Range of pathologies diagnosed using a minimally invasive capsule sponge to evaluate patients with reflux symptoms

Anna L Paterson*[1,2], Pierre Lao-Sirieix*[1], Maria O'Donovan[1,2], Irene Debiram-Beecham[1],

Massimiliano di Pietro[1], Ahmad Miremadi[1,2], Stephen E Attwood[3], Fiona M Walter[4], Peter D

Sasieni[5], Rebecca C Fitzgerald[1] on behalf of the BEST and BEST2 study groups

[1]MRC Cancer Unit, Hutchison-MRC Research Centre, Cambridge, UK; [2]Department of

Histopathology, Addenbrooke's Hospital, Cambridge, UK; [3]Department of Surgery, North

Tyneside Hospital, North Shields, UK; [4]Department of Public Health and Primary Care,

University of Cambridge, Cambridge, UK; [5]Cancer Prevention Trials Unit, Wolfson Institute of

Preventative Medicine, London, UK.

*contributed equally to the work

Correspondence to:

Rebecca Fitzgerald rcf@hutchison-mrc.cam.ac.uk.

MRC Cancer Unit, Hutchison-MRC Research Centre, Cambridge, UK, CB2 0QQ.

Tel: 00 44 1223 763240

Short title: Cytosponge utility for evaluating reflux symptoms

ABSTRACT

AIMS: Reflux symptoms are highly prevalent and non-specific hence, in the absence of alarm symptoms, endoscopy referral decisions are challenging. This study evaluated whether a non-endoscopic Cytosponge could detect benign oesophageal pathologies and thus have future potential in triaging patients with persistent symptoms.

METHODS AND RESULTS: Two complementary cohorts were recruited: 1) Patients with reflux symptoms and no prior endoscopy (n=409); 2) Patients with reflux symptoms referred for endoscopy (n=411). All patients were investigated using the Cytosponge and endoscopy.

Significant epithelial inflammation was present in 130 (16%) Cytosponge samples of which 32 had ulcer slough. Candida and significant inflammation was detected in a further 22 (2.3%) cases; epithelial infiltration with >15 eosinophils/hpf reflecting possible eosinophilic oesophagitis (EOE) in five (0.6%); and viral inclusions suggestive of herpes oesophagitis in one (0.1%). No significant pathology was detected in the majority, 662 (81%), of Cytosponge samples.

Cytosponge and endoscopy findings were in agreement in 574 (70%) cases, in most discordant cases, 165 (67%), one investigation showed mild inflammation whilst the other was negative; with an additional 22 (8.9%) differing on the inflammation extent. Eighteen cases with severe inflammation, six with candida and two with EOE were detected only at endoscopy; whilst 18 with candida and significant inflammation, 13 with ulcer slough, one probable EOE, and one viral oesophagitis were identified on the Cytosponge only.

CONCLUSIONS: The Cytosponge detects a range of benign oesophageal pathologies, and therefore has potential clinical utility in the triaging of patients with troublesome reflux symptoms. This warrants further investigation.

INTRODUCTION

Gastro-oesophageal reflux symptoms are highly prevalent in the westernised world, affecting 10-44% of the general population, and continue to increase in incidence (1). They are the most common gastrointestinal symptoms resulting in physician office visits in the US and accrue the highest annual costs (2, 3). US and UK guidelines suggest that patients reporting classical symptoms of dyspepsia, heartburn and/or regurgitation should be managed as presumptive gastrointestinal reflux disease (GORD) with advice on life-style modifications, reviewing any contributory drugs, and a trial of proton pump inhibitors (PPI) (4, 5). If symptoms return on initial discontinuation of PPI therapy, it is recommended to restart the drug and to tailor the dosing schedule to maximise symptom control (4, 5). In the majority of cases this would be expected to fully-control symptoms, however 17-32% of patients in a Primary Care setting remain symptomatic (6). Currently it is recommended that this group requires further optimisation of medical therapy followed by a referral to specialist services with consideration of an upper gastro-intestinal endoscopy (OGD) to identify patients with erosive esophagitis, Barrett's oesophagus or other benign diagnosis requiring further management and/or disease surveillance (4, 5, 7, 8).

