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Review Article

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Cytosponge and Methylation-Based Markers for Detection of Dysplasia in Patients with Barrett's Oesophagus: A Review

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Abstract

Barrett's oesophagus is the only established precursor of oesophageal adenocarcinoma, and most people who have it do not know. Endoscopy finds it reliably and cannot be offered to everyone at risk, which is why swallowable sampling devices paired with a biomarker have been developed. Whether they detect dysplasia, as opposed to detecting the metaplasia itself, is a separate question that the evidence answers less completely.

Objectives: To review the published evidence on non-endoscopic cell collection combined with trefoil factor 3 immunocytochemistry or DNA methylation markers in Barrett's oesophagus: reported accuracy, how performance varies with segment length and sampling, what has been shown specifically for dysplasia, and where these tests sit against endoscopy.

Material and Methods: Narrative review of device development studies, case-control diagnostic studies, randomised trials, cohort studies of risk stratification, cost-effectiveness analyses and guidelines indexed in PubMed and the major gastroenterology journals. Studies were eligible if they reported the accuracy or acceptability of a non-endoscopic sampling device with a biomarker readout, outcomes from its use in a defined population, or guidance on its place in practice. No new patient data were generated; every accuracy estimate quoted is one published by the original investigators.

Results: In a multicentre case-control study of 1,110 participants, 93.9 per cent successfully swallowed the device and no serious adverse events were attributed to it. Sensitivity of Cytosponge-TFF3 for Barrett's oesophagus was 79.9 per cent overall, rising to 87.2 per cent for segments of at least 3 centimetres, which carry the higher cancer risk, and to 89.7 per cent when the device was swallowed twice; specificity was 92.4 per cent, and there was no loss of sensitivity in patients with dysplasia. A separate series reported 90.0 per cent sensitivity and 93.5 per cent specificity for circumferential segments of at least 2 centimetres. A balloon-based device paired with methylated vimentin and cyclin A1 achieved sensitivity and specificity both above 90 per cent. In a pragmatic randomised trial across 109 primary care practices, offering the test increased the number of patients diagnosed with Barrett's oesophagus.

Conclusion: Non-endoscopic sampling with either readout detects Barrett's oesophagus well enough to serve as a triage to endoscopy, and a randomised trial has shown it changes how many diagnoses are made. The evidence for detecting dysplasia specifically is thinner: sensitivity is reported as preserved in dysplastic patients rather than measured against dysplasia as the primary target, and the biomarker panels that stratify risk within a known Barrett's segment are a different application again.

Abbreviations: BE – Barrett's Oesophagus, EAC – Oesophageal Adenocarcinoma, TFF3 – Trefoil Factor 3, VIM – Vimentin, CCNA1 – Cyclin A1, IM – Intestinal Metaplasia, LGD – Low-Grade Dysplasia, HGD – High-Grade Dysplasia, GORD – Gastro-Oesophageal Reflux Disease, EGD – Oesophagogastrroduodenoscopy, NGS – Next-Generation Sequencing, FFPE – Formalin-Fixed Paraffin-Embedded, NPV – Negative Predictive Value.

Keywords: Barrett's Oesophagus; Cytosponge; DNA Methylation; Trefoil Factor 3; Non-Endoscopic Screening

Introduction

Oesophageal adenocarcinoma has risen sharply in incidence across western populations over recent decades, and projections to 2030 suggest the trend continues [1,2]. Survival remains poor once the disease is symptomatic, which places the emphasis on its precursor. Barrett's oesophagus, in which the squamous lining is replaced by intestinal metaplasia under the influence of reflux, is the only established precursor lesion [3,4].

The screening problem is one of scale. Heartburn affects up to a fifth of adults in the populations where adenocarcinoma is rising, and only a small fraction of those individuals have Barrett's oesophagus [5]. Endoscopy identifies it accurately but cannot be offered to everyone with reflux without imposing an unsustainable burden, and the guidelines reflect that by restricting screening to those with multiple risk factors [6,7,8,9].

Non-endoscopic cell collection was developed as a way through. The approach pairs a swallowable device that samples the oesophageal surface with a biomarker applied to the collected cells. Two readouts have reached clinical evaluation: immunocytochemistry for trefoil factor 3, a marker of intestinal metaplasia [10,11], and DNA methylation panels measured by sequencing [12,13,14].

The distinction that matters for this review is between detecting Barrett's oesophagus and detecting dysplasia within it. The first is a screening question: who should have an endoscopy at all. The second is a surveillance question: among people already known to have Barrett's oesophagus, who is progressing. These are different targets with different prevalences and different consequences for error, and the literature addresses the first far more completely than the second [15,16].

