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Title: Safety and Acceptability of Esophageal Cytosponge Cell Collection Device in a Pooled Analysis of Data from Individual Patients

Short title: Safety and acceptability of the Cytosponge

Wladyslaw Januszewicz[1,2*], **Wei Keith Tan**[1,3*], Katie Lehovsky[4], Irene Debiram-Beecham[1], Tara Nuckcheddy[1], Susan Moist[5], Sudarshan Kadri[6], Massimiliano di Pietro[1], Alex Boussioutas[7], Nicholas J. Shaheen[8], David A. Katzka[9], Evan S. Dellon[8], Rebecca C. Fitzgerald[1] on behalf of the BEST 1 and BEST2 study investigators

* first two authors contributed equally, and both should be regarded as first authors

1. MRC Cancer Unit, University of Cambridge, Cambridge UK

2. Department of Gastroenterology, Hepatology and Clinical Oncology, Medical Centre for Postgraduate Education, Warsaw, Poland

3. Department of Gastroenterology, Addenbrookes Hospital, Cambridge University NHS Foundation Trust, Cambridge, UK

4. School of Clinical Medicine, University of Cambridge, Cambridge, UK

5. Center for Esophageal Diseases and Swallowing, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA

6. Department of Gastroenterology, University Hospital Leicester, Leicester, UK

7. Sir Peter MacCallum Department of Oncology, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Melbourne, Victoria, Australia.

8. Center for Esophageal Diseases and Swallowing, University of North Carolina School of Medicine, Chapel Hill, USA.

9. Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota.

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Abbreviations

AE, adverse events

BE, Barrett's esophagus

EAC, esophageal adenocarcinoma

EGD, esophagogastroduodenoscopy

EMR, endoscopic mucosal resection

EoE, eosinophilic esophagitis

GERD, gastro-esophageal reflux disease

HGD, high-grade dysplasia

LGD, low-grade dysplasia

RFA, radiofrequency ablation

SAE, serious adverse events

VAS, visual analogue scale

Corresponding author:

Rebecca C. Fitzgerald

MRC Cancer Unit

University of Cambridge,

CB2 0XZ, Cambridge, UK

Phone: (+44)122376 63287

e-mail: rcf29@mrc-cu.cam.ac.uk

Conflict of Interest: The data presented herein all relate to the in-house (design and local manufacture) produced Cytosponge®. The Cytosponge® is licensed by the Medical Research Council (MRC) to Covidien GI Solutions (now owned by Medtronic) and they are in the process of developing an FDA approved and CE marked device. Medtronic are not privy to the contents of this manuscript prior to publication. Rebecca Fitzgerald, Sudarshan Kadri and Pierre Lao-Sirieix are named inventors on patents related to the Cytosponge®. Evan S. Dellon has received research funding from NIH, ACG, AGA, Adare, Meritage, Miraca, Nutricia, Celgene/Receptos, Regeneron, Shire, CURED, UNC/NC TraCS, consulting fees from Adare, Alivio, Banner, Enumeral, GSK, Celegene/Receptos, Regeneron, Robarts Clnical Trials, Shire and educational grant from Banner, Holoclara. All of the remaining authors disclose no conflict of interest.

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ABSTRACT

Background & Aims: Non-endoscopic methods for diagnosis and surveillance of Barrett's esophagus (BE) and eosinophilic esophagitis are needed. Cytosponge is a minimally invasive device for esophageal cell sampling. We aimed to assess safety and acceptability of this device.

Methods: We collected data from 5 prospective trials from patients with reflux disease, BE, or eosinophilic esophagitis in primary and secondary care. We analyzed data from 2,672 Cytosponge procedures, performed in 2,418 individuals from 2008 through 2017. Acceptability of the Cytosponge and subsequent endoscopy were calculated using the visual analogue scale (VAS; score of 0 for the lowest and 10 for highest level of acceptability) and compared using a Mann Whitney test. The number of attempts, failures in swallowing the device, and occurrence of adverse events were analyzed. Risk factors for failure in swallowing were analyzed using a multivariate regression model.

Results: There were 2 adverse events related to the device: a pharyngeal bleed and 1 case of detachment (<1:2000). The median acceptability score for Cytosponge was 6.0 (inter-quartile range [IQR], 5.0–8.0), which was higher than for endoscopy without sedation (median 5.0, IQR, 3.0–7.0; *P*<.001) and lower than for endoscopy with sedation (median 8.0, IQR, 5.0–9.0; *P*<.001). Nearly all patients (96.5%) successfully swallowed the Cytosponge, most often on the first swallow attempt (90.1%). Failure to swallow the device was more likely to occur in secondary care (odds ratio, 5.13, 95% CI, 1.48–17.79; *P*<.01).

Conclusion: Cytosponge is safe and well accepted for esophageal tissue collection, in a variety of health care settings.

KEY WORDS: EoE; clinical trials; acceptability; cytology

INTRODUCTION

Two chronic esophageal diseases - Barrett's esophagus (BE) and eosinophilic esophagitis (EoE) - have become emerging issues in the public health over the last several decades[1,2,3].

BE develops on the background of long-standing gastro-esophageal reflux disease (GERD) and is defined as a metaplastic change in the esophageal lining, from a squamous-type epithelium to a specialized columnar epithelium. The estimated population prevalence of BE is 1- 2%[4]. BE is a major risk factor for esophageal adenocarcinoma (EAC) - a cancer with rapidly increasing incidence in the Western world[5]. Patients with chronic GERD and other risk factors (male sex, age of ≥ 50 years, white race, family history of BE or EAC) may be offered endoscopic screening for the presence of BE[6], however most BE cases remain undiagnosed. Patients with the benefit of a BE diagnosis undergo endoscopic surveillance with the aim to identify neoplastic changes within BE segment at the earliest possible stage[7,8,9]. Such patients are candidates for endoscopic treatment with either endoscopic mucosal resection (EMR) or radiofrequency ablation (RFA)[10,11] with excellent survival results for intra-mucosal disease[12].