Erosive oesophagitis is the most common positive OGD finding in patients with reflux symptoms (9), identified in 12-34% (10, 11). However reflux symptoms may be secondary to alternative pathologies which are non-responsive to PPIs and therefore require alternative management (12). The most prevalent is candida oesophagitis identified in 2.3-3.8% of all OGDs (13-15). When symptomatic, it presents predominantly with reflux symptoms (14), and requires treatment with anti-fungal medication. Eosinophilic oesophagitis (EOE) is a condition of uncertain aetiology which is increasing in incidence (16). Acute dysphagia is the

classical presentation (17), however EOE is identified in 1% of patients who are investigated for reflux symptoms and requires management with a trial of PPI, topical or systemic steroids, and/or dietary elimination (18, 19).

It is estimated however, that at least two-thirds of OGDs in patients with reflux symptoms will not reveal any diagnostic pathology. An OGD requires patients to be referred to secondary care, to undergo a relatively invasive procedure, and has associated costs of approximately $685 or £400 per out-patient investigation (2, 20). Previous studies have demonstrated a non-endoscopic cell sampling method, the Cytosponge, to be a safe and well-tolerated method to sample the oesophageal lining which can be undertaken in a primary-care setting by nurse practitioners (21-23). The device yields a combination of single cells and micro-biopsies which quantitatively exceeds that achieved by a biopy (Supplementary Figure 1). Compared to an OGD, it offers a cost-effective method to diagnose Barrett's oesophagus in high-risk patients (24) with a high sensitivity and specificity when combined with TFF3 immunohistochemistry (23, 25), and has also demonstrated utility in monitoring disease activity in patients with EOE (26).

The aim of this study was to explore in symptomatic patients with no prior oesophageal diagnosis, the ability of the Cytosponge to detect benign oesophageal pathology aside from Barrett's oesophagus.

METHODS

Study cohort

The study group comprised two complementary cohorts, patients enrolled in the BEST study (21) and the control group from the BEST2 study (23). To summarise, the BEST cohort was identified from a Primary Care setting, and had received acid-suppression medication for at least three consecutive months in the five years prior to being enrolled in the study. These patients were not considered to require an OGD by their Primary Care Physician. Patients were excluded if they were known to have Barrett's oesophagus or if they had had an OGD in the twelve months prior to enrolment. The BEST2 control group were patients who had been referred for an OGD to investigate dyspepsia and/or reflux symptoms refractory to medical therapy. Patients with a prior diagnosis of Barrett's oesophagus were excluded.

The UK Medical Health Regulatory Agency issued a letter of no objection for the use of the Cytosponge (Medical Research Council, London, UK) in both studies. The BEST and BEST2 studies were approved by Cambridge Regional Ethics Committee (LREC 06/Q0108/272) and the East of England–Cambridge Central Research Ethics Committee (No: 10/H0308/71) respectively. BEST2 was registered in the UK Clinical Research Network Study Portfolio (9461). Each patient provided written informed consent.

Cytosponge administration and processing

The Cytosponge was administered by a research nurse and following retrieval was stored at 4^oC in SurePath preservative (TriPath Imaging, Burlington, North Carolina, US) before being transferred to the laboratory for processing to paraffin blocks (21, 23). One haematoxylin and eosin (H&E) stained slide was generated per sample and all cases were reviewed by at least two independent observers (MoD & PLS), one of whom (MoD) is a specialist cytopathologist and gastrointestinal pathologist. Any features suggestive of benign pathology were recorded. In a subset of cases (n=7) where both Candida and significant

inflammation was present on the H&E stained slide, a periodic acid-Schiff (PAS) stain was undertaken to improve diagnostic certainty.

