessing assay performance on sample types that sample a greater surface area of the BE mucosa: such as from endoscopic brushings or swallowed nonendoscopic cell collection devices.[19]

The management strategy for those with high or low TissueCypher progression risk classes remains to be defined. Given the risks and costs associated with endoscopic eradication therapy (EET), high assay specificity is critical for considering proactive EET in those with NDBE and a TissueCypher high risk result, particularly if combined with other clinical risk factors predicting increased risk, such as confirmed LGD. With a modest 38% sensitivity in predicting progression with a high-risk class, it may be more reasonable to perform intensive surveillance using advanced imaging techniques to detect prevalent dysplasia (which would justify EET) following a high-risk class result. In contrast, a low-risk class may open the possibility of extending surveillance particularly in combination with other clinical variables such as female sex and short-segment BE, which would lower progression rates (see risk calculator results). Similarly, a low-risk class result could help recommending surveillance in LGD, if the patient is a female with a short BE segment (see risk calculator results). In a recent study, an initial change in the clinical management was instituted in a majority of 60 patients with BE utilizing TissueCypher results, though a change in final outcome was not reported.[20] Implementation of such management changes on the basis of biomarker panel results combined with clinical variables will require additional prospective studies more precisely defining risk estimates, in a true surveillance population.

The British Society of Gastroenterology recommends p53 immunostaining to improve diagnostic reproducibility of a BE dysplasia diagnosis.[6] In a systematic review and meta-analysis, aberrant p53 expression was associated with 4- to 17-fold increased risk of progression.[21] The TissueCypher assay incorporates p53 expression as one of the biomarkers. In a prior study, the multimarker TissueCypher assay demonstrated superior risk stratification (hazard ratio, 4.7; $P < .0001$) than p53 results alone (hazard ratio, 1.6; $P = .19$).[14] It is likely that the multiple biomarkers and morphological variables incorporated in the TissueCypher assay provide more predictive information than p53 alone.

The strengths of this study include the large sample size, allowing a priori planned analyses. Inclusion of patients from multiple institutions from the United States and Europe render the results more generalizable. All biomarker analyses were conducted in a blinded fashion, using set cutoffs and standardized methods. Limitations include the retrospective design of 3 studies included in the primary analysis. Variables influencing progression, such as smoking history, body mass index, and medication history were not available, and their incorporation into models could alter results. The absence of central expert pathology read may be viewed as a limitation, although this is likely reflective of real-world practice. We also acknowledge that this was not a true nested case-control study, as controls were selected based on knowledge that at last follow-up they were event-free, but given the overall low incidence of progression, this is unlikely to have a significant impact. Although it is conceivable that some of the non-progressors could have progressed after the end of follow-up, the nonprogressor follow-up was substantially longer than that of the progressors. The assay predicts progression risk within 5 years of the baseline biopsy analyzed. Hence, prediction beyond 5 years cannot be assessed. Finally, some data were missing for BE length and hiatal hernia. However, the concordance of results between analyses with and without imputed data and those incorporating BE segment length as continuous and dichotomous (long- vs short-segment) variables are reassuring. Lastly, most studies included in this analysis are case-control studies leading to higher than clinically observed progression rates, precluding calculation of positive and negative predictive values.

Conclusions

In summary, the TissueCypher assay significantly increases the accuracy of prediction of BE progression over that proferred by clinical variables alone. Assay sensitivity remains modest, and strategies to improve assay sensitivity need to be explored. However, assay specificity is high and could assist in clinical decision making particularly if combined with clinical variables. Prospective studies in patients with NDBE are needed to determine true positive and negative predictive values of the assay and validate the integrated clinical and biomarker risk score developed in this study.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2022.02.033.