EoE, on the other hand, is a relatively newly defined immune-mediated disease characterized by predominant eosinophilic inflammation of the esophagus (a peak count of ≥ 15 eosinophil per high-power field of biopsy tissue)[13]. EoE is seen predominantly in younger men, however it affects all age groups and both sexes[14,15]. It is one of the most common condition in adult patients leading to food bolus impaction. As with BE, most cases of EoE are undiagnosed, and its incidence rate is reaching up to 12.8 /100,000 / year in some regions of the US[16]. The aim of diagnosis and treatment is to control the symptoms, resolve esophageal eosinophilia, and reduce complications.

Although the nature of these two entities is highly disparate, both require long-term, endoscopic monitoring and repeated collection of mucosal samples to optimize and monitor the treatment. To perform systematic screening and surveillance for these conditions would constitute a huge burden on health care systems. A survey study analyzing trends in endoscopic volume in the US showed that there was a 54% increase in upper GI endoscopy between 2000 and 2009, with an estimated number of 6.9 million of these procedures performed in 2009[17]. The rising incidence of BE and EoE may have contributed to these numbers. Patients with EoE alone have an estimated annual health-care cost of as much as $1.4 billion in the US[18].

While diagnostic esophagogastroduodenoscopy (EGD) is considered to be a safe procedure, it is not devoid of complications. The overall mortality rates for EGD are ranging from none to 1 in 2,000 in various studies[19]. Perforation, a potentially life-threatening complication, is reported to occur from 1 in every 2,500 to 1 in every 11,000 procedures[20,21]. Moreover, many of the EGDs in the US and Europe are performed under sedation, exposing patients to additional risks. These include cardiopulmonary complications, which account for as much as 60% of endoscopy adverse events and an incidence ranging between 1 in 170 and 1 in 10,000[22].

Therefore, new, less invasive methods of esophageal mucosal sampling are being investigated. Cytosponge® is a minimally invasive cell collection device that consists of a 30-mm polyurethane sponge, contained within a capsule attached to a string. When withdrawn, the device collects esophageal cells for analysis (*Figure 1A*). Cytosponge has already been successfully used in several studies to identify BE and EoE[23,24,25]. The cells retrieved from the sponge are spun down and embedded to produce a pseudo-biopsy suitable for routine laboratory analysis (*Figure 1B-D*). To aid the identification of BE, the histopathological analysis is coupled with a diagnostic

biomarker, Trefoil Factor-3 (TFF-3); *Figure 1C*. Of note, the utility of the Cytosponge goes beyond the confines of BE and EoE diagnosis since a range of pathologies affecting the esophagus and proximal stomach, such as esophageal candidiasis, esophageal ulcers, *H.pylori* infection, intestinal metaplasia at the cardia and viral esophagitis can also be diagnosed[26].

The aim of this study was to combine data from 5 large trials on Cytosponge performed in patients with chronic GERD, BE and EoE in 3 different countries (UK, USA and Australia) to assess the overall safety and acceptability of this test.

METHODS

Study design and study participants

This was a retrospective, patient-level technical review of prospectively collected data. Studies included in the analyses were the Barrett's ESophagus Trial 1 (BEST1)[24], BEST2[25], BEST-Australia, the ongoing BEST2-RFA study (ClinicalTrials.gov number NCT02106910) and Cytosponge Eosinophilic Esophagitis study (EoE Study, NCT02114606)[23]. Principal investigators of each trial shared the original trial databases. All studies were conducted with the use of Cytosponge approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA).

Briefly, the setting and patients' eligibility criteria of each study were as follows:

- BEST1: individuals with chronic GERD managed in primary care with long-term PPI (>3 months).
- BEST2: patients with previously diagnosed BE (cases) and patients with GERD without BE (control group) referred to the secondary care unit for endoscopy.
- BEST-Australia: patients with chronic GERD symptoms referred for endoscopy in a secondary care unit.

- BEST2-RFA: patients with BE with low-grade dysplasia (LGD) or high-grade dysplasia (HGD), who received radiofrequency ablation (RFA) or are under surveillance following ablative treatment.
- EoE study: patients with EoE referred for the secondary care unit to undergo clinically indicated endoscopy.

Exclusion criteria were generally consistent between studies and included bleeding disorders, known cirrhosis +/- varices, history of esophageal surgery, dysphagia and esophageal stricture. An overview of study characteristics is presented in *Table 1*.

Cytosponge Procedure

The Cytosponge was administered in a similar fashion in each trial by trained research nurses, research fellows or study investigators. All participants were given the option of having a local anesthetic (1% lignocaine throat spray) before having the test. After swallowing the device in sitting position, the capsule coating disintegrates within 5 minutes upon reaching the stomach, revealing a 3-cm diameter spherical mesh that is withdrawn by pulling the string. Following its retrieval, the string is cut andthe Cytosponge is immersed in SurePath Preservative Fluid (TriPath Imaging, Burlington, North Carolina, USA) and kept at 4^{o}C until transported to the laboratory for processing. Hematoxilin Eosin (H&E) staining and immunohistochemistry for TFF-3 is then performed on paraffin-embedded Cytosponge specimens by adhering to standard H&E and TFF3 protocols on a BOND-MAX autostainer (Leica Biosystems, Newcastle Upon Tyne, UK).

Outcome measures

Acceptability of the Cytosponge and subsequent endoscopy (regardless of sedation) was recorded using a visual analogue scale (VAS), wherein 10 indicated the

best and 0 the worst experience[27]. The acceptability scores were collected immediately after Cytosponge procedure and after each endoscopy procedure (within 30 minutes). Patients in secondary care studies (BEST2, BEST-Australia, EoE Study, BEST2-RFA) underwent the Cytosponge and endoscopy on the same day, whereas patients from BEST1 (primary care) had their endoscopy scheduled within three weeks and the acceptability score for endoscopy was not recorded. Number of swallow attempts and failure in swallowing the Cytosponge were noted. 'Failure to swallow' was stated when the device could not be swallowed despite three attempts. Patients in BEST2 and EoE study had repeated Cytosponge tests. All serious adverse events (SAE) were reported in accordance to the Good Clinical Practice guidelines. Minor events, such as sore throat, were not systematically recorded.

Cytosponge abrasions grading system

An abrasion grading system was introduced to categorize the severity of abrasions following the Cytosponge procedure. The presence and degree of abrasions were recorded during subsequent EGD. Abrasions provide useful information on the most distal passage of the device (important for diagnosing BE) as well as a comparator with biopsies for the bleeding risk. The grading system is presented in *Figure 2*.