Patients then underwent an OGD with biopsies taken where clinically indicated according to usual clinical care. All the endoscopists in the study were Consultant-level with a particular interest in oesophageal disease and followed a study protocol. Endoscopic findings were categorised based on the endoscopy report and supplemented by histopathology findings when biopsies were undertaken. Mild oesophagitis was defined as Los Angeles oesophagitis grades A or B, or inflammation only identified on a biopsy, whilst severe oesophagitis was defined as the presence of Los Angeles grades C or D erosions (4), and/or ulceration. This was an observational study and no therapy was initiated as a result of Cytosponge findings; patient management was adjusted as per current recommendations based on the endoscopic findings.

The sensitivity and specificity of the Cytosponge as a screening tool for Barrett's oesophagus and associated dysplasia has been reported previously (23), therefore cases where Barrett's oesophagus was identified using the Cytosponge and/or OGD were excluded (n=94), unless an additional significant benign pathology was also present (n=9).

Statistical Analysis

Statistics for continuous variables were expressed as medians and interquartile ranges. The Mann-Whitney test was used to compare continuous variables between groups, and a Chi-square test was used to compare counts between categorical variables.

RESULTS

A total of 820 patients met the inclusion criteria for the study, 409 patients from BEST and 411 from BEST2. The demographic data is summarised in Table 1. The BEST group reported less well controlled symptoms compared with the BEST2 cohort, were older and more likely to have a high BMI and/or waist:hip ratio.

Cytosponge findings

A range of benign oesophageal pathologies were identified using the Cytosponge. 662/820 (81%) of Cytosponge samples were considered to be within normal limits (Figure 1 a,b), including occasional samples containing Aspergillus species, a recognised commensal in tonsillar tissue (Figure 1 c,d). In the majority of cases, only occasional acute and chronic inflammatory cells were present, usually separate from epithelial fragments, and therefore were deemed not to be clinically significant. However in a subgroup of patients, 98/820 (12%), abundant acute and chronic inflammatory cells were seen to permeate the epithelial fragments (Figure 1, e,f), or to be present in dense clusters separate from the epithelial fragments. In both situations, this was felt to represent clinically significant inflammation. In 32 (3.9%) additional cases, dense aggregates of predominantly acute inflammatory cells were observed with abundant associated fibrin (Figure 1, g,h), and interpreted as sampling from the surface of an ulcer.

Fungal spores and/or pseudo-hyphae with an appearance consistent with Candida species were identified in 35/820 (4.3%) cases. Since Candida is a known commensal within the oropharynx, its presence was only judged to be potentially pathogenic in 22/820 (2.7%) patients where significant inflammation was also present (Figure 1, i,l).

In 5/820 (0.6%) cases eosinophils were seen to infiltrate between epithelial cells with a density of >15 eosinophils per high power field, and with microabscess formation in some cases (Figure 1, m,n). Therefore these samples met multiple histological criteria used for the diagnosis of EOE on biopsy (18), and were designated possible EOE.

Viral nuclear inclusions, representing herpes oesophagitis, were present in a single case (Figure 1, o,p).

Endoscopic findings and comparison with the Cytosponge

In the majority of cases, 638/820 (78%), no significant pathology was identified at endoscopy. Oesophagitis was the most common pathology and present in 166/820 (20%) cases, the majority of which, 101/166 (61%), were Grade A oesophagitis with only 30/166 (18%) having severe oesophagitis. Candida oesophagitis and EOE were identified in 10/820 (1.2%) and 6/820 (0.7%) cases respectively.