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Conflicts of interest
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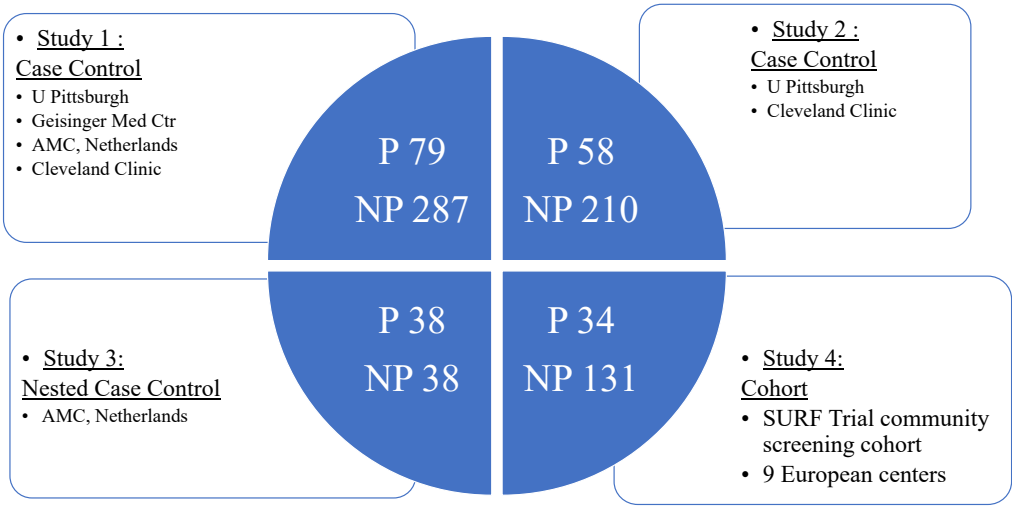
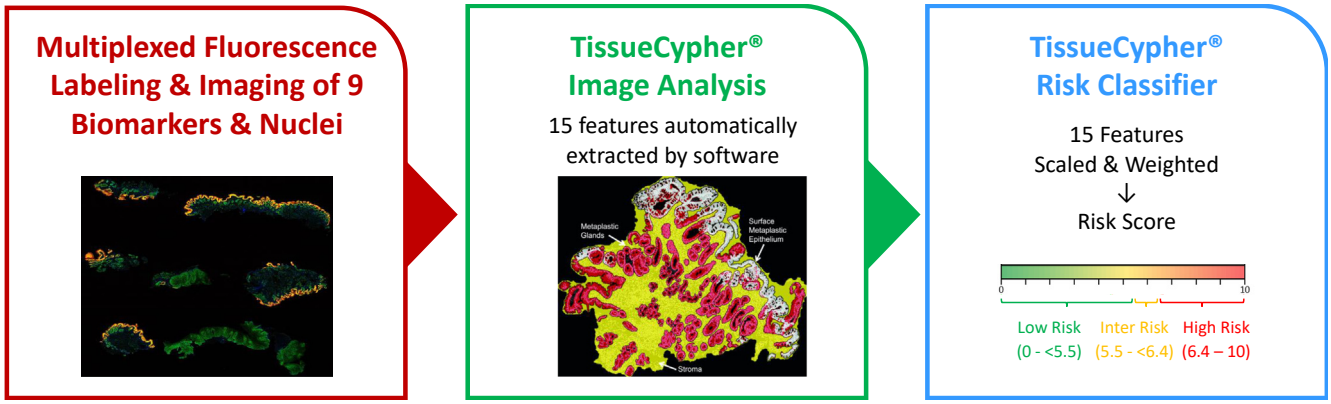


Supplementary Figure 1. Studies from which patient-level data were included in the primary analyses. *NP*, Non-progressors; *P*, progressors.



Supplementary Figure 2. Overview of the TissueCypher Barrett's esophagus assay. Serial sections of FFPE Barrett's esophagus biopsies are fluorescently immunolabeled for p16, AMACR, p53, HER2, K20, CD68, COX-2, HIF-1a, and CD45RO, plus Hoechst using an autostainer. The labeled slides undergo whole-slide fluorescence scanning to generate image data on each protein-based biomarker and nuclei. The whole-slide images are then analyzed by the TissueCypher Image Analysis Platform that automatically extracts 15 pre-defined features, which are quantitative measurements of the protein-based biomarkers and nuclear morphology. The TissueCypher continuous risk score (0–10) is calculated from the scaled and coefficient-weighted sum of the 15 features. Cutoffs are applied to classify patients for risk of progression to HGD/EAC within 5 years as follows: risk class = low if score 0 to <5.5, intermediate if score 5.5 to <6.4, and high if score 6.4 to 10. The scaling parameters, coefficients, and cutoffs were derived and locked in a training study.