Statistical Analysis

Statistics for continuous variables were expressed as medians and interquartile ranges (IQRs). The Mann-Whitney test was used to compare continuous variables between groups. The association between failure in swallowing the Cytosponge and risk factors was analyzed using multivariable regression model. We reported odds ratios (OR) and 95% confidence intervals (CI) adjusted for patient's sex, study setting,

BMI and indication. All statistical tests were two-sided. For all analyses, *P* value of less than 0.05 was considered statistically significant. All analyses were performed using R Statistics version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient Demographics

In total, data on 2,418 patients from 5 studies between May 2008 and August 2017 were analyzed. Eighty-four patients were unable to swallow the Cytosponge and 50 were withdrawn due to study eligibility (2.0%), leaving 2,284 patients who successfully underwent the Cytosponge test (94.5%) and a successful swallow rate of 96.5%. The study cohort comprised of 518 BEST1 patients (21.4%), 1,498 BEST2 patients (62.0%), 224 BEST-Australia patients (9.3%), 76 BEST2-RFA patients (3.1%), and 102 EoE study patients (4.2%).

There were 1,329 patients with GERD (56.7%), 987 patients with BE (40.8%; 911 from BEST2 and 76 from BEST2-RFA) and 102 patients with EoE (4.2%). The median age was 62 years (IQR 54-68) and the male to female ratio was 1.7:1.0. The median body mass index (BMI) was 28.2 kg/m^2 (IQR 25.1-31.5), indicating that most patients were overweight. The median waist-to-hip ratio for females was 0.86 (IQR 0.81-0.91) and for males it was 0.96 (IQR 0.92-0.99). Smoking status was recorded for 1,971 patients. Of these, 809 were reported as lifetime non-smokers (41.0%), 971 as former smokers (49.2%) and 191 as active smokers (9.7%). More than half of patients who underwent endoscopy had been diagnosed with hiatus hernia (53.7%). Combined demographic data is presented in *Table 2*.

Cytosponge Acceptability

Overall, 2,672 Cytosponge test were performed, of which 2,289 had acceptability score recorded (85.7%). The length of the procedure was only recorded in the BEST-Australia study (n=166; 58 missing), indicating that the median time of the procedure was 7.0 minutes (range:3.0-9.0). Anesthetic throat spray was only used in 190 cases (7.1%), however, this data was not routinely recorded and is therefore missing for nearly half of procedures (n=1316, n=49.3%). The endoscopy acceptability score was not recorded in BEST1 due to the gap between the two procedures which would make a comparison difficult. Overall, acceptability was recorded for 1,406 endoscopy procedures in 1,221 patients. Therefore, for 2,672 Cytosponge procedures we had 1,406 corresponding acceptability scores for subsequent endoscopies (52.6%). Of these, 1,175 endoscopies included data on sedation (96.2%), such that 402 EGD's were performed without sedation (34.2%) and 773 with sedation (65.8%), which inevitably affected the rating.

The overall acceptability for the Cytosponge was satisfactory, with a median score of 6.0 (IQR 5.0-8.0). This was significantly higher when compared to endoscopy without sedation with median VAS score of 5.0 (IQR 3.0-7.0) (P<0.001), but still comparatively lower than endoscopy with sedation (VAS 8.0, IQR 5.0-9.0)(P<0.001); see *Figure 3*. EoE patients had the highest acceptability for the test (VAS 8.0, IQR 5.1-9.0), as compared to patients with BE [VAS 7.0 (IQR 5.0-8.0)] and GERD [VAS 6.0 (IQR 4.9-8.0)]; P<0.001 for both comparisons. The presence of hiatus hernia did not influence the acceptability score (P=0.109). Males had higher acceptability than females [median 7.0 (IQR 5.0-8.0) vs 6.0 (IQR 5.0-8.0), P=0.003], as did patients in primary care setting, when compared to patients in secondary care (7.0 [IQR 5.0-8.0] vs. 6.0 [IQR 5.0-8.0], P<0.001). See *Figure 4.*

Failure to swallow the Cytosponge

Eighty-four patients failed to swallow the Cytosponge (3.5%). All EoE patients successfully swallowed the device. The proportion of patients who were unable to swallow the device was over 2-times higher within BE patients than in GERD patients (5.7% vs 2.1%) and slightly higher within males as compared to females (3.9% vs 2.7%), however, in a multivariable regression model, the risk of swallow failure in patients with previously diagnosed BE was not significantly different, when compared to patients with GERD (OR=0.63, 95%CI 0.35-1.14, P=0.13). Moreover, high BMI and gender were not associated with different rates of failure in swallowing the device. Patients examined in secondary care setting were over 5-times more likely to fail swallowing the Cytosponge, as compared to primary care setting (OR= 5.13, 95% CI 1.48-17.79, *P*<0.01). *Supplementary table 1* presents the multivariable regression model results. Most successful tests were achieved with the first swallow attempt (90.1%).

Cytosponge adverse events

Overall, of the 2,672 Cytosponge tests performed, there were 12 SAE reported, of which only 2 could be directly attributed to the Cytosponge (<1: 2,000). These included one detachment of the sponge and one pharyngeal bleeding after Cytosponge withdrawal. The others were related to endoscopic therapy performed immediately after the Cytosponge test (see *Supplementary table 2*). As sore throat is a frequent event following endoscopy, we did not consider it an AE and the data was not collected systematically across all studies. No late AE, such as strictures have been reported.

Cytosponge detachment occurred in a 76-year-old male patient with BE in the BEST2-RFA study at the University of North Carolina. The patient did not report any

discomfort when the device was retained. Since the Cytosponge test was performed in the secondary care setting, it was retrieved endoscopically on the same day. The detached device was found in the pylorus and was successfully retrieved with a Roth net without further adverse consequences for the patient.

There was one case of mild pharyngeal bleeding in a patient from BEST2 study. The patient was on warfarin for atrial fibrillation, that was stopped prior to the procedure (INR was 1.2). The bleeding resolved spontaneously and there was no drop in Haemoglobin levels. He was hospitalized as a precautionary measure and was discharged home the next day.