The aim of this review is to set out what has been demonstrated for each device and biomarker combination, how performance depends on the length of the segment and the number of samples taken, and what the evidence supports specifically in relation to dysplasia.

Material and Methods

Search strategy: PubMed and the archives of the major gastroenterology journals were searched for studies of non-endoscopic detection of Barrett's oesophagus. Search terms combined Cytosponge, EsoCheck, non-endoscopic, cell collection device and swallowable capsule with Barrett's oesophagus, oesophageal adenocarcinoma, trefoil factor 3, DNA methylation, vimentin, cyclin A1, dysplasia and screening. Reference lists of retrieved trials, guidelines and practice updates were screened by hand.

Eligibility: Studies were included if they reported the diagnostic accuracy, acceptability or safety of a non-endoscopic sampling device with a biomarker readout in a defined population; randomised or cohort evaluation of such a device in practice; risk stratification within known Barrett's oesophagus using biomarkers from such a device; or cost-effectiveness modelling of the resulting pathway. Guidelines and practice updates were included where they define the current place of these tests.

Data extraction and handling: Design, sample size, device, biomarker, reference standard, the definition of a positive result and reported accuracy estimates with confidence intervals were extracted as published. Accuracy figures are reported together with the segment definition used, because sensitivity in this field depends heavily on how Barrett's oesophagus is defined and on the length of segment present. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 3 and Tables 1 and 2 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

How the approach developed: The concept began with the observation that immunocytological markers could identify Barrett's cells in non-endoscopically collected samples [10], followed by microarray-based selection of candidate biomarkers which identified trefoil factor 3 as the most discriminating [11]. A primary care cohort study then established that the sampling device was acceptable to patients and produced usable material [17], and the approach was formalised as a screening test in development [18].

Accuracy in the pivotal case-control study: The definitive accuracy data come from a multicentre case-control study conducted

across eleven UK hospitals, in which 647 patients with known Barrett's oesophagus and 463 controls with reflux symptoms swallowed the device before endoscopy. Of 1,110 participants, 1,042 or 93.9 per cent swallowed it successfully, and no serious adverse events were attributed to the device. Patients rated it favourably against endoscopy, and those who had not been sedated for endoscopy rated it more favourably still [19].

Overall sensitivity for Barrett's oesophagus was 79.9 per cent (95% CI 76.4-83.0), rising to 87.2 per cent (83.0-90.6) in patients with at least 3 centimetres of circumferential disease, the group at higher cancer risk, and to 89.7 per cent (82.3-94.8) among 107 patients who swallowed the device twice. Specificity was 92.4 per cent (89.5-94.7) (Figure 1) [19]. An independent series using the same approach reported 90.0 per cent sensitivity and 93.5 per cent specificity for circumferential segments of at least 2 centimetres [20].

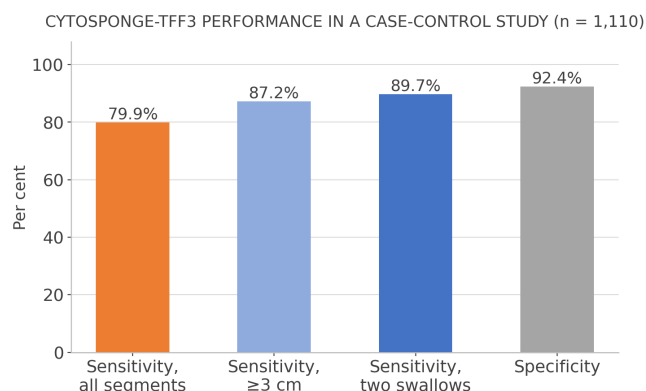


Figure 1: Cytosponge-TFF3 accuracy in a multicentre case-control study of 1,110 participants [19]

Sensitivity depends on how much Barrett's there is: The gradient across segment definitions is the most practically important feature of these data. A test that finds four in five short segments and roughly nine in ten long ones is behaving in a clinically convenient way, because cancer risk rises with segment length and the lesions most likely to be missed are those least likely to progress (Figure 3) [19,20]. It is nonetheless a limitation, and it means that quoted sensitivity figures are not comparable unless the segment definition is stated alongside them.

Sampling twice recovers much of the shortfall, raising sensitivity to close to 90 per cent [19]. Work quantifying trefoil factor 3 expression rather than reading it categorically has been pursued as a route to distinguishing clinically relevant segments from focal metaplasia at the junction [21].