There was agreement between the findings on endoscopy and Cytosponge in 596/820 (73%) cases, with both showing no significant pathology in 541/820 (66%) (Table 2). Both investigations led to a diagnosis in some cases which was not corroborated by the other test. In 121/820 (15%) cases the Cytosponge was negative when a pathology was identified at endoscopy (Table 2). This was predominantly mild inflammation, 97/121 (80%), as well as 18 cases (15%) with severe oesophagitis and 6 cases (5.0%) of candida oesophagitis. In 97/820 (11%) cases the endoscopy was negative when the Cytosponge identified pathology (Table 2). The missed diagnoses at endoscopy were significant inflammation or ulceration in 81/97 (84%) patients, candida with significant accompanying inflammation in 15/97 (15%),

and a single case with viral inclusions suggestive of herpes oesophagitis. In 80/97 (82%) cases where pathology was noted on the Cytosponge but not at endoscopy, no biopsy had been taken at endoscopy as it was not felt to be clinically indicated. In three cases mild atypia was noted on the Cytosponge sample however since significant inflammation related to reflux, Candida or EOE was also present, the significance is unclear and this may represent reactive changes. No squamous dysplasia was seen in the single case where a biopsy was also taken.

In a further 6/820 (0.7%) cases, there was disagreement between the OGD and Cytosponge findings. In two cases the Cytosponge detected mild inflammation whilst the OGD suggested that this was due to EOE; and in four cases the OGD found mild oesophagitis whilst the Cytosponge examination suggested that this was secondary to candida oesophagitis (three cases) and EOE (one case).

DISCUSSION

This study has demonstrated the ability of a minimally invasive sampling device, the Cytosponge, to detect a broad range of benign oesophageal pathologies. This is a preliminary study, however it does suggest that this test has future potential for triaging and possibly even guiding the management of patients with reflux symptoms, and warrants further research to explore the clinical utility of such a strategy.

The Cytosponge can be administered by a nurse practitioner in a primary-care or specialist clinic setting within a fifteen minute appointment. From the patient's perspective this may be more convenient than an out-patient endoscopy and previous studies have shown that

most patients do not find the Cytosponge to be an unpleasant or anxiety provoking investigation, preferring it to endoscopy (21, 23). From a health-economics perspective, the Cytosponge is anticipated to be cheaper to deliver than an endoscopy, mainly because it does not require specialist services or equipment.

In the majority of cases, 66%, both the Cytosponge and endoscopy showed no significant pathology. Alternative diagnoses to GORD were identified in 36 (4.4%) patients, when the findings from the OGD and Cytosponge are combined, consistent with previous findings (14, 15, 19). The main diagnosis was Candida with significant associated inflammation, which was detected more than twice as frequently using the Cytosponge compared to an OGD. A PAS stain was also shown to be effective in highlighting Candida species on Cytosponge samples, and could be used selectively in future studies to increase the sensitivity of the Cytosponge to detect Candida in cases with severe inflammation and ulceration. The clinical significance of candida identified using the Cytosponge needs further study, however such patients could be treated by a short-course of anti-fungal therapy which is well tolerated and it is likely that such cases would not have symptom control with PPIs alone.

The majority of patients with EOE present with dysphagia, and so would be referred directly for endoscopy (17), however a sub-set have predominantly reflux-type symptoms and the Cytosponge detected five cases of probable EOE in our study groups. Diagnostic criteria applied to biopsies, the presence of >15 eosinophils per high power field infiltrating the epithelium and/or the presence of eosinophilic micro-abscesses (18), are equally applicable to the Cytosponge samples due to the micro-biopsies that are obtained. A recent small study of patients with known EOE found that the Cytosponge had a sensitivity of 85% for identifying active disease (26). EOE is known to have a patchy distribution and may not be

visible endoscopically (12). A major advantage of the Cytosponge is that it samples the entire length of the oesophagus and so is less susceptible to sampling errors. However, eosinophils are frequently seen in biopsies from the distal oesophagus in reflux oesophagitis, hence to diagnose EOE at endoscopy, eosinophils should be present in biopsies taken from both the distal and proximal oesophagus (18). Therefore patients suspected of having EOE based on the Cytosponge findings would require endoscopic confirmation prior to starting treatment.

