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DNA Methylation biomarkers for the diagnosis of Barrett's Oesophagus

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To the Editor

With the recent publication by Iyer *et al.*¹, it is very encouraging to see the considerable effort being made to develop non-endoscopic, non-invasive and cost-effective approaches for early diagnosis of Barrett's oesophagus, with potential applicability to primary care. In this new study, combined DNA hypermethylation of VAV3 and ZNF682 was used with an EsophaCap™ device for detection of Barrett's¹. Previous studies investigated the potential of utilising non-endoscopic cell collection devices coupled with DNA methylation markers for Barrett's diagnosis^{2,3}, neither of which were cited¹. Biomarkers identified by Chettouh *et al.*² were based on discovery assays as part of the upfront study design, whereas Moinova *et al.*³ and Iyer *et al.*¹ also included markers from previous studies and the samples size of these studies varied substantially.

Utilising a two-step discovery process ($n=31$ Barrett's; $n=7$ controls), Chettouh *et al.* identified four candidates (TFPI2, TWIST1, ZNF345, ZNF569) and validated them in non-endoscopic Cytosponge™ samples ($n=149$ Barrett's; $n=129$ controls) with matched TFF3 immunohistochemistry data⁴. These markers yielded AUC values 78.7-87.7% which positively correlated with the length of the Barrett's segment; a combined marker analysis was not performed to avoid overfitting of the data.

Moinova *et al.* reported 412 differentially methylated regions (DMRs) in a discovery cohort ($n=14$ Barrett's; $n=26$ oesophageal adenocarcinoma (OAC); $n=26$ controls). Following their filtering process, CCNA1 was selected for validation in oesophageal brushings and non-endoscopic encapsulated balloon devices. VIM was then added to the validation phase following positive results in their previous studies. In balloon devices ($n=50$ Barrett's; $n=36$ controls) CCNA1 and VIM revealed specificities of 100% and 91.7% and sensitivities of 72% and 80% respectively.

Iyer *et al.* identified 188 DMRs in a discovery cohort ($n=54$ Barrett's; $n=18$ controls), from which 25 were selected for validation along with other 35 OAC methylation markers

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Conflict of Interest

RCF is named on patents pertaining to the cytosponge and associated assays that have been licensed by the Medical Research Council to Covidien (now Medtronic). RCF is a chief investigator on the BEST3 trial.

reported in their previously published abstract⁵. ELMO1 and CD1D were the top performers with AUCs 0.94 and 0.93 respectively. 19 methylation candidates were further validated in oesophageal brushings (16 OAC, 3 Barrett's) and EsophaCapTM samples ($n=19$ Barrett's; $n=20$ controls). VAV3 and ZNF682 were shown to be the best candidates for detecting Barrett's through EsophaCapTM with a perfect area under receiver operator characteristics curve (AUC) of 1.0 ($n=19$ Barrett's; $n=20$ controls). The marker sensitivity was dependent on segment length (short versus long), although the cut-off was not provided. Interestingly, the reason for selection of VAV3 is not apparent from the earlier publications or in the abstract cited by the authors to justify the choice⁵ and ZNF682 was not a discriminant biomarker in their oesophageal brushings cohort.

One drawback of non-endoscopic devices is the limited control over their passage through the distal oesophagus; if devices fail to retrieve cells from the junction biomarkers may not succeed in diagnosing Barrett's. In the clinical trial BEST2 ($n=1,110$) the Cytosponge-TFF3 test's overall sensitivity was 79.9% and increased to 87.2% with longer segments (3cm)⁴ and TFF3 false negatives were largely due to the lack of columnar cells in the specimen rather than failure of the TFF3 antibody. Exclusion of cases with <5 columnar cell groups resulted in sensitivities of 91-98%, across five different centres⁴. Although Iyer *et al.* mention TFF3 is expressed in normal gastric cells, it is only expressed in intestinal metaplasia⁶ a precursor to gastric carcinogenesis.

These promising non-endoscopic approaches require testing in large-scale clinical trials to assess tolerability, sensitivity and specificity in the relevant population. BEST3 is an example of such trial with a cluster randomised design ($n=13,000$), currently mid-way through recruitment (www.best3trial.org).

References

1. Iyer PG, Taylor WR, Johnson ML, et al. Highly Discriminant Methylated DNA Markers for the Non-endoscopic Detection of Barrett's Esophagus. *Am J Gastroenterol*. 2018; doi: 10.1038/s41395-018-0107-7
2. Chettouh H, Mowforth O, Galeano-Dalmau N, et al. Methylation panel is a diagnostic biomarker for Barrett's oesophagus in endoscopic biopsies and non-endoscopic cytology specimens. *Gut*. 2017; 0:1–8.
3. Moinova HR, LaFramboise T, Lutterbaugh JD, et al. Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett's esophagus. *Sci Transl Med*. 2018; 10:1–11.
4. Ross-Innes CS, Debiram-Beecham I, O'Donovan M, et al. Evaluation of a Minimally Invasive Cell Sampling Device Coupled with Assessment of Trefoil Factor 3 Expression for Diagnosing Barrett's Esophagus: A Multi-Center Case-Control Study. *PLoS Med*. 2015; 12:e10001780.
5. Taylor WR, K J, Yab TC, Cao X, et al. Novel Epigenetic Markers for Detection of Esophageal Cancer: Selection by Whole Methylome Sequencing and Tissue Validation. *Gastroenterology*. 2015; 148:S–550. Zong L, Hattori N, Yoda Y, et al. Establishment of DNA methylation marker to evaluate cancer cell fraction in gastric cancer. *Gastric Cancer*. 2016; 19:361–369. [PubMed: 25678126]
6. Lao-Sirieix P, Boussioutas A, Kadri SR, et al. Non-endoscopic screening biomarkers for Barrett's oesophagus: from microarray analysis to the clinic. *Gut*. 2009; 58:1451–1459. [PubMed: 19651633]