Role of TFF3 as an adjunct in the diagnosis of Barrett's esophagus using a minimally invasive esophageal sampling device—The Cytosponge™

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Abstract
The incidence of esophageal carcinoma continues to increase whilst its prognosis remains poor. The most dramatic reduction in mortality is likely to follow early diagnosis of the preinvasive precursor lesion, Barrett's esophagus (BE), coupled with treatment of dysplastic lesions. The major risk factor for BE is gastroesophageal reflux disease, however this is highly prevalent and only a small proportion of individuals have BE, therefore an endoscopy-based screening strategy to detect BE is unfeasible. Minimally invasive esophageal sampling devices offer an alternative, cost-effective strategy which can be deployed within an at-risk population in a primary care setting to identify individuals with probable BE who can then be referred for endoscopic confirmation. The device that has currently progressed furthest in clinical trials is the Cytosponge™ which collects cells from the gastric cardia, gastroesophageal junction and along the whole esophageal length. The cell sample is processed into a formalin-fixed paraffin-embedded block and sections assessed for the presence of intestinal metaplasia. TFF3 immunohistochemistry has consistently been shown to be a valuable adjunct that increases the accuracy of the Cytosponge™ test by highlighting early goblet cells which may be missed on morphological assessment and by allowing pseudogoblet cells to be differentiated from true goblet cells.

KEYWORDS
Barrett's esophagus, biomarkers, esophageal adenocarcinoma, screening

1 | INTRODUCTION

The incidence of esophageal adenocarcinoma (EAC) has increased dramatically over the last 30 years and is now the dominant subtype of esophageal carcinoma (EC) in most of the western world.¹,² Although there has been a steady, if minor, improvement in the prognosis of patients with EC overall it remains poor with a 15% overall five-year survival.³ A marked improvement in survival is seen if EAC is detected at an early stage,⁴ however most patients first present with dysphagia and weight loss which is associated with advanced local disease.⁵ Therefore for significant improvements in patient survival to occur EAC needs to be consistently detected at an earlier stage, and ideally before invasive disease develops.

Barrett's esophagus (BE) is a precursor lesion for EAC, therefore it is recommended that all patients diagnosed with BE are enrolled onto endoscopic surveillance programs with regular
systematic biopsy sampling of the BE segment to detect dysplasia development.9 The risk of cancer developing in dysplastic segments is up to 30% and therefore endoscopic therapy such as radiofrequency ablation (RFA) combined with endoscopic mucosal resection (EMR) of any focal lesion is recommended once dysplasia is detected.7,9

However, identifying patients in the general population with BE is challenging. BE is thought to affect between 3-6% of the adult population with the main risk factors being male sex, chronic gastroesophageal reflux (GERD) and obesity. The diagnosis is currently most commonly made when patients are referred for endoscopy to investigate persistent GERD or other non-specific upper gastrointestinal symptoms. However using this strategy most patients with BE remain undiagnosed since it is not feasible to endoscope all those with these common risk factors, for example, GERD symptoms are estimated to affect 10-44% of the general population in the Westernized world10 however only a small minority of these patients will have BE or intestinal metaplasia (IM) of the cardia at endoscopy.11

An alternative screening approach is to have a two-stage strategy consisting of an initial less invasive triage test to identify a subset of patients with a high probability of having BE followed by endoscopic confirmation. The different screening options for BE, including a traditional endoscopy, transnasal endoscopy, video capsule endoscopy, minimally invasive sampling devices, and serum biomarkers have recently been reviewed12,13 and considered against a range of factors including patient convenience, test sensitivity and specificity, cost, equipment transportability and patient acceptance. Tests with the highest accuracy such as endoscopy are also associated with the highest costs, lowest convenience for the patient, lowest transportability and lower patient acceptance.12

Large population-based studies have demonstrated the ability of this device to detect a spectrum of predominantly squamous dysplasia and malignancy, reflecting the dominant pathology in this region, however occasional cases of columnar dysplasia and EAC were also reported showing that in at least some cases the lower esophagus/GEJ was sampled.14,15