Moreover, there was a single case of variceal bleeding in BEST2 study patient, however this event was more likely to be related with subsequent endoscopy procedure than with the Cytosponge. In this case, there was no signs of bleeding after withdrawal of the device and the subsequent endoscopy (on the same day) revealed esophageal varices (patient had no previous history of varices). Since there were no signs of bleeding at that time, endoscopy was performed as per usual practice and the patient was discharged, however had to be re-admitted in the early hours of the following day with haematemesis. Gastroscopy was performed again, and 2 bleeding varices were banded.

Cytosponge abrasions

A Cytosponge abrasions grading system was devised in November 2011. It categorizes abrasions into five categories based on visual appearance of abrasions during endoscopy. This grading system was used in BEST2, BEST2-RFA and EoE Study. Overall, 1,075 Cytosponge procedures were followed by an endoscopy with abrasion score assessment. In most of the cases (919/1075; 85.5%) Cytosponge

caused no or only mild abrasions (grade 0-2). Precisely, there were 74 cases with no abrasions noted after Cytosponge procedure (6.9%), 433 cases of grade 1 abrasions (40.3%), 412 cases of grade 2 abrasions (38.3%), 132 cases grade 3 abrasions (12.3%) and only 24 cases (2.2%) of severe post- Cytosponge abrasions (grade 4). There were no cases of grade 5 abrasions that required endoscopic or surgical intervention. Of note, Cytosponge abrasions, even at the highest grade of 4, appear less severe when compared to current standard of care (quadrantic biopsies obtained every 2 cm - Seattle protocol[28]), as shown in *Figure 2*.

DISCUSSION

This technical review of five large prospective studies on the performance of the Cytosponge showed that it is a safe procedure with good acceptability ratings. The test can be safely performed by a nurse in both the primary and secondary care setting, with minimal risk of AE. The Cytosponge test was feasible when used for screening purposes (GERD patients with high-risk for BE), as well as for surveillance (EoE and BE after endoscopic treatment).

Safety is paramount for any procedure especially when being performed in the primary care setting. Our review showed that of 2,672 Cytosponge procedures there were only two SAE that could be directly attributed to the device (<1: 2,000) and both resolved without any ill-effects for the patient. The detachment is the most concerning risk factor to both clinicians and patients[29]. However, a retained sponge in the stomach would not be expected to cause any symptoms as was the case in the patient reported here. Since objects greater than 2–2.5cm in diameter do not pass through the pylorus[30], we expect the expanded sponge (which has a diameter of 3 cm) to stay in

348 the stomach after detachment. In case of this unlikely event, endoscopy retrieval
349 should be easily arranged.

350 In a recent perspective article, it was reported that the Cytosponge had been
351 recalled due to two cases of detachment in the CASE1 study (FDA Recall Z-2123-
352 2016)[31]. We would like to emphasize that the above article refers to an alternative
353 prototype device developed by Covidien GI Solutions (now Medtronic), not the original
354 prototype patented by the Medical Research Council (MRC) UK, which was used in all
355 the studies reported here. FDA and CE marking of the original device is underway
356 [Cytosponge received 510(k) clearance from the FDA on November 26, 2014
357 (K142695)].

358 Previous interview-based, quality study on 33 participants with GERD showed
359 that Cytosponge is acceptable for most participants, as well as being preferred to
360 endoscopy[29]. In our study, most patients (79.3%) scored their experience as at least
361 “neutral” (VAS≥5) and the median VAS score was 6.0 (IQR 5.0-8.0). This was
362 significantly higher when compared to endoscopy without sedation (VAS 5.0, IQR 3.0-
363 7.0), however lower than endoscopy with sedation (VAS 8.0, IQR 5.0-9.0, $P<0.001$ for
364 both comparisons). It must be stressed, that the Cytosponge has other advantages as
365 a screening tool, when compared to the latter. Endoscopy with sedation is an invasive,
366 time-consuming procedure (usually several hours including recovery time), that
367 requires the patient to avoid work and operating machinery for the subsequent 24
368 hours. Cytosponge can be performed in 5-7 minutes, within a primary care office, and
369 (usually) does not involve any restrictions for the remaining part of the day.

370 Our review shows that patients with previously diagnosed BE and EoE have a
371 higher acceptability rating for Cytosponge as compared to patients with GERD

(*P*<0.001). Supposably, these patients are more aware of the importance of undergoing regular monitoring and are more used to repeated endoscopic examinations, which might explain the higher degree of acceptability. Patients examined in the primary care setting (n= 518), had markedly higher acceptance, as compared to patients examined in the secondary care (n=2,154). The unequal size of the groups could, however, be a confounding factor. Nevertheless, we postulate that the more patient-friendly environment and individual approach of a primary care setting benefits the overall acceptability of the test and it also possible that in secondary care patients are more keen to undergo the current gold-standard endoscopy procedure. These results are promising, since the Cytosponge was developed with aim to be a minimally invasive test for use in a primary-care offices.

Prior to implementation in clinical practice, randomized trial data is required to fully evaluate the diagnostic yield of Cytosponge and its safety, acceptability and health economic outcomes. This is currently underway in the Barrett's ESophagus Trial 3 [(BEST3); trial ID ISRCTN68382401], a 10,000-patient cluster randomized controlled trial which is being conducted in multiple UK primary care surgeries (more information: https://www.best3trial.org/the-best3-trial, funded by Cancer Research UK).

The main strength of the study is the direct access to original dataset to minimize missing data and ensure high quality of the statistical analyses. The studies were undertaken in several countries, for different indications and in different health care settings, however with the use of same Cytosponge device (design and model) and standard operating procedure for administration. This study does have some limitations. There were comparatively fewer acceptability scores recorded for endoscopy than the Cytosponge. This was because patients enrolled onto the BEST1 trial did not have the acceptability score recorded following endoscopy. Furthermore,

the VAS scale is a crude measure of acceptability and further quantitative and qualitative interviews will be required to fully understand the patient experience. Some of the studies included in this analysis had more complex tools to measure patients' experience, such as Impact Event Score or Spielberger state trait anxiety inventory, however we did not include it in this analysis since they were. not used across all the studies. Moreover, we could not conclude whether the use of local anesthetic had any influence on the acceptability ratings of the Cytosponge test, as its use wasn't routinely recorded and the data is missing for nearly half of the procedures.

