

Algorithm Training and Independent Test Set Performance for a Molecular Non-Endoscopic Test for Detection of **Esophageal Adenocarcinoma and Barrett's Esophagus in Multicenter Cohorts**

Prasad Iver, MD, FACG¹, Seth Slettedahl, MS¹, Douglas Mahoney, MS¹, Martin Krockenberger, PhD², Maria Giakoumopoulos, PhD², Carla Volkmann, MS², Jacob Otto, BS², Adam Solsrud, BS², William Taylor, MS¹, Ramona Lansing, BSN¹, Erin Gibbons, BS¹, Melissa Passe, BSN¹, Molly Stewart, BS⁸, Shamel Brown, BS⁷, Katelyn Valdez, MBA³, Kristen Lozano, BS⁶, Karen Doering, MBA¹, Kelli Burger, BS¹, Daniel Kim, MS⁷, Calise Berger, BS¹, Cadman Leggett, MD¹, Kenneth Wang, MD, FACG¹, Gary Falk, MD, MS⁴, Tsung-Teh Wu, MD, PhD¹, Julian Abrams, MD, MS⁵, Francisco Ramirez, MD, FACG³, Allon Kahn, MD³, Herbert Wolfsen, MD⁶, Chanakyaram A. Reddy, MD⁷, Anh D. Nguyen, MD⁷, Vani Konda, MD⁷, Arvind Trindade, MD⁸, and John Kisiel, MD, MSc¹

¹ Mayo Clinic, Rochester Minnesota, ³ Scottsdale, Arizona, ⁶ Jacksonville, Florida, ² Exact Sciences Corporation, Madison, Wisconsin, ⁴ University, New York, ⁷ Baylor University Medical Center, Dallas, Texas, ⁸ Northwell Health, New Hyde Park, New York

INTRODUCTION

- \succ Sedated endoscopy for Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) detection is invasive and expensive.
- > Non-endoscopic BE/EAC detection tools have been guideline-endorsed to facilitate higher patient participation at lower cost.¹
- \succ We previously described a promising panel of 5 methylated DNA markers (MDMs) assayed on esophageal specimens obtained by a sponge-on-astring cell collection device in phase II studies.^{2,3}
- \succ We aimed to train an algorithm (establish cutoff, to adjudicate samples as elevated/negative) using a final MDM panel followed by testing in an independent sample set.

METHODS

- (N=352) training samples > Algorithm were prospectively collected from patients seen at 6 US medical centers. Test samples (N=125) were obtained from an independent, NIH-funded study conducted at 3 US medical centers. Both training and test sets were case control studies.
- > Cases had endoscopic columnar metaplasia with histological intestinal metaplasia; controls had no endoscopic evidence of BE. Histology was reviewed by expert GI pathologists.
- > The EsophaCap (Lucid, New York City, NY) cell collection device (25 mm, 10 pores per inch) was swallowed and withdrawn after 6-8 minutes followed by criterion standard endoscopy within 24 hours.
- \succ DNA was extracted from collected cells and then bisulfite treated. Five MDMs were blindly assayed using the long probe quantitative amplified signal (LQAS) method.
- The algorithm was set using cross-validated logistic regression. The algorithm performance was evaluated with an independent test set.

Baseline characteristics of patients in training and test sets are described in **Table 1**. Training and test sets were comparable.