Therefore balloon-based esophageal sampling combined with cytological assessment is a potential screening and/or surveillance tool for BE, however only a limited number of studies have been undertaken to date. In a pilot study of 10 patients with known BE, none of the samples obtained contained identifiable goblet cells,16 whilst in a larger study of 63 patients with known BE, 83% of samples contained columnar epithelium however the cell yield was low and goblet cells were only identified in 24% of cases overall.17 The latest approach has combined an equivalent balloon-based sampling device with a DNA-methylation biomarker for BE rather than cytological assessment.18 Initial analysis of esophageal brushing samples identified aberrant cytosine methylation at the CCNA1 locus as a potential biomarker for BE, dysplasia and EAC. When combined with aberrant vimentin methylation19 these biomarkers together showed a sensitivity and specificity of 95% and 91% respectively for BE, which was replicated in an independent cohort of brushing samples.18 Balloon-based esophageal sampling combined with the methylation biomarkers was then undertaken in a cohort of 156 patients with a sampling success rate of 82% and high patient tolerance. An adequate yield of DNA for methylation analysis was obtained in 91% of successful samples, equating to 74% of the total cohort. A further 30 cases were excluded due to patients having had previous ablation, gastric IM only or a BE segment length of <1 cm. In the remaining selected group of 86 individuals consisting of 50 cases and 36 controls aberrant methylation of CCNA1 and/or vimentin showed a sensitivity and specificity of 90% and 92% respectively. The test had a lower sensitivity, 22%, for detecting IM of the cardia most likely reflecting differences in its pathogenesis compared to BE. The test specificity may also be lower in smokers who can show aberrant methylation of CCNA1 and/or vimentin in esophageal squamous epithelium. No aberrant methylation of these genes was detected in patients with chronic inflammation of the cardia or eosinophilic esophagitis (EOE).

An alternative esophageal sampling method consists of a sponge contained within a dissolvable capsule and attached to a string. The first such device to be developed was the Cytosponge™, which has been used in at least five prospective studies undertaken to date in the United Kingdom, United States, and Australia.13 A recent comprehensive and independent review summarizes all of the studies which have been undertaken using the Cytosponge™.20 The largest study, BEST2, a case-control study with 1110 patients, combined cytological assessment of the Cytosponge™ sample with TFF3 immunohistochemistry. It demonstrated a sensitivity of 80% for detecting BE of any length, and 87% for circumferential BE segments measuring >3 cm; and a specificity of 92% compared to endoscopy and biopsy.21 This performance is comparable to values quoted for the sensitivity and specificity in meta-analyses of established screening tests; 79% and 95% respectively for distal colorectal adenocarcinomas using the

2 | MINIMALLY INVASIVE ESOPHAGEAL SAMPLING DEVICES AS SCREENING TOOLS FOR BARRETT’S ESOPHAGUS

An alternative to endoscopy and biopsy is a minimally invasive device which samples the surface of the esophageal epithelium combined with cytology-based and/or molecular-based assessment of the sample to identify probable BE, which can then be confirmed on subsequent endoscopy. A balloon-based, minimally invasive esophageal sampling tool has been used for many decades in Northern China which has a high population prevalence of esophageal squamous cell carcinoma14,15. A collapsed balloon attached to a catheter is swallowed and introduced into the esophagus. The balloon is inflated with air and withdrawn along the esophagus sampling the epithelium due to the device’s irregular surface. Cytological smear preparations are made from the sample and categorized by a Cytopathologist based on the presence of inflammation, hyperplasia, atypia or probable malignancy. In order to sample the gastroesophageal junction (GEJ) which is the start of any BE segment, the balloon needs to be inflated when it is estimated to be the correct distance from the incisors, this takes operator skill and experience.