CONCLUSIONS

In conclusion, in this first review of clinical data on safety and acceptability of the Cytosponge, we have demonstrated that this device has a favourable safety and acceptability profile. The relative ease of administration and the higher safety profile as compared to endoscopy makes it a promising tool to be used in the primary care setting as a screening and surveillance test for esophageal disorders such as BE or EoE. Results from the ongoing BEST3 randomized trial (www.best3trial.org) will be critical prior to implementing the Cytosponge test for widespread use.

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Table 1. Characteristics of Cytosponge studies included in the analysis

Table 2. Demographic characteristics of patients from Cytosponge studies. Values are numbers (percentages) unless stated otherwise

Figure 1

A. Cytosponge in gelatin capsule (right) and expanded (left).

B, C. Haematoxylin and eosin (B) and trefoil-factor 3 (C) staining (20x) from patient with Barrett's oesophagus showing columnar lined epithelium with goblet cells (arrowheads) (courtesy of dr Maria O'Donovan)

D. Haematoxylin and eosin staining (200x) from patient with eosinophilic oesophagitis showing squamous epithelium with admixed eosinophils (arrowheads)

Figure 2. The abrasion grading system after Cytosponge

Figure 3. Cytosponge and endoscopy acceptability (per-procedure)

Figure 4. Acceptability scores for the Cytosponge in different groups of patients (per-procedure).

Supplementary Table 1. Multivariate analysis model for failure of swallowing the Cytosponge

Supplementary Table 2. Combined adverse events from all studies included in the analysis

537 **Table 1**. Characteristics of Cytosponge® studies included in the analysis

	Study 1 (BEST1)	Study 2 (BEST2)	Study 3 (BEST-Australia)	Study 4 (BEST2-RFA)	Study 5 (EoE)
Country:	UK	UK	Australia	USA	USA
Disease:	GERD	GERD and BE	GERD	BE after RFA treatment	EoE
No. of patients (%):	518 (21.4%)	1,498 (62.0%)	224 (9.3%)	76 (3.1%)	102 (4.2%)
No. of Cytosponge® procedures (%):	518 (19.4%)	1,752 (65.6%)	224 (8.4%)	76 (2.8%)	102 (3.8%)
Time of recruitment:	May 2008 – Dec 2009	July 2011 – Dec 2013	May 2010 – August 2014	October 2014 –present (ongoing)	December 2012– present (ongoing)
Inclusion criteria:	• 50 – 70 yrs. • Prescription of acid suppressants for>3 months	• Cases: BE under surveillance • Controls: GERD referred for endoscopy	• 50 – 70 yrs. • Prescription of acid suppressants for>3 months	• 18 – 80 yrs. • BE with LGD / HGD after successful RFA treatment	• 18 - 65 yrs. • EoE undergoing endoscopy

Setting:	Primary care (12 general practices)	Secondary care (11 hospitals)	Secondary care (1 hospital)	Secondary care (1 hospital)	Secondary care (2 hospitals)
Time between Cytosponge® and endoscopy	Up to 3 weeks	Same day (within an hour)	Same day	Same day	Same day (2 hours prior to endoscopy)

BE, Barrett's esophagus; EoE, eosinophilic esophagitis; GERD, gastro-esophageal reflux disease; HGD, high-grade dysplasia; LGD, low-grade dysplasia; RFA, radio-frequency ablation

570 **Table 2.** Demographic characteristics of patients from Cytosponge® studies. Values

571 are numbers (percentages) unless stated otherwise

Characteristics	All participants*	Men**	Women**
Age (years) - median (IQR)	62 (54-68)	63 (54-69)	61 (54-67)
Missing data	153 (6.3)	119 (12.8)	36 (2.4)
Number of participants			
All studies	2,418 (100)	1,486 (61.5)	932 (38.5)
Study 1 (BEST1 Study)	518 (21.4)	240 (46.3)	278 (56.7)
Study 2 (BEST2 Study)	1,498 (62.0)	1,035 (69.1)	463 (30.9)
Study 3 (BEST Study Australia)	224 (9.3)	95 (42.4)	129 (57.6)
Study 4 (POST-RFA Study)	76 (3.1)	58 (76.3)	18 (23.7)
Study 5 (EoE Study)	102 (4.2)	58 (56.9)	44 (43.1)
Indication to Cytosponge®			
GERD	1,329 (55.0)	632 (47.6)	697 (52.4)
BE	987 (40.8)	796 (80.6)	191 (19.4)
EoE	102 (4.2)	58 (56.9)	44 (43.1)
Body Mass Index (BMI, kg/m^2)			
Median (IQR)	28.3 (25.3-31.6)	28.1 (25.6-31.0)	28.6 (24.8-33.1)
Underweight (<18.5)	14 (0.6)	12 (85.7)	2 (14.3)
Normal (18.5 to 24.9)	447 (18.5)	185 (41.4)	262 (58.6)
Overweight (25.0 to 29.9)	853 (35.3)	236 (27.7)	617 (72.3)
Obese (≥30.0)	739 (30.6)	313 (42.4)	426 (57.6)
Missing data	365 (15.0)	186 (51.0)	179 (49.0)
Waist to Hip Ratio***			

Median (IQR)	0.93 (0.87-0.98)	0.96 (0.92-0.99)	0.86 (0.81-0.91)
Low Risk	786 (32.5)	622 (79.1)	164 (20.9)
Moderate Risk	558 (23.1)	379 (67.9)	179 (32.1)
High Risk	626 (25.9)	244 (39.0)	382 (61.0)
Missing data	448 (18.5)	241 (53.8)	207 (46.2)
Smoking Status			
Never	809 (33.5)	466 (57.6)	343 (42.4)
Former	191 (7.9)	133 (69.6)	58 (30.4)
Active	971 (40.2)	630 (64.9)	341 (35.1)
Missing data	447 (18.5)	257 (57.5)	190 (42.5)
Hiatus hernia			
Present	1,191 (49.3)	825 (69.3)	366 (30.7)
Absent	1,025 (42.4)	538 (52.5)	487 (47.5)
Missing data	202 (8.3)	123 (60.9)	79 (39.1)
Previous endoscopic treatment (EMR, RFA, PDT)			
Yes	243 (10.0)	204 (84.0)	39 (16.0)
No	2,175 (90.0)	1,282 (58.9)	893 (41.1)

