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

4 Short title: Safety and acceptability of the Cytosponge

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## 28 **Abbreviations**

29 AE, adverse events

30 BE, Barrett's esophagus

31 EAC, esophageal adenocarcinoma

32 EGD, esophagogastroduodenoscopy

33 EMR, endoscopic mucosal resection

34 EoE, eosinophilic esophagitis

35 GERD, gastro-esophageal reflux disease

36 HGD, high-grade dysplasia

37 LGD, low-grade dysplasia

38 RFA, radiofrequency ablation

39 SAE, serious adverse events

40 VAS, visual analogue scale

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48 **Conflict of Interest:** The data presented herein all relate to the in-house (design and  
49 local manufacture) produced Cytosponge<sup>®</sup>. The Cytosponge<sup>®</sup> is licensed by the  
50 Medical Research Council (MRC) to Covidien GI Solutions (now owned by  
51 Medtronic) and they are in the process of developing an FDA approved and CE  
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70 **ABSTRACT**

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

75

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

85

86 **Results:** There were 2 adverse events related to the device: a pharyngeal bleed and  
87 1 case of detachment (<1:2000). The median acceptability score for Cytosponge was  
88 6.0 (inter-quartile range [IQR], 5.0–8.0), which was higher than for endoscopy without  
89 sedation (median 5.0, IQR, 3.0–7.0;  $P<.001$ ) and lower than for endoscopy with  
90 sedation (median 8.0, IQR, 5.0–9.0;  $P<.001$ ). Nearly all patients (96.5%) successfully  
91 swallowed the Cytosponge, most often on the first swallow attempt (90.1%). Failure  
92 to swallow the device was more likely to occur in secondary care (odds ratio, 5.13,  
93 95% CI, 1.48–17.79;  $P<.01$ ).

94

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

97 **KEY WORDS:** EoE; clinical trials; acceptability; cytology

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106 **INTRODUCTION**

107 Two chronic esophageal diseases - Barrett's esophagus (BE) and eosinophilic  
108 esophagitis (EoE) - have become emerging issues in the public health over the last  
109 several decades<sup>1,2,3</sup>.

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

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

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

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

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

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

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

## 165 **METHODS**

### 166 **Study design and study participants**

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

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

- 175 • BEST1: individuals with chronic GERD managed in primary care with long-term PPI  
176 (>3 months).
- 177 • BEST2: patients with previously diagnosed BE (cases) and patients with GERD  
178 without BE (control group) referred to the secondary care unit for endoscopy.
- 179 • BEST-Australia: patients with chronic GERD symptoms referred for endoscopy in  
180 a secondary care unit.



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

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

### 189 **Cytosponge Procedure**

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

### 202 **Outcome measures**

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

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

### 216 **Cytosponge abrasions grading system**

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

### 223 **Statistical Analysis**

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

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

## 232 **RESULTS**

### 233 **Patient Demographics**

234 In total, data on 2,418 patients from 5 studies between May 2008 and August  
235 2017 were analyzed. Eighty-four patients were unable to swallow the Cytosponge and  
236 50 were withdrawn due to study eligibility (2.0%), leaving 2,284 patients who  
237 successfully underwent the Cytosponge test (94.5%) and a successful swallow rate of  
238 96.5%. The study cohort comprised of 518 BEST1 patients (21.4%), 1,498 BEST2  
239 patients (62.0%), 224 BEST-Australia patients (9.3%), 76 BEST2-RFA patients (3.1%),  
240 and 102 EoE study patients (4.2%).

241 There were 1,329 patients with GERD (56.7%), 987 patients with BE (40.8%;  
242 911 from BEST2 and 76 from BEST2-RFA) and 102 patients with EoE (4.2%). The  
243 median age was 62 years (IQR 54-68) and the male to female ratio was 1.7:1.0. The  
244 median body mass index (BMI) was 28.2 kg/m<sup>2</sup> (IQR 25.1-31.5), indicating that most  
245 patients were overweight. The median waist-to-hip ratio for females was 0.86 (IQR  
246 0.81-0.91) and for males it was 0.96 (IQR 0.92-0.99). Smoking status was recorded for  
247 1,971 patients. Of these, 809 were reported as lifetime non-smokers (41.0%), 971 as  
248 former smokers (49.2%) and 191 as active smokers (9.7%). More than half of patients  
249 who underwent endoscopy had been diagnosed with hiatus hernia (53.7%). Combined  
250 demographic data is presented in *Table 2*.

### 251 **Cytosponge Acceptability**

252 Overall, 2,672 Cytosponge test were performed, of which 2,289 had  
253 acceptability score recorded (85.7%). The length of the procedure was only recorded  
254 in the BEST-Australia study (n=166; 58 missing), indicating that the median time of the  
255 procedure was 7.0 minutes (range:3.0-9.0). Anesthetic throat spray was only used in  
256 190 cases (7.1%), however, this data was not routinely recorded and is therefore  
257 missing for nearly half of procedures (n=1316, n=49.3%). The endoscopy acceptability  
258 score was not recorded in BEST1 due to the gap between the two procedures which  
259 would make a comparison difficult. Overall, acceptability was recorded for 1,406  
260 endoscopy procedures in 1,221 patients. Therefore, for 2,672 Cytosponge procedures  
261 we had 1,406 corresponding acceptability scores for subsequent endoscopies  
262 (52.6%). Of these, 1,175 endoscopies included data on sedation (96.2%), such that  
263 402 EGD's were performed without sedation (34.2%) and 773 with sedation (65.8%),  
264 which inevitably affected the rating.