Table 1 Baseline Characteristics of BE Cases and Controls

Variable	Training Set		Test Set		P value
	(N=198 controls,154 cases)		(N= 44 controls,81 cases)		
	Control	Case	Control	Case	
Mean age, (SD)	55 (13)	65 (10)	52 (15)	65 (11)	0.312
Male Sex (%)	102 (52)	119 (77)	17 (39)	64 (79)	0.992
Mean BMI (SD)	29 (7)	30 (6)	30 (7)	30 (6)	0.283
Ever Smokers (%)	77 (39)	87 (56)	18 (41)	47 (58)	0.733
Mean BE length, cm (SD)	-	4 (3)	-	5 (3)	0.070
Long segment BE, N, (%)	-	97 (63)	-	56 (69)	0.426
Short segment BE, N (%)	-	57 (37)	-	25 (31)	
BE dysplasia grade, N (%)					
- EAC	-	12 (8)	-	2 (2)	
- HGD	-	18 (12)	-	11 (14)	
- LGD	-	7 (4)	-	10 (12)	
- IND	-	18 (12)	-	14 (17)	
- NDBE (long segment)	-	57 (37)	-	25 (31)	
- NDBE (short segment)	-	42 (27)	-	19 (24)	

BE, Barrett's esophagus; BMI, body mass index; cm, centimeter; EAC, esophageal adenocarcinoma; HGD, high grade dysplasia; IND, indefinite for dysplasia; LGD, low grade dysplasia; NDBE, non-dysplastic Barrett's esophagus; SD, standard deviation.

- marker B3GALT6.
- segment NDBE in the test set was 63% (38-84%) (Table 2).

Table 2 Algorithm Performance in Training and Test Datasets

	Training Set		Test Set			
	n Positive	% Sensitivity (95% CI)	n Positive	% Sensitivity (95% CI)		
Overall	125	81 (68-94)	71	88 (78-94)		
- EAC	12	100 (100-100)	2	100 (16-100)		
- HGD	18	100 (100-100)	11	100 (72-100)		
- LGD	5	71 (0-100)	9	90 (55-100)		
- IND	13	74 (0-100)	13	93 (66-100)		
- NDBE (long segment)	52	91 (73-100)	24	96 (80-100)		
- NDBE (short segment)	25	61 (25-100)	12	63 (38-84)		
	n Positive	% Specificity (95% CI)	n Positive	% Specificity (95% CI)		
Control (No BE)	20	90 (79-98)	7	84 (70-93)		

BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; HGD, high grade dysplasia; IND, indefinite for dysplasia; LGD, low grade dysplasia; NDBE, non-dysplastic Barrett's esophagus.

RESULTS

> The final assay included 3 MDMs (NDRG4, VAV3, ZNF682) and a reference

> Overall sensitivity for BE/EAC detection in the training set was 81% (68-94%) with specificity of 90% (79-98%). BE/EAC sensitivity in the test set was 88% (78-94%) at 84% (70-93%) specificity. Sensitivity for HGD/EAC was 100% in the training and test sets. Sensitivity for short

sets, respectively (Figure 1).



- \succ The algorithm was not influenced by age, sex, or smoking history.
- the cell collection device, which was well tolerated and safe.

CONCLUSION

- BE cases in multi-center case control training and test sets.

REFERENCES

Acknowledgements

Medical writing and editorial support was provided by Carolyn Hall, PhD, and Feyza Sancar, PhD (Exact Sciences, Madison, WI).

This study was sponsored by Exact Sciences Corp., Madison, WI and NIH grants U54CA163004, R01CA241164.

> Areas under the receiver operating characteristic (AUROC) curves for BE/EAC detection were 0.92 (95% CI 0.89-0.95) and 0.94 (0.90-0.98) in the training and test

Figure 1. Area under the receiver operating characteristic curves in training and test sets.

> 97% of participants in the training set and 85% in the test set successfully swallowed

> A 3-MDM panel for BE/EAC detection demonstrated excellent sensitivity for high risk

 \succ The performance of this panel and algorithm will be validated in ongoing studies.

1. Shaheen NJ, Falk GW, Iyer PG, Souza RF, Wani S. Am J Gastroenterol. Apr 2022;117(4):559-587. 2. Iyer PG, Taylor WR, Johnson ML, et al. Am J Gastroenterol. Aug 2018;113(8):1156-1166. 3. Iyer PG, Taylor WR, Johnson ML, et al.. Am J Gastroenterol. Aug. 2020;115(8):1201-1209.