Supplementary Table 1. TissueCypher Results in Patients Who Progressed and did not Progress to HGD/EAC During Surveillance

			TissueCypher result n (%)			
Patient group	Patient outcome	N	Low-risk	Intermediate-risk	High-risk	Intermediate- or high-risk
All patients (baseline diagnoses of NDBE, IND, and LGD)	Progressed to HGD/EAC during available surveillance time	152	69 (45.4)	25 (16.4)	58 (38.2)	83 (54.6)
	Did not progress to HGD/EAC during available surveillance time	400	328 (82.0)	48 (12.0)	24 (6.0)	72 (18.0)
NDBE subset	Progressed to HGD/EAC during available surveillance time	112	54 (48.2)	17 (15.2)	41 (36.6)	58 (51.8)
	Did not progress to HGD/EAC during available surveillance time	360	305 (84.7)	42 (11.7)	13 (3.6)	55 (15.3)

EAC, Esophageal adenocarcinoma; *HGD*, high-grade dysplasia; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *NDBE*, nondysplastic Barrett's esophagus.

Supplementary Table 2. Multivariable Conditional Logistic Regression Model and C-Statistic Using TissueCypher Score as Continuous Variable for Prediction of Incident Progression in the Entire Cohort

	OR	95% CI		*P* value
Age	1.07	0.99	1.14	.07
Sex (male vs female)	3.15	0.70	14.24	.14
Expert diagnosis (IND vs ND)	1.77	0.65	4.77	.26
Expert diagnosis (LGD vs ND)	3.18	1.29	7.87	.01
Hiatal hernia (yes vs no)	0.66	0.31	1.38	.27
Segment length in cm	1.10	0.95	1.27	.19
TissueCypher risk score	1.47	1.25	1.73	< .001

	C-statistic	95% CI		*P* value[a]
	0.77	0.67	0.84	< .001

C-statistic, Concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *OR*, odds ratio.
[a]For the likelihood ratio test comparison between the c-statistic of this model vs the model without TissueCypher.

Supplementary Table 3. Multivariable Conditional Logistic Regression Model and Concordance Statistic Using TissueCypher Class Merging High + Intermediate vs Low-risk Classes for Prediction of Incident Progression in the Entire Cohort

	OR	95% CI		*P* value
Age	1.07	0.99	1.15	.07
Sex (male vs female)	2.72	0.60	12.38	.20
Expert diagnosis (IND vs ND)	2.03	0.73	5.67	.17
Expert diagnosis (LGD vs ND)	3.42	1.39	8.43	.01
Hiatal hernia (yes vs no)	0.71	0.34	1.48	.36
Segment length in cm	1.12	0.97	1.29	.11
TissueCypher risk class (high/intermediate vs low)	3.18	1.941	5.21	< .001

	C-statistic	95% CI		*P* value[a]
	0.74	0.66	0.82	< .001

C-statistic, Concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *OR*, odds ratio.
[a]For the likelihood ratio test comparison between the c-statistic of this model vs the model without TissueCypher.

Supplementary Table 3A. Multivariable Conditional Logistic Regression Model and C-statistic Using TissueCypher Class, Merging High- vs Intermediate-/Low-Risk Classes for Prediction of Incident Progression in All Patients (With Baseline NDBE, IND, and LGD)

	OR	95% CI		*P* value
Age	1.062	0.990	1.139	.0458
Sex (male vs female)	2.921	0.642	13.286	.0827
Expert diagnosis (IND vs ND)	2.233	0.809	6.161	.0604
Expert diagnosis (LGD vs ND)	3.149	1.277	7.768	.0064
Hiatal hernia (yes vs no)	0.727	0.337	1.568	.7918
Segment length in cm	1.152	1.003	1.323	.0227
Tissue cypher risk class (high vs intermediate/low)	5.671	2.841	11.320	< .0001

	C-statistic	95% CI		*P* value[a]
	0.7329	0.6457	0.8200	< .0001

C-statistic, Concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *NDBE*, nondysplastic Barrett's esophagus; *OR*, odds ratio.
[a]For the likelihood ratio test comparison between the c-statistic of this model vs the model without TissueCypher.