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fecal occult blood test and 71% and 95% respectively for proximal tumours,\textsuperscript{22} 94% and 88% respectively for human papilloma virus testing\textsuperscript{23} and 84% and 88% respectively for cervical smear cytology.\textsuperscript{23}

The Cytosponge\textsuperscript{TM} test can be delivered in a primary care setting by a nurse in a short, approximately thirty-minute appointment, minimizing any inconvenience for the patient and requiring minimal assistance compared with balloon inflation devices. The Cytosponge\textsuperscript{TM} is a 3 cm diameter polyester mesh sphere contained within a gelatin-coated capsule and attached to a string. The patient swallows the capsule whilst the nurse holds the attached string. The gelatin capsule dissolves in the stomach allowing the sponge to deploy. After 7 to 8 minutes the sponge is then pulled up from the stomach along the esophagus where it collects cells from the surface epithelium, sampling along the length of the esophageal mucosa, to the mouth (Figure 1). The sponge is placed in preservative fluid and transported at room temperature to the processing laboratory. A meta-analysis of pooled data from five prospective trials including 2418 individuals and 2672 Cytosponge\textsuperscript{TM} procedures, has shown that over 96% of patients were able to successfully swallow the Cytosponge\textsuperscript{TM} with only two adverse events and a median acceptability higher than endoscopy without sedation.\textsuperscript{13}

There are distinct advantages to a cytology-based sample assessment since the Cytosponge\textsuperscript{TM} and other non-endoscopic devices, sample a range of cells as they transition from the stomach to the mouth. This affords the potential to detect a range of benign inflammatory conditions in addition to IM of the cardia or esophagus including EOE, erosive esophagitis, and local infections such as candida esophagitis or herpes simplex esophagitis.\textsuperscript{24} In order to reduce the overall workload of screening and to maximize the sensitivity of the test to detect IM it was realized at the first inception of the Cytosponge\textsuperscript{TM} that it would be ideal to use a combination of cytological screening and biomarker assessment.\textsuperscript{25} Subsequently investigators have explored a range of biomarkers to diagnose and risk stratify

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\caption{Schematic outlining the steps of the Cytosponge\textsuperscript{TM}-TFF3 assay. Step 1—the sponge is swallowed, the coating dissolves in the stomach and the sponge deploys. The Cytosponge\textsuperscript{TM} is then withdrawn using the attached string collecting cells from the gastroesophageal junction and the length of the esophagus. Step 2—the sampled cells are retrieved from the sponge by a series of washing steps then a cell pellet is made by centrifugation. Plasma-thrombin is added to the cell pellet making a cell clot. Step 3—the cell clot is fixed in formalin and processed into a paraffin block using standard laboratory protocols. Step 4—sections are cut from the paraffin block and stained for assessment by a pathologist.}
\end{figure}
cases of BE on a range of tissue sample types including miRNAs, methylation, protein expression and the presence of specific mutations.19,26-28

Modeling has shown the Cytosponge™ to have an acceptable associated cost as a first-line screening tool in populations at increased risk of BE29,30 with a predicted 25-27% cost reduction compared to standard endoscopy and an incremental cost-effectiveness ratio (ICER) of $26 358-33 307 compared to no screening.29 Microsimulation studies have also suggested a greater reduction in the incidence of symptomatic EACs in the at-risk population using Cytosponge™ based screening compared to endoscopic screening, 19% vs 17%, due to a higher predicted uptake of the Cytosponge™ test due to its increased convenience and acceptability compared to a standard endoscopy.30

Another cell sampling device called the EsophaCap™ has also been developed and early data from small cohorts has recently been published31,32 One study has explored the ability of the EsophaCap™ to diagnose BE when the cytology sample is combined with methylation biomarkers.33 An initial training set of 52 patients allowed the development of a four-gene methylation biomarker panel which when combined with age in a test set of 28 patients with adequate samples showed a sensitivity and specificity of 78.6% and 92.8% for detecting BE. A second study explored the ability of cytological assessment of the EsophaCap™ sample combined with MUC2 immunohistochemistry to detect BE as well as dysplasia and EAC.32 Diagnostic criteria and categories were developed using a pilot set of 28 samples, then the performance of the test was determined in a test set of 136 cases demonstrating a sensitivity of 68% and a specificity of 91%. However, the negative predictive value was low at 20%, which was hypothesized to be due to 37% of the samples having less than four gland groups suggestive of suboptimal sampling of the GEJ.