The majority of missed diagnoses for both the sponge and endoscopy related to oesophagitis and/or ulceration. This disagreement is not unique to this setting since the scoring of oesophagitis and ulceration at endoscopy is known to have limited inter-observer agreement, k score 0.40-0.65 (27, 28). Furthermore the majority of medical adjustments to acid-suppression therapy are guided by patient symptoms, not by endoscopic findings (4), as patients can report severe symptoms in the absence of oesophagitis on endoscopy (27).

A major challenge in the broader interpretation of this study is accurately calculating the specificity and sensitivity of the Cytosponge investigation (29). The endoscopy protocol reflected standard clinical practice, hence in the majority cases where pathology was identified on the Cytosponge but not macroscopically at endoscopy a biopsy was not taken, so it is not possible to confirm or refute the Cytosponge findings microscopically. Furthermore biopsies were not taken as standard to confirm the macroscopic impression of inflammation, therefore "false-negative" Cytosponge findings cannot be confirmed, particularly as concordance between endoscopists is known to be low for mild oesophagitis (27, 28).

Therefore given that the ability of the Cytosponge to detect benign oesophageal pathologies has now been established, future studies now need to be undertaken to accurately establish the sensitivity and specificity of this investigation compared to endoscopy and hence its potential clinical utility. For example, the Cytosponge may offer a means to triage patients with troublesome reflux symptoms, without alarm features, away from first-line endoscopic investigation. Patients in whom the Cytosponge demonstrates no significant pathology, epithelial inflammation without ulceration, or candida with associated inflammation, representing 95% of our cohort, could continue to be managed in primary care with adjustment of acid suppression therapy or anti-fungal medication as appropriate. Whilst those with evidence of ulceration, possible EOE or viral oesophagitis on the Cytosponge, in addition to those with troublesome, persistent or unexplained symptoms following a negative Cytosponge investigation would be referred for a diagnostic or follow-up endoscopy. An OGD would remain the investigation of choice for all patients presenting with alarm symptoms, or dyspepsia since the Cytosponge is not intended to be used to detect gastric pathology.

To conclude, this research demonstrates the ability of the Cytosponge to detect benign oesophageal pathologies. Prospective studies are now required to explore its potential as a triage tool in patients with troublesome reflux symptoms.

FIGURE LEGENDS

Figure 1: *Representative H&E examples of each of the main diagnoses identified on the Cytosponge shown at x100 magnification with the boxed area also shown at x400 magnification. (a,b) normal stratified squamous epithelium; (c,d) Aspergillus species representing normal colonisation of tonsillar tissue; (e,f) significant inflammation, indicated by inflammatory cells infiltrating between the epithelial cells; (g,h) ulceration comprising abundant inflammatory cells admixed with fibrin; (i-l) candida oesophagitis indicated by inflamed epithelium with fungal spores and pseudohyphae which can be highlighted using a periodic-acid Schiff (PAS) stain (k, l); (m, n) eosinophilic oesophagitis indicated by abundant eosinophils infiltrating between epithelial cells; (o,p) herpes oesophagitis shown by the present of an epithelial cell with nuclear inclusions.*

	BEST	BEST2	Combined	
Individuals	409	411	820	
Age – median (IQR)	62 (56-66) [#]	56 (44-66)	60 (52-66) [#]	p < 0.01
Ethnicity – White Caucasian (%)	384 (94%) [$]	378 (92%)	762 (93%) [$]	p = 0.05
M:F*	1.0:1.2 [$]	1.0:1.3	1.0:1.3 [$]	p = 0.65
BMI - median (IQR)	29.0 (26.2-32.6) [+]	26.8 (24.0-30.0) [$]	28.0 (24.8-31.6) [&]	p < 0.01
Waist:Hip ratio – median (IQR)	0.91 (0.85-0.96) [#]	0.88 (0.83-0.94) [#]	0.89 (0.84-0.95) [%]	p < 0.01
Symptom control (BEST)/ symptom frequency (BEST2)				
Well-controlled/never symptoms	25 (6.2%) [$]	55 (13%)[^]	80 (9.9%)[#]	p < 0.01
Fairly well controlled /sometimes	85 (21%)	125 (31%)	210 (26%)	
Uncontrolled/often	111 (28%)	75 (18%)	186 (23%)	
Very poor or poor control/daily	181 (45%)	154 (38%)	335 (41%)	