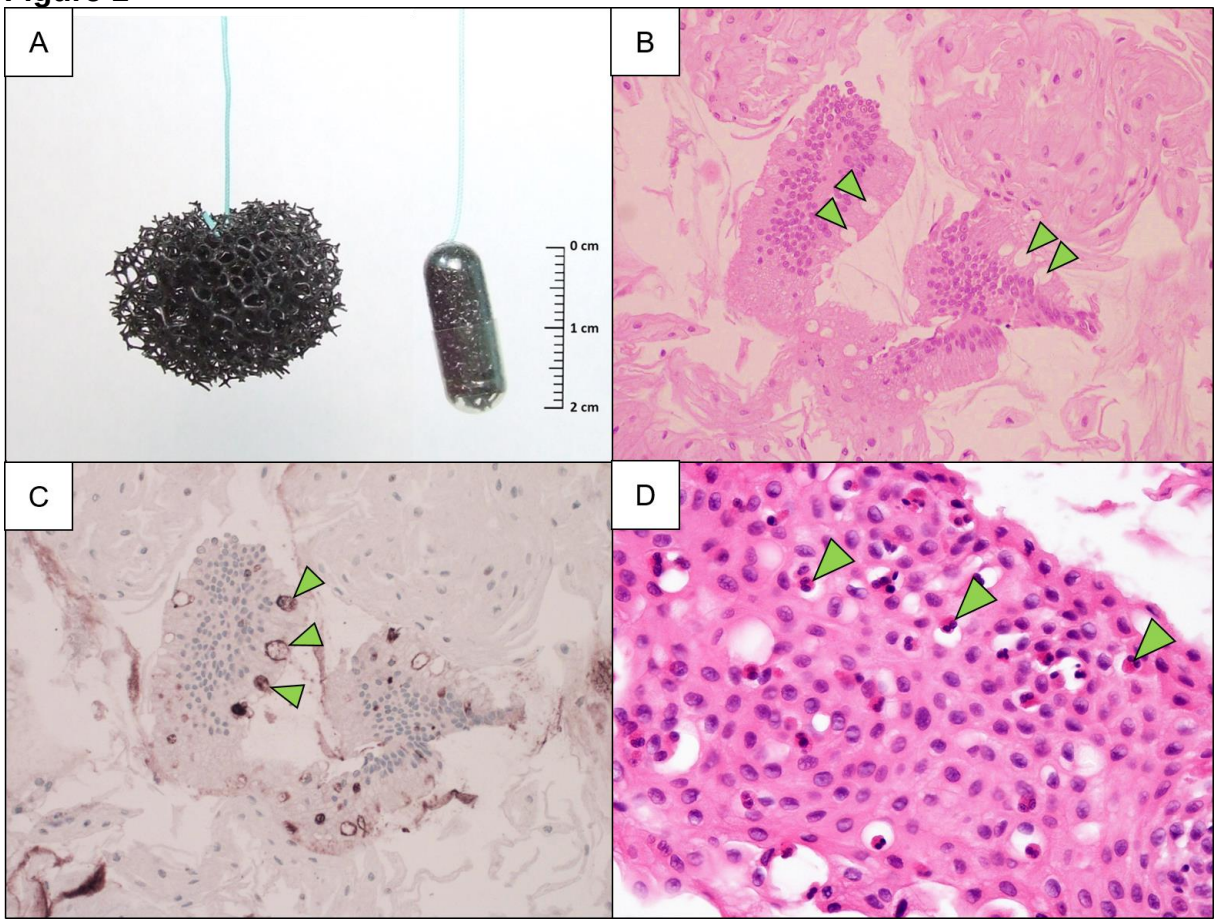
572 EMR, endoscopic mucosal resection; PDT, photo-dynamic therapy; RFA, radio-
573 frequency ablation;

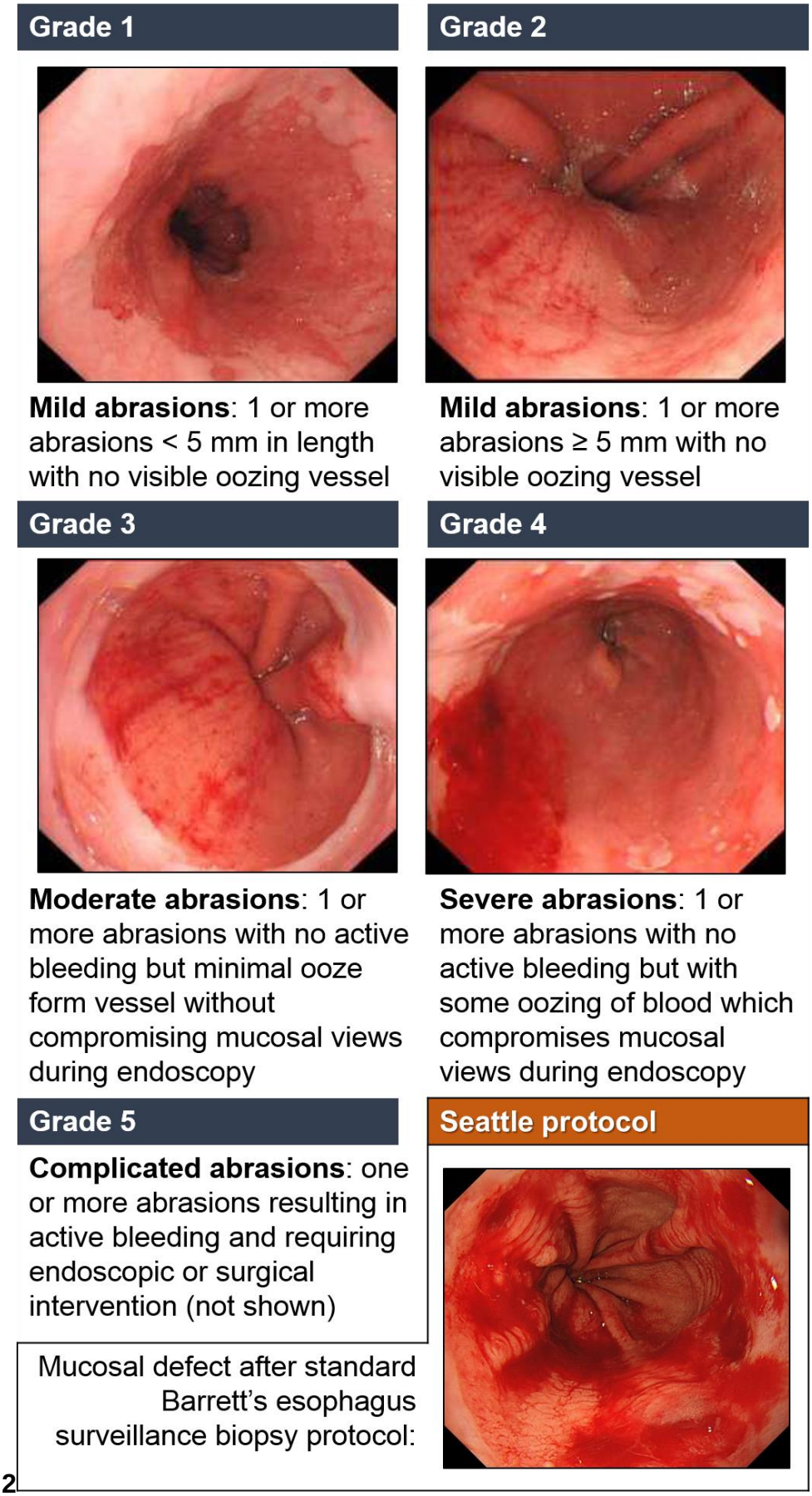
574 * The proportion (%) of patients from each group in the first column refers to the total
575 participant number