Study	n	Design	Principal reported finding
Kadri et al. 2010 [17]	504	Cohort	Device acceptable and accurate in primary care
Ross-Innes et al. 2015 [19]	1,110	Case-ctrl	Sensitivity 79.9%, specificity 92.4%; 93.9% swallowed
Ross-Innes et al. 2017 [22]	—	Cohort	Biomarker panel stratifies risk within known BE
Moinova et al. 2018 [13]	408	Case-ctrl	Methylated VIM and CCNA1 above 90% sensitivity and specificity
Fitzgerald et al. 2020 [23]	109	RCT	Offer of the test increased BE diagnoses in primary care
Chak et al. 2024 [24]	—	Cohort	Biomarker-driven non-endoscopic detection of BE and EAC

Table 1: Principal studies reviewed, with design and the finding as reported by the original investigators

The methylation route: A parallel line of work replaced the immunocytochemical readout with DNA methylation. Aberrant methylation of vimentin had already been established as a marker of gastrointestinal neoplasia in stool [25] and as characteristic of upper gastrointestinal pathology [26]. Combining methylated vimentin with methylated cyclin A1 and applying the panel to cells collected by a swallowable balloon-capsule device produced sensitivity and specificity both above 90 per cent for Barrett's oesophagus and adenocarcinoma against endoscopy with biopsy (Figure 2) [13]. The assay reads methylation at 31 sites within the two genes by targeted sequencing and returns a binary result [13,14].

A subsequent multicentre prospective trial evaluated the same biomarker-driven approach in a screening rather than case-control setting [24]. Practice guidance from the major societies now lists non-endoscopic cell collection with biomarker testing as an acceptable alternative to endoscopy for screening in appropriately selected patients [7,16].

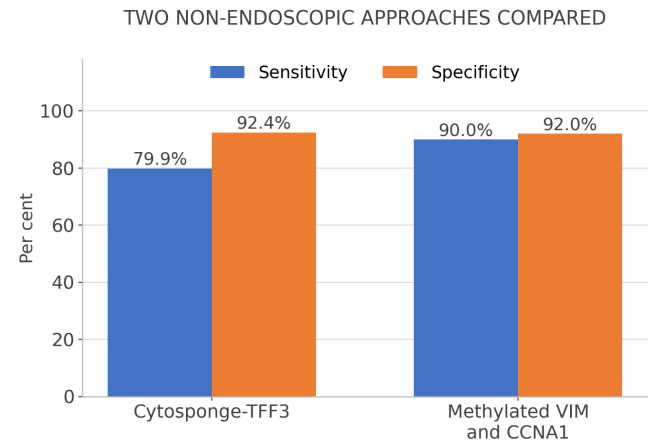


Figure 2: Reported sensitivity and specificity for the two non-endoscopic approaches against endoscopy with biopsy [13,19]

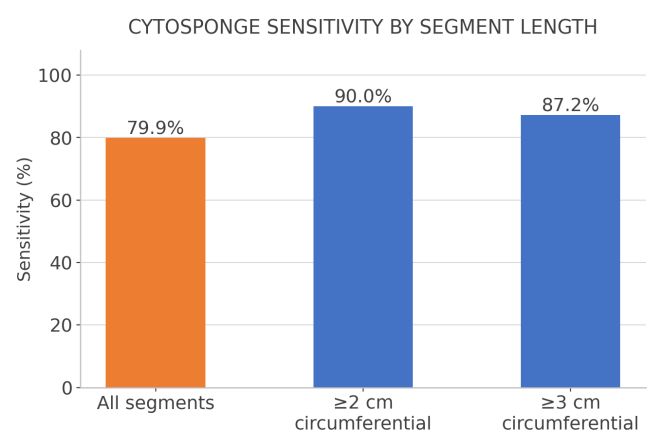


Figure 3: Cytosponge-TFF3 sensitivity by the segment definition applied, across two studies [19,20]

What has been shown for dysplasia: Here the evidence is thinner than the screening data, and the distinction is easily lost. The pivotal case-control study reported no loss of sensitivity in patients with dysplasia, which establishes that dysplastic segments are detected as reliably as non-dysplastic ones [19]. It does not establish that the test distinguishes dysplastic from non-dysplastic Barrett's oesophagus, which is the question surveillance asks.

That second question has been addressed separately, by applying a biomarker panel to non-endoscopically collected cells in patients already known to have Barrett's oesophagus, in order to stratify their risk of progression [22]. This is a genuinely different application: the population is defined, the prevalence of the target is low, and the purpose is to lengthen or shorten a surveillance interval rather than to trigger a first endoscopy. Reporting screening accuracy figures as though they answered the surveillance question overstates what has been shown.