Table 1: *Demographics of the BEST and BEST2 study cohorts used in this study, and the two cohorts combined. * Sex ratio rounded to the nearest tenth. Data was unavailable for [^]2, [$]7, [+]8, [#]9, [&]15 and [%]18 patients respectively.*

		Cytosponge Diagnosis						
		Negative	Epith Inflam	Ulcer	Sig Candida	EOE	Viral	Total
	Negative	541	68	13	15	0	1	638 (78%)
	Mild inflam	97	21	15	2	1	0	136 (17%)
Endoscopy diagnosis	Severe inflam	18	7	4	1	0	0	30 (3.7%)
	Candida	6	0	0	4	0	0	10 (1.2%)
	EOE	0	2	0	0	4	0	6 (0.7%)
	Viral	0	0	0	0	0	0	0
	Total	662 (81%)	98 (12%)	32 (3.9%)	22(2.7%)	5 (0.6%)	1 (0.1%)	

Table 2: *Comparison of the findings on OGD and using the Cytosponge. Cases with a shared diagnosis are shaded in blue, those where only the Cytosponge detected the dominant pathology are shaded green, and those when the main pathology was only identified by OGD are shaded red.*

Research Centre and the Cambridge Experimental Medicine Centre. ALP is an NIHR Academic Clinical Fellow and holds the Pang Kam Ping Fellowship in Medicine at Queens' College, Cambridge. The Addenbrooke's Hospital Tissue Bank is supported by the NIHR Cambridge Biomedical Research Centre.

REFERENCES

1.   El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2014;63:871-80.

2.   Peery AF, Dellon ES, Lund J, et al. Burden of Gastrointestinal Disease in the United States: 2012 Update. Gastroenterology. 2012;143:1179-87.

3.   Sandler RS, Everhart JE, Donowitz M, et al. The Burden of Selected Digestive Diseases in the United States. Gastroenterology. 2002;122:1500-11.

4.   Katz PO, Gerson LB, Vela VF. Guidelines for the Diagnosis and Management of Gastroesophageal Refl ux Disease. Am J Gastroenterol. 2012;108:308-28.

5.   Dyspepsia and gastro-oesophageal reflux disease. NICE clinical guideline 184 2014.

6.   El-Serag H, Becher A, Jones R. Systematic review: persistent reflux symptoms on proton pump inhibitor therapy in primary care and community studies. Aliment Pharmacol Ther. 2010;32:720-37.

7.   Fitzgerald RC, di Pietro M, Ragunath K, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut. 2014;63(7-42):7.

8.   Suspected cancer: recognition and referral. Available at http://www.nice.org.uk/guidance/NG12/chapter/1-recommendations#upper-gastrointestinal-tract-cancers last accessed November 2015. NICE guidelines [NG12]. 2015.

9.   Wo JM, Mendez C, Harrell S, Joubran R, Bressoud PF, McKinney WP. Clinical Impact of Upper Endoscopy in the Management of Patients with Gastroesophageal Reflux Disease. Am J Gastroenterol. 2004;99:2311-6.

10.   Ronkainen J, Aro P, Storskrubb T, et al. High prevalence of gastroesophageal reflux symptoms and esophagitis with or without symptoms in the general adult Swedish population: A Kalixanda study report. Scand J Gastroenterol. 2005;40:275-85.

11.   Dent J, Becher A, Sung J, Zou D, Agreus L, Bazzoli F. Systematic Review: Patterns of Reflux-Induced Symptoms and Esophageal Endoscopic Findings in Large-Scale Surveys. Clin Gastroenterol Hepatol. 2012;10:863-73.