576 * The proportion (%) of male and female patients refers to the number of participants
577 from each group (first row), not the total participant number

****** Waist to hip ratio was considered low risk for male <0.95 and female <0.80, moderate risk for male 0.95-1, female 0.81-0.85 and high risk for male >1, female >0.85

Figure 1





652 **Figure 2**
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Figure 3

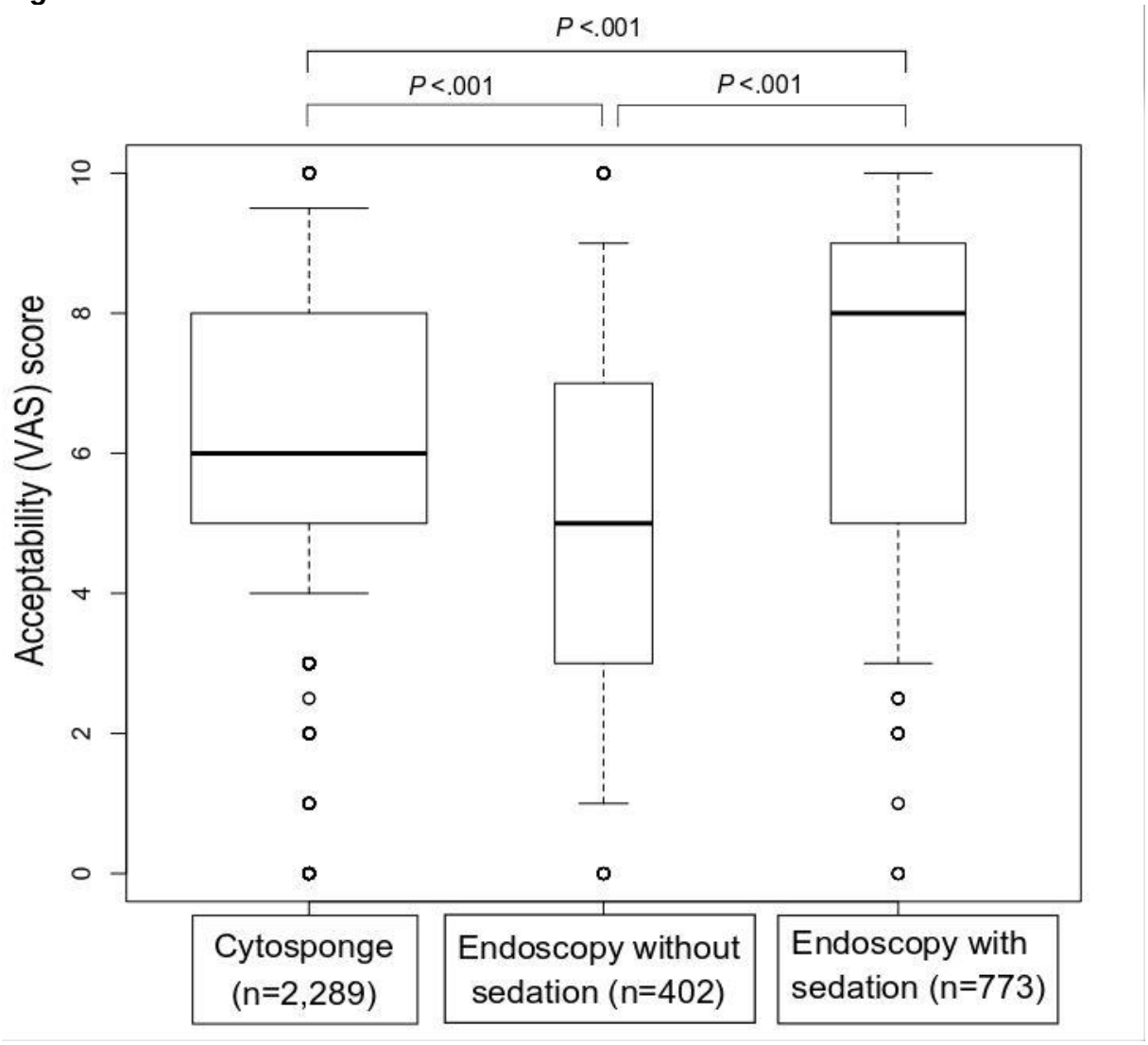
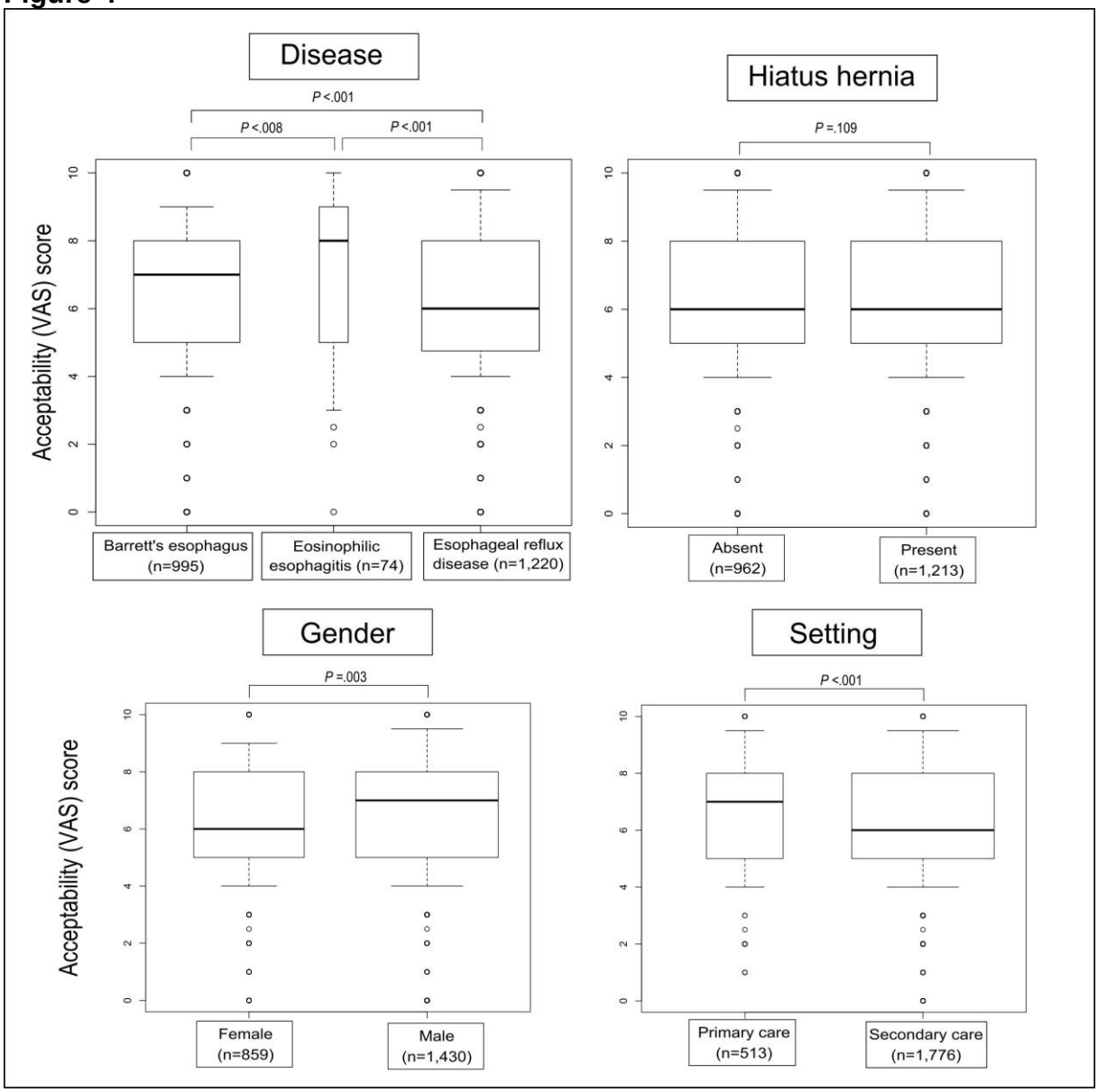