While further data regarding the sensitivity and specificity of the EsophaCap™ in larger cohorts is awaited, to date the Cytosponge™-TFF3 test has accumulated the most published data20 with a multicenter cluster-randomized controlled trial also currently on-going. The main limitation of the current data regarding the Cytosponge™-TFF3 test is that whilst many of these trials have been multicenter and/or multinational in terms of patient recruitment, for studies exploring the sensitivity and specificity of the Cytosponge™ to detect BE, the processing and interpretation of the samples has been undertaken at a single center. Although this has allowed the protocol for processing the specimen to be optimized and expertise in the interpretation of the cytological samples to be developed, including an appreciation of potential diagnostic pitfalls, independent studies using this device are required. The remainder of this review will focus on the pivotal studies specifically in the development and refinement of the Cytosponge™-TFF3 test and important practical considerations when implementing and interpreting the cytopathological aspects of the test. This will therefore provide guidance for laboratories using the test in the routine clinical setting, and facilitate other centers in undertaking studies using the Cytosponge™-TFF3 test which, provided the same protocols are followed, will allow their results to be directly compared to the BEST series of trials.

Section 3: Processing of the Cytosponge™-TFF3 Test Sample

In order to allow a combination of cytological and biomarker assessment the Cytosponge™ sample is processed into a formalin-fixed paraffin-embedded cell block. Preparation of the Cytosponge™ sample should follow a standard, optimized protocol to maximize the quality and homogeneity of the material for assessment. The process is summarized in Figure 1. Serial sections are cut and consecutive sections stained with hematoxylin and eosin (H&E) and an immunohistochemical biomarker to allow a direct comparison between the morphological appearances and biomarker status. Additional sections and the remainder of the block can be stored for ancillary studies if required. This method has benefits over liquid-based cytology where the whole sample is often processed, usually across multiple slides to minimize cell crowding and overlap, making screening labor intensive. It is also more challenging to combine biomarkers with liquid based cytology and since each slide is a separate preparation, direct comparison between the Pap-stained and immunohistochemical-stained slide is not possible. In addition, often no diagnostic material remains after the initial slide preparation preventing subsequent risk stratification assessment in positive cases.

Section 4: Expected Components in a Cytosponge™ Sample

A standard Cytosponge™ sample will contain squamous epithelium from the esophagus and oropharynx (Figure 2A) and gastric-type columnar epithelium from the stomach and/or a hiatus hernia (Figure 2B). There are also background neutrophils, lymphocytes and eosinophils, and provided that they are distinct from the epithelial groups (Figure 2C), they are not considered to be of pathological significance. Respiratory-type columnar epithelium from the oropharynx may also be seen and can be recognized by the presence of terminal bars and cilia on the apical side of pseudostratified columnar cells (Figure 2D). It is important to recognize their respiratory nature as the epithelial strips can contain admixed goblet cells which may otherwise result in a false-positive Cytosponge™ test result, and the pseudostratified cellular arrangement may result in an erroneous impression of columnar atypia. Tonsillar sampling is occasionally present and is recognized by squamous epithelium with associated lymphoid cells or keratinous material, together with actinomyces organisms (Figure 2E). Isolated fungal spores and hyphae can sometimes be seen, however in the absence of acutely inflamed squamous epithelium they are considered to represent oropharyngeal commensals (Figure 2F).

Identification of true goblet cells on the H&E sample can be challenging for a number of reasons. Firstly, depending on the preservation of the material it may not always be possible to identify grey mucin with confidence within potential goblet cells. Secondly, whilst mature goblet cells have a classical appearance and are unlikely to be missed on systematic screening of a H&E slide (Figure 3A), early or crosscut goblet cells can be subtle (Figure 3C) and lead to a false
FIGURE 2  Legend on next page.