Supplementary Table 4. Multivariable Conditional Logistic Regression Model and C-statistic Using TissueCypher Score as Continuous Variable in NDBE Subgroup for Prediction of Incident Progression

	OR	95% CI		*P* value
Age	1.06	0.98	1.14	.1310
Sex (male vs female)	1.46	0.29	7.46	.6506
Hiatal hernia (yes vs no)	0.56	0.22	1.39	.2101
Segment length in cm	1.11	0.95	1.29	.1819
TissueCypher risk score	1.55	1.21	1.87	< .0010

	C-statistic	95% CI		*P* value[a]
	0.72	0.62	0.82	< .001

C-statistic, Concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *ND*, no dysplasia; *NDBE*, nondysplastic Barrett's esophagus; *OR*, odds ratio.
[a]For the likelihood ratio test comparison between the c-statistic of this model vs the model without TissueCypher for the ND subgroup.

Supplementary Table 5. Multivariable Conditional Logistic Regression Model and C-statistic Using TissueCypher Class Merging High and Intermediate Categories for the NDBE Subgroup for Prediction of Incident Progression

	OR	95% CI		*P* value
Age	1.08	0.99	1.17	.083
Sex (male vs female)	1.16	0.22	6.05	.856
Hiatal hernia (yes vs no)	0.69	0.28	1.69	.41
Segment length in cm	1.14	0.98	1.33	.09
TissueCypher risk (high/intermediate vs low)	4.69	2.54	8.67	< .001

	C-statistic	95% CI		*P* value[a]
	0.71	0.61	0.81	< .001

C-statistic, Concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *ND*, no dysplasia; *NDBE*, nondysplastic Barrett's esophagus; *OR*, odds ratio.
[a]For the likelihood ratio test comparison between the C-statistic of this model vs the model without TissueCypher for the ND subgroup.

Supplementary Table 5A. Multivariable Conditional Logistic Regression Model and C-statistic Using TissueCypher Class, Merging High vs Intermediate/Low Risk Classes for Prediction of Incident Progression in Patients With Only NDBE

	OR	95% CI		*P* value
Age	1.064	0.980	1.155	.0689
Sex (male vs female)	1.096	0.212	5.657	.4565
Hiatal hernia (yes vs no)	0.517	0.185	1.445	.8957
Segment length in cm	1.177	1.013	1.368	.0167
Tissue cypher risk (high vs intermediate/low)	13.549	4.904	37.433	< .0001

	C-statistic	95% CI		*P* value[a]
	0.7100	0.6043	0.8153	< .0001

C-statistic, Concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *ND*, no dysplasia; *NDBE*, nondysplastic Barrett's esophagus; *OR*, odds ratio.
[a]For the likelihood ratio test comparison between the c-statistic of this model vs the model without tissue cypher for the ND subgroup.

Supplementary Table 6. Multivariable Conditional Logistic Regression Model and C-statistic Using BE Segment Length Dichotomized Into Long Segment vs Short Segment BE for Entire Cohort

	OR	95% CI		*P* value
Age	1.05	0.99	1.10	.0722
Sex (male vs female)	3.08	0.77	12.26	.1108
Expert diagnosis (IND vs ND)	2.13	0.87	5.22	.0979
Expert diagnosis (LGD vs ND)	8.00	3.84	16.65	< .001
Hiatal hernia (yes vs no)	0.77	0.42	1.42	.41
Segment length in cm	1.15	1.03	1.28	.01

	C-statistic	95% CI	
	0.69	0.61	0.77

BE, Barrett's esophagus; *C-statistic*, concordance statistic; *CI*, confidence interval; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *OR*, odds ratio.

Supplementary Table 7. Multivariable Conditional Logistic Regression Model Without TissueCypher Class in Patients With Complete Data Without the Need for Imputation

	OR	95% CI		*P* value
Age	1.0556	0.9745	1.1435	.1848
Sex (male vs female)	0.9180	0.1616	5.2140	.9231
Expert diagnosis (IND vs ND)	1.1221	0.2859	4.4043	.8689
Expert diagnosis (LGD vs ND)	3.9097	1.1481	13.3138	.0292
Hiatal hernia (yes vs no)	0.8041	0.2825	2.2887	.6828
Segment length in cm	1.2728	1.0727	1.5101	.0057

CI, Confidence interval; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *OR*, odds ratio.