P <.001
P <.001
P <.001
Acceptability (VAS) score
0
2
4
6
8
10
Cytosponge (n=2,289)
Endoscopy without sedation (n=402)
Endoscopy with sedation (n=773)

Figure 4


Disease
P <.001
P <.008
P <.001
Acceptability (VAS) score
Barrett's esophagus (n=995)
Eosinophilic esophagitis (n=74)
Esophageal reflux disease (n=1,220)
Hiatus hernia
P =.109
Absent (n=962)
Present (n=1,213)
Gender
P =.003
Acceptability (VAS) score
Female (n=859)
Male (n=1,430)
Setting
P <.001
Primary care (n=513)
Secondary care (n=1,776)

675 **Supplementary Table 1.** Multivariate analysis model for failure of swallowing the
676 Cytosponge®

Factor	OR	95% CI	*P* value
Gender			
Female (n=932)	1.00	-	-
Male (n=1,486)	1.08	0.61-1.90	*P*=0.79
Study setting			
Primary care (n=518)	1.00	-	-
Secondary care (n=1,900)	5.13	1.48-17.79	***P*<0.01**
Body mass index*			
Normal BMI (n=447)	1.00	-	-
Overweight (n=854)	1.02	0.52-2.03	*P*=0.94
Obese (n=739)	1.75	0.91-3.36	*P*=0.09
Indication			
BE + EoE (n=987+102)	1.00	-	-
GERD (n=1,329)	0.63	0.35-1.14	*P*=0.13

677 * Since there were only 14 cases (0.6%) of underweight patients we did not include
678 them in this analysis.

679 BE, Barrett's esophagus; CI, confidence interval; EoE, eosinophilic esophagitis;
680 GERD, gastroesophageal reflux disease; OR, Odds ratio

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685 **Supplementary Table 2.** Combined adverse events from all studies included in the

686 analysis

Serious Adverse Events	Study	Number of events
Cytosponge®adverse events		
Cytosponge®detachment from string	BEST2-RFA	1
Laceration at the back of the throat	BEST2	1
Endoscopy adverse events		
Bleeding post-EMR and biopsy	BEST2	1
Chest pain post-EMR and syncope	BEST2	1
Post-RFA atrial fibrillation	BEST2	1
RFA-induced ulceration and bleeding	BEST2	2
Syncope	BEST2	1
Haematemesis from esophageal varices	BEST2	1
Epigastric pain	BEST2	1
Diarrhoea and coffee-ground vomiting post procedure	BEST2	1
Central chest pain and melena	BEST2	1
Total		12

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