(A) Squamous epithelium  (B) Gastric-type columnar epithelium

(C) Background inflammatory cells  (D) Respiratory epithelium

(E) Tonsillar sampling  (F) Isolated fungal spores/hyphae
negative result. Thirdly, occasional columnar cells known as "pseudogoblet" cells may appear enlarged with features resembling true goblet cells and lead to false positive result (Figure 3E). To increase the sensitivity and specificity of the Cytosponge™ test for IM it is therefore recommended that a biomarker is used in combination with H&E staining. A biomarker also speeds up the identification of IM in cases where there are few goblet cells, such that H&E screening can focus on looking for cytological atypia and additional pathologies.

5 | DEVELOPMENT OF THE TFF3 IMMUNOHISTOCHEMICAL BIOMARKER

In order to identify a suitable biomarker for IM a microarray experiment was undertaken which compared the mRNA expression of a broad range of genes in normal squamous esophagus, BE and normal gastric cardia.25 A total of 14 candidates were identified of which two, trefoil factor 3 (TFF3) and dopa decarboxylase (DDC), were confirmed to have increased expression at the mRNA level by RT-PCR in BE relative to esophageal and gastric mucosa. Immunohistochemistry for TFF3 showed strong staining at the mucosal surface in biopsies of BE, the compartment sampled by the Cytosponge™, and thus makes it the optimal biomarker candidate to facilitate the identification of true IM. In contrast DDC expression was patchy, often weak, and predominantly located in the deeper glands which would not be sampled by the Cytosponge™, hence it was not taken further.

TFF3 is a member of the trefoil factor family that contains a characteristic trefoil motif consisting of a 40 amino acid domain with three conserved disulphides.33 It is expressed on goblet cells and is therefore a normal component that may be seen in a Cytosponge™ sample. Normal components that may be seen in a Cytosponge™ sample, all images at 10× magnification. A, Squamous epithelial cells from the esophagus and oropharynx. B, Gastric-type columnar epithelium from the stomach and/or a hiatus hernia. C, Mixed inflammatory cells which provide that they are separate from epithelial cell groups are not considered to indicate clinically significant esophagitis. D, Strips of respiratory epithelium recognized by their terminal bars and cilia. E, Tonsillar sampling characterized by keratinous material and actinomyces organisms. F, Fungal spores and occasional hyphae (arrow) with appearances consistent with Candida. As there is no associated significant acute inflammation they are considered to represent commensal organisms rather than being suggestive of Candida esophagitis.

Further investigation of false-positive cases from this study showed an association with the presence of Helicobacter Pylori, a recognized risk factor of gastric IM, suggesting that in some cases the Cytosponge™-TFF3 test may have detected gastric IM that was not sampled by the gastric cardia biopsy taken at endoscopy. These patients also warrant endoscopic assessment and clinical follow up as gastric IM is associated with an increased risk of gastric intestinal-type adenocarcinoma and the extent of the risk is related to the extent of atrophy and IM.36 The 2019 European Guidelines for the management of precancerous epithelial lesions of the stomach highlight the need for patients with advanced gastritis defined as the presence of atrophy and/or IM affecting more than one site within the stomach to be identified via a high quality endoscopy with gastric biopsies from the antrum and corpus to detect advanced gastritis and Helicobacter pylori infection.36

In the aforementioned studies TFF3 immunohistochemistry was undertaken on two sections from the paraffin block. The TFF3 score was binary with positive TFF3 staining on either or both sections being scored as positive. However there was wide variation between samples in the total number of TFF3 positive gland groups and this may indicate the likelihood of a diagnosis of BE at subsequent endoscopy. Cases were retrospectively classified as being positive on the
**FIGURE 3** Legend on next page.
first TFF3 stained section only, on the second TFF3 stained section only, or on both sections; and this compared to the endoscopic result. There was a difference between the incidence of BE at endoscopy when only the first section (65.9%), or only the second section was positive (75.0%). When both sections contained TFF3 positive groups the incidence of BE at endoscopy was 89.8% compared to 70.5% when only one section was positive. In future this may allow the generation of a TFF3 data algorithm which divides patients into “low confidence” and “high confidence” positive results with different management.