Performance in practice rather than in a case-control study: The case-control design that produced the accuracy estimates enriches for disease and does not generalise directly to primary care, as the investigators noted [19]. That gap was addressed by a pragmatic cluster-randomised trial across 109 primary care practices in England, in which patients on long-term acid suppression were offered the test or usual care [23,27]. Offering it increased the number of patients diagnosed with Barrett's oesophagus, and the trial also identified treatable dysplasia and early cancer, at the cost of additional endoscopies and some false positive results [23].

Pooled safety and acceptability data across individual patients from multiple studies support the device's tolerability [28], and separate work has examined where adequacy of the sample falls short and what can be done about it [29]. Economic modelling has suggested the pathway can be cost-effective relative to endoscopic screening [30,31].

Question	What the evidence supports	Source
Who should have an endoscopy	Non-endoscopic sampling with either readout is an adequate triage	[13,19,23]
Does offering it change practice	Randomised evidence of increased BE diagnosis in primary care	[23]
Are dysplastic segments detected	Sensitivity reported as preserved in dysplastic patients	[19]
Can it separate dysplastic from non-dysplastic BE	Addressed only by biomarker panels applied within known BE	[22]
Is the pathway affordable	Modelling supports cost-effectiveness against endoscopic screening	[30,31]

Table 2: The distinct questions in this literature and what has been demonstrated for each

Discussion

The case for non-endoscopic sampling as a screening triage is now strong. Both readouts detect Barrett's oesophagus with sensitivity in the region of 80 to 90 per cent and specificity above 90 per cent [13,19,20], the device is acceptable to the large majority of patients who are offered it [19,28], and a randomised trial has shown that offering it changes how many diagnoses are made in the population where screening would actually happen [23]. Few biomarker-based screening proposals in gastroenterology reach that standard of evidence.

The case for these tests as dysplasia detection is weaker, and the title of this field invites the confusion. Preserved sensitivity in patients who happen to have dysplasia is not the same as discrimination between dysplastic and non-dysplastic disease [19]. The work that addresses progression risk directly uses a biomarker panel within an already diagnosed population, which is a surveillance application with different requirements [22].

Where the two readouts differ

Trefoil factor 3 immunocytochemistry and methylation sequencing are not simply alternatives. The former is a morphological assessment that requires a pathologist and yields information about sample adequacy alongside the result [20,29]. The latter is an automated sequencing assay returning a binary output, which is easier to scale but discards the cytological context [13,14]. Quantitative and machine-learning approaches to trefoil factor 3 scoring narrow that gap by automating the read [21].

For a health system deciding between them, the relevant considerations are therefore laboratory infrastructure and throughput rather than headline accuracy, since the reported accuracy figures overlap substantially [13,19].

Study limitations

The limitations belong to the underlying literature. The accuracy estimates derive from case-control studies in which cases had known Barrett's oesophagus and controls had reflux symptoms, a design the investigators explicitly noted does not generalise to primary care [19]. Sensitivity varies with segment length, so a single quoted figure conceals a range [19,20]. The methylation panel data come predominantly from populations that were not demographically diverse, and performance in other groups is under evaluation [13,24]. The randomised trial measured diagnosis of Barrett's oesophagus rather than cancer incidence or mortality, which are the outcomes that would establish screening benefit [23]. Several of the commercial assay validation reports come from groups with declared intellectual property interests [24].

Two gaps deserve naming. No randomised trial has yet reported whether non-endoscopic screening reduces oesophageal adenocarcinoma incidence or mortality, which is the endpoint that matters. And no study reviewed here reports the accuracy of either readout for dysplasia as a prespecified primary target in a surveillance population, which is the question the field is often described as having answered [19,22].

Conclusion

Non-endoscopic cell collection paired with either trefoil factor 3 immunocytochemistry or a methylated vimentin and cyclin A1 panel detects Barrett's oesophagus with accuracy adequate for triage, is acceptable to patients, and has randomised evidence that offering it increases diagnosis in primary care. Sensitivity rises with segment length and with repeat sampling, so quoted figures should always carry the segment definition. For dysplasia the position is different and should be stated as such: what has been demonstrated is that dysplastic segments are not missed preferentially, not that these tests distinguish dysplasia from non-dysplastic Barrett's oesophagus. Risk stratification within a known segment is a separate application with its own, smaller, evidence base.

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Conflict of Interest

Not one of the authors has a competing interest to disclose.

Ethics Statement

Published studies alone are examined here. Since no fresh patient data were gathered and no one took part as a subject, the question of ethical approval did not arise.

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