Supplementary Table 8. Multivariable Conditional Logistic Regression Model With TissueCypher Class in Patients With Complete Data Without the Need for Imputation

	OR	95% CI		*P* value
Age	1.0388	0.9474	1.1390	.4177
Sex (male vs female)	0.4861	0.0771	3.0657	.4426
Expert diagnosis (IND vs ND)	1.1081	0.2375	5.1697	.8961
Expert diagnosis (LGD vs ND)	2.7006	0.7105	10.2649	.1448
Hiatal hernia (yes vs no)	0.6936	0.2189	2.1980	.5342
Segment length in cm	1.2497	1.0403	1.5012	.0172
TissueCypher risk class (high vs low)	6.9651	2.7065	17.9246	.0001
TissueCypher risk class (intermediate vs low)	2.1948	0.8760	5.4988	.0934

CI, Confidence interval; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *OR*, odds ratio.

Supplementary Table 9. Multivariable Conditional Lsogistic Regression Model and C-statistic NOT Using TissueCypher Information for Prediction of Incident and Prevalent HGD/EAC

	OR	95% CI		*P* value
Age	1.05	0.99	1.10	.0722
Sex (male vs female)	3.08	0.77	12.26	.1108
Expert diagnosis (IND vs ND)	2.13	0.87	5.22	.0979
Expert diagnosis (LGD vs ND)	8.00	3.84	16.65	< .001
Hiatal hernia (yes vs no)	0.77	0.42	1.42	.41
Segment length in cm	1.15	1.03	1.28	.01
	C-statistic	95% CI		
	0.69	0.61	0.77	

C, Concordance; *CI*, confidence interval; *EAC*, esophageal adenocarcinoma; *HGD*, high-grade dysplasia; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *OR*, odds ratio.

Supplementary Table 10. Multivariable Conditional Logistic Regression Model and C-statistic Using TissueCypher Class for Prediction of Incident and Prevalent HGD/EAC

	OR	95% CI		*P* value
Age	1.03	0.97	1.09	.34
Sex (male vs female)	2.36	0.54	10.23	.25
Expert diagnosis (IND vs ND)	1.87	0.69	5.05	.22
Expert diagnosis (LGD vs ND)	3.50	1.59	7.67	.002
Hiatal hernia (yes vs no)	0.77	0.40	1.40	.44
Segment length in cm	1.14	1.01	1.28	.04
TissueCypher risk class (high vs low)	7.81	4.06	15.03	< .001
TissueCypher risk class (intermediate vs low)	1.81	1.01	3.24	.05
	C-statistic	95% CI		*P* value[a]
	0.76	0.68	0.83	< .001

C, Concordance; *CI*, confidence interval; *EAC*, esophageal adenocarcinoma; *HGD*, high-grade dysplasia; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *OR*, odds ratio.

[a]For the likelihood ratio test comparison between the c-stat of this model vs the model without TissueCypher for the ND subgroup.

Supplementary Table 11. Multivariable Conditional Logistic Regression Model and C-statistic Using TissueCypher Class for the NDBE Subgroup for Prediction of Incident and Prevalent HGD/EAC

	OR	95% CI		*P* value
Age	1.04	0.97	1.11	.32
Sex (male vs female)	1.02	0.20	5.29	.98
Hiatal hernia (yes vs no)	0.51	0.21	1.23	.14
Segment length in cm	1.15	0.99	1.34	.06
TissueCypher risk class (high vs low)	18.07	6.57	49.71	< .001
TissueCypher risk class (intermediate vs low)	1.94	0.93	4.03	.0776
	C-statistic	95% CI		*P* value[a]
	0.72	0.62	0.82	< .001

C-statistic, Concordance statistic; *CI*, confidence interval; *EAC*, esophageal adenocarcinoma; *NDBE*, nondysplastic Barrett's esophagus; *ND*, no dysplasia; *OR*, odds ratio.

[a]For the likelihood ratio test comparison between the c-statistic of this model vs the model without TissueCypher for the ND subgroup.