Further validation of the Cytosponge™-TFF3 test is now underway in a multicenter cluster-randomized controlled trial, BEST3, which aims to recruit 9000 participants over a 3 year period. In this trial the use of high and low confidence scores for both negative and positive results are being evaluated. If no glandular cells are identified, suggesting that the device may not have reached the stomach, the case is reported as “negative with low confidence” and the patient is invited for a repeat test. While TFF3 positive cases are subdivided into low or high confidence depending on how many glands groups are positive to inform the endoscopist of the expected likelihood of BE vs gastric cardia IM, with the latter having a different endoscopic follow-up strategy.

6 | PATHOLOGICAL QUALITY CONTROL AND REPORTING PROCEDURES FOR THE CYTOSPONGE™-TFF3 TEST

In the current protocol, slides are reviewed by a single pathologist who has received specific training in the assessment and scoring of Cytosponge™-TFF3 samples. Double-reporting is not considered necessary as standard for the Cytosponge™-TFF3 assay due to the substantial agreement obtained in the BEST1 and BEST2 studies between assessors. This has the advantage of reducing both the reporting workload and turnaround time.

It is important that sample assessment is undertaken in a systematic manner and that the data is captured in a standard way. The first step is to assess whether the sample is adequate. Clot preparations that are less than 5 mm in size and do not contain columnar cells are classified as “inadequate” and repeat testing is recommended. The second step is to count the number of columnar cell groups that are present, excluding any respiratory groups. The sample is categorized as containing no columnar cell groups, one to four columnar cell groups, or greater than five columnar cell groups. Samples that are greater than 5 mm in size but do not contain columnar cells groups are classified as “negative with low confidence” and the clinician is advised to consider repeat Cytosponge™-TFF3 testing. Samples with greater than five columnar cell groups and without IM are categorized as “negative with high confidence”.

If goblet cells are seen in columnar cell groups this is classified as “positive” and suggestive of IM of the esophagus (BE), GEJ or cardia. In occasional samples the columnar epithelium may also be atypical with nuclear pleomorphism, pseudostratification of the nuclei, an abnormal chromatin pattern, and the presence of mitotic figures. The differential diagnosis is between reactive atypia and true dysplasia. These samples are reported as “positive with columnar atypia” and the patient should undergo endoscopy with biopsy sampling to clarify the nature of the atypia detected by the Cytosponge™-TFF3 test.

7 | AVOIDING POTENTIAL DIAGNOSTIC PITFALLS IN INTERPRETING THE CYTOSPONGE™-TFF3 TEST

The Cytosponge™ sample is processed in such a way as to ensure that the H&E and TFF3 sections are directly adjacent to each other to allow direct comparisons between the two slides. It is recommended that the TFF3 slide is screened first to rapidly identify positive columnar groups before screening the H&E slide. It is important that the final status of the case takes into account both the H&E and TFF3 slide appearances to minimize both false negative and false positive results.

The expected TFF3 staining pattern is dense, dark brown, round to oval intracellular staining with a smooth outline which may show peripheral accentuation (Figure 3A,B and Figure 4A). The goblet cells can be of varying size from small, early goblet cells which could be missed on the H&E stain (Figure 3C,D), to traditionally fully formed oval cells (Figure 3A,B), whilst pseudogoblet cells seen on the H&E slide will be negative for TFF3 (Figure 3E,F).

Non-specific background cytoplasmic staining can sometimes be seen which lacks the crisp outline seen in true goblet stains (Figure 4A) whilst non-specific staining of extracellular mucin is usually separate from the cell groups so does not present an interpretative challenge. Weak non-specific cytoplasmic staining of columnar cells can occasionally be seen (Figure 4B) however it lacks the strong, well-defined pattern seen in true goblet cells. The nonspecific staining pattern is particularly prevalent in cases with true goblet cells elsewhere; therefore every area of staining should be carefully scrutinized for goblet cells. Any area thought to represent true positive TFF3 staining should be compared to the equivalent cell cluster on the H&E slide to ensure that it corresponds to IM within gastrointestinal-type columnar epithelium rather than respiratory epithelium (Figure 4C), as discussed previously.