12.   Pace F, Pallotta S, Antinori S. Nongastroesophageal reflux disease-related infectious, inflammatory and injurious disorders of the esophagus. Curr opin gastroenterol. 2007;23:446-51.

13.   Wilson A, Delport J, Ponich T. Candida glabrata Esophagitis: Are We Seeing the Emergence of a New Azole-Resistant Pathogen? Int J Microbiol. 2014;2014:1-4.

14.   Asayama N, Nagata N, Shimbo T, et al. Relationship between clinical factors and severity of esophageal candidiasis according to Kodsi's classification. Dis esophagus. 2014;27:214-9.

15.   Weerasuriya N, Snape J. A study of candida esophagitis in elderly patients attending a district general hospital in the UK. Dis esophagus. 2006;19:189-92.

16.   Dellon ES, Erichsen R, Baron JA, et al. The increasing incidence and prevalence of eosinophilic oesophagitis outpaces changes in endoscopic and biopsy practice: national population-based estimates from Denmark. Aliment Pharmacol Ther. 2015;41:662-70.

17.   Sealock RJ, Rendon G, El-Serag HB. Systematic review: the epidemiology of eosinophilic oesophagitis in adults. Aliment Pharmacol Ther. 2010;32:712-9.

18.   Dellon ES, Gonsalves N, Hirana I, Furuta GT, Liacouras CA, Katzka DA. ACG Clinical Guideline: Evidenced Based Approach to the Diagnosis and Management of Esophageal Eosinophilia and Eosinophilic Esophagitis (EoE). Am J Gastroenterol. 2013;108:679-92.

19.   Ludvigsson JF, Aro P, Walker MM, et al. Coeliac disease, eosinophilic oesophagitis and gastrooesophageal reflux disease, an adult population-based study. Scand J Gastroenterol. 2013;48(7):808-14.

20.   2015/16 National Tariff Payment System: Draft national prices. Accessed from https://www.gov.uk/government/publications/nhs-national-tariff-payment-system-201516-draft-price-setting-models (October 2015).

21.   Kadri SR, Lao-Sirieix P, O'Donovan M, et al. Acceptability and accuracy of a non-endoscopic screening test for Barrett's oesophagus in primary care: cohort study. BMJ. 2010;341:4372.

22.   Lao-Sirieix P, Rous R, O'Donovan M, Hardwick RH, Debiram I, Fitzgerald RC. Non-endoscopic immunocytological screening test for Barrett's oesophagus. Gut. 2007;56(1033-1034).

23.   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 Medicine. 2015;12(1):1-19.

24.   Benaglia T, Sharples LD, Fitzgerald RC, Lyratzopoulos G. Health Benefits and Cost Effectiveness of Endoscopic and Nonendoscopic Cytosponge Screening for Barrett's Esophagus. Gastroenterology. 2013;144:62-73.

25.   Lao-Sirieix P, Boussioustas A, Kadri SR, et al. Non-endoscopic screening biomarkers for Barrett's oesophagus: from microarray analysis to the clinic. Gut. 2009;58:1451-9.

26.   Katzka DA, Geno DM, Ravi A, et al. Accuracy, Safety, and Tolerability of Tissue Collection by Cytosponge vs Endoscopy for Evaluation of Eosinophilic Esophagitis. Clin Gastroenterol Hepatol. 2015;13:77-83.

27.   Lundell LR, Dent J, Bennett JR, et al. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. Gut. 1999;45:172-80.

28.   Rath HC, Timmer A, Kunkel C, et al. Comparison of interobserver agreement for different scoring systems for reflux esophagitis: impact of level of experience. Gastrointest Endosc. 2004;60:44-9.

29.   Rutjes AWS, Retisma JB, Coomarasamy A, Khan KS, Bossuyt PMM. Evaluation of diagnostic tests when there is no gold standard. A review of methods. Health Technol Assess. 2007;11(50):1-86.