As would be expected there will be rare cases with equivocal TFF3 staining. This is usually seen in a single group and may be

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**FIGURE 3** Challenges in identifying true goblet cells using haematoxylin and eosin stained preparations alone and the benefits of TFF3 immunohistochemistry, all images at 20x magnification. A and B, Classic, mature goblet cell. C and D, Subtle goblet cells which are highlighted on TFF3 staining. E and F, Columnar cells with features suggestive of an associated goblet cell however no staining is seen with TFF3 showing that this is not a true goblet cell but a “pseudogoblet” cell.
resolved if definite goblet cells are identified elsewhere. These cases may benefit from double reporting and/or discussion at a consensus meeting to determine if a definite positive or negative result can be given. It is useful to consider whether the staining is in the same plane of focus as the rest of the material and confined to the cell, favoring true staining, rather than extending beyond the limits of the cell or out of the plane of section, favoring background staining. Reviewing the group on the H&E stain and additional levels may help to identify diagnostic clusters. However ultimately the case may best be regarded as equivocal and the patient offered a repeat Cytosponge™-TFF3 test.

FIGURE 4 Interpreting TFF3 staining patterns. A, TFF3 staining in true goblet cells should be dense dark brown with a crisp edge. B, Weak non-specific cytoplasmic TFF3 staining can occasionally be seen in strips of columnar cells however it lacks the dense, well-defined pattern of staining in individual cells seen in true goblet cell staining. C, Strips of respiratory epithelium can contain goblet cells which will also be TFF3 positive. The respiratory nature of these groups can be recognized on the parallel haematoxylin and eosin stain by the presence of terminal bars and cilia.

8 | DIAGNOSIS OF BENIGN ESOPHAGEAL PATHOLOGIES ON CYTOSPONGE™ SAMPLES

Although not a primary aim of the Cytosponge™-TFF3 test, the cytological appearances may suggest the presence of benign esophageal pathology in up to 20% of cases such as significant epithelial inflammation with or without ulceration, EOE, viral esophagitis and/or candida esophagitis. Studies in patients with known EOE have shown the Cytosponge™ to have an accuracy of 80% for disease activity when using endoscopic assessment and biopsies as the gold
standard, and of note the Cytosponge™ identified active disease in number of cases missed on endoscopic biopsies. Patients with significant epithelial inflammation, defined as neutrophils infiltrating squamous epithelial groups, ulcer slough and/or granulation tissue, may benefit from increased acid suppression; whilst the presence of eosinophils within squamous epithelial groups at a density of >15 eosinophils per high power field and/or forming microabscesses is suggestive of EOE which should be investigated further. Fungal hyphae permeating squamous groups with associated inflammation is suggestive of candida esophagitis and the identification of the classical viral nuclear inclusions may indicate herpes simplex esophagitis both of which need further investigation and/or treatment. Therefore features suggestive of a significant benign esophageal pathology are included on the reporting proforma for BEST3 so that the patient’s primary care practitioner can undertake appropriate management. This is analogous to the cervical screening program in the United Kingdom where HPV-related pathologies are managed within the screening program, whilst the patient’s primary care practice is made aware other findings such as local infections and are responsible for their confirmation, investigation and management.

9 | POTENTIAL FUTURE DIRECTIONS

The availability of a sensitive, safe, acceptable and cost effective minimally invasive esophageal sampling device has the potential to make screening for BE feasible in a large at-risk population such as all patients over the age of 50 years with long-standing severe reflux symptoms and to obviate the need for endoscopy in all those referred to secondary care centers with reflux symptoms. However apart from the ease of administration, performance, and costs of the test, there are a number of other factors that need to be considered such as the likely uptake of the test in the at-risk population, whether the test can be delivered with acceptable turnaround times, the quality control of the laboratory test, and how best to manage patients with a positive test both in the short and long term. These factors will all be explored as part of the BEST3 study.

In the current protocol all patients with a positive test are referred for endoscopic confirmation. However additional tests could be undertaken on positive cases to allow further patient risk-stratification. Those with a moderate to high risk of dysplasia within a BE segment would require an endoscopy within a short timeframe, whilst those with a low risk of dysplasia could have a longer interval between the positive Cytosponge™-TFF3 test and endoscopy, or even avoid endoscopy altogether. A panel of glandular atypia, abnormal immunohistochemical phenotype for p53, and positive immunohistochemical staining for aurora kinase A combined with clinical parameters of age and waist: hip ratio was able to subclassify patients with a positive Cytosponge™-TFF3 test into low, moderate and high-risk groups which corresponded with their risk of high grade dysplasia/intramucosal carcinoma. In a validation cohort, 38% of patients were classified as being low-risk and 96% of these had no dysplasia on endoscopic biopsies, whilst 8% were classified as being high-risk and all had biopsy-proven high-grade dysplasia. A multigene next-generation sequencing panel can also be undertaken on Cytosponge™ samples to screen for hotspot mutations in 50 oncogenes and tumor suppressor genes, and therefore identify cases with probable dysplasia. Further work is ongoing to identify the most steam-lined workflow to allow risk stratification of TFF3 positive cases so that low risk individuals could have on-going surveillance using the Cytosponge™, thus avoiding having to endoscope all patients with BE on a regular basis which may to lead to psychological stress and impair quality of life. This strategy of an initial screening test followed by a risk stratification to guide the urgency and nature of subsequent assessment could help to reduce the burden on endoscopy services and potentially the risk of overtreatment.

The strong, well-defined staining seen with TFF3 and the binary nature of the scoring system makes scoring of the Cytosponge™-TFF3 test amenable for automation with initial screening using an artificial intelligence (AI) algorithm to highlight the areas of interest on the TFF3 slide for subsequent confirmation by a pathologist. Validation in a large cohort would be required to confirm that AI assessment of negative cases had a sufficiently high negative predictive value for these cases not to be screened by a pathologist. Additional biomarkers could also be added to the Cytosponge™-TFF3 test to further increase its sensitivity and specificity for detecting BE, a panel of three miRNAs or alternatively a methylation panel have shown potential in a pilot study and require further validation.

The Cytosponge™-TFF3 test has been developed to screen for BE in Western populations where EAC is the dominant histological subtype of esophageal carcinoma. Squamous neoplasia was not identified in any of the BEST series of studies reflecting its very low incidence in the targeted patient populations. However, in other parts of the world squamous cell carcinoma of the esophagus is more prevalent and represents a significant public health problem. A preliminary study in a population with a high prevalence of squamous cell carcinoma has shown the Cytosponge™ to be a feasible, safe and acceptable test for esophageal squamous dysplasia (ESD) with a sensitivity and specificity of 100% and 97% respectively when combined with p53 immunohistochemistry. Further studies are on-going to explore the potential of the Cytosponge™ test combined with molecular and/or immunohistochemical biomarkers to detect ESD.

10 | CONCLUSION

In summary the Cytosponge™-TFF3 assay is a safe, sensitive, and specific minimally invasive test for IM of the esophagus (BE) and stomach which can be undertaken in a community setting with high patient acceptability scores. Quality assurance of the laboratory processing and histopathological evaluation is essential to maximize the accuracy of the test and TFF3 immunohistochemistry is an essential component. Provided interpretative guidelines are followed the assay can be scored by a single pathologist in less than 5 minutes giving reproducible results. There is significant future potential for
automation of aspects of scoring, making the workflow more efficient and reducing the pathologists’ workload. The future addition of a risk-stratification step in positive tests may guide the urgency and/or nature of subsequent patient investigations optimizing the use of endoscopy services. Such efforts to improve the early detection of cancer are badly needed given the poor outcomes of esophageo-gastric cancer.

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CONFLICT OF INTEREST

Patents and a trademark were filed on the Cytosponge™ in 2010 by the Medical Research Council (MRC). RCF and MOD are named inventors on patents pertaining to the Cytosponge™ and related assays. In 2013 the MRC licensed the technology to Covidien GI Solutions (now Medtronic).

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REFERENCES