265 The overall acceptability for the Cytosponge was satisfactory, with a median  
266 score of 6.0 (IQR 5.0-8.0). This was significantly higher when compared to endoscopy  
267 without sedation with median VAS score of 5.0 (IQR 3.0-7.0) ( $P<0.001$ ), but still  
268 comparatively lower than endoscopy with sedation (VAS 8.0, IQR 5.0-9.0)( $P<0.001$ );  
269 see *Figure 3*. EoE patients had the highest acceptability for the test (VAS 8.0, IQR 5.1-  
270 9.0), as compared to patients with BE [VAS 7.0 (IQR 5.0-8.0)] and GERD [VAS 6.0  
271 (IQR 4.9-8.0)];  $P<0.001$  for both comparisons. The presence of hiatus hernia did not  
272 influence the acceptability score ( $P=0.109$ ). Males had higher acceptability than  
273 females [median 7.0 (IQR 5.0-8.0) vs 6.0 (IQR 5.0-8.0),  $P=0.003$ ], as did patients in  
274 primary care setting, when compared to patients in secondary care (7.0 [IQR 5.0-8.0]  
275 vs. 6.0 [IQR 5.0-8.0],  $P<0.001$ ). See *Figure 4*.

## 276 **Failure to swallow the Cytosponge**

277 Eighty-four patients failed to swallow the Cytosponge (3.5%). All EoE patients  
278 successfully swallowed the device. The proportion of patients who were unable to  
279 swallow the device was over 2-times higher within BE patients than in GERD patients  
280 (5.7% vs 2.1%) and slightly higher within males as compared to females (3.9% vs  
281 2.7%), however, in a multivariable regression model, the risk of swallow failure in  
282 patients with previously diagnosed BE was not significantly different, when compared  
283 to patients with GERD (OR=0.63, 95%CI 0.35-1.14, P=0.13). Moreover, high BMI and  
284 gender were not associated with different rates of failure in swallowing the device.  
285 Patients examined in secondary care setting were over 5-times more likely to fail  
286 swallowing the Cytosponge, as compared to primary care setting (OR= 5.13, 95% CI  
287 1.48-17.79,  $P<0.01$ ). *Supplementary table 1* presents the multivariable regression  
288 model results. Most successful tests were achieved with the first swallow attempt  
289 (90.1%).

### 290 **Cytosponge adverse events**

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

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

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

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

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

### 319 **Cytosponge abrasions**

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

325 caused no or only mild abrasions (grade 0-2). Precisely, there were 74 cases with no  
326 abrasions noted after Cytosponge procedure (6.9%), 433 cases of grade 1 abrasions  
327 (40.3%), 412 cases of grade 2 abrasions (38.3%), 132 cases grade 3 abrasions  
328 (12.3%) and only 24 cases (2.2%) of severe post- Cytosponge abrasions (grade 4).  
329 There were no cases of grade 5 abrasions that required endoscopic or surgical  
330 intervention. Of note, Cytosponge abrasions, even at the highest grade of 4, appear  
331 less severe when compared to current standard of care (quadrantic biopsies obtained  
332 every 2 cm - Seattle protocol<sup>28</sup>), as shown in *Figure 2*.

### 333 **DISCUSSION**

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

340 Safety is paramount for any procedure especially when being performed in the  
341 primary care setting. Our review showed that of 2,672 Cytosponge procedures there  
342 were only two SAE that could be directly attributed to the device (<1: 2,000) and both  
343 resolved without any ill-effects for the patient. The detachment is the most concerning  
344 risk factor to both clinicians and patients<sup>29</sup>. However, a retained sponge in the stomach  
345 would not be expected to cause any symptoms as was the case in the patient reported  
346 here. Since objects greater than 2–2.5cm in diameter do not pass through the  
347 pylorus<sup>30</sup>, 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)<sup>31</sup>. 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<sup>29</sup>. In our study, most patients (79.3%) scored their experience as at least  
361 “neutral” (VAS $\geq$ 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



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

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

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

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

## 405 **CONCLUSIONS**

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

## 413 **Acknowledgement**

414 We would like to thank Natasha Di Costanzo, Ashan Green and Alberto Stella for help  
415 in data extraction and Paulina Wieszczy and Sarah Killcoyne and for help with statistics  
416 analyses.

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

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

520 **Figure 1**

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

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

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

527 **Figure 2.** The abrasion grading system after Cytosponge

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

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

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

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

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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:	<ul style="list-style-type: none"> <li>• 50 – 70 yrs.</li> <li>• Prescription of acid suppressants for &gt;3 months</li> </ul>	<ul style="list-style-type: none"> <li>• Cases: BE under surveillance</li> <li>• Controls: GERD referred for endoscopy</li> </ul>	<ul style="list-style-type: none"> <li>• 50 – 70 yrs.</li> <li>• Prescription of acid suppressants for &gt;3 months</li> </ul>	<ul style="list-style-type: none"> <li>• 18 – 80 yrs.</li> <li>• BE with LGD / HGD after successful RFA treatment</li> </ul>	<ul style="list-style-type: none"> <li>• 18 - 65 yrs.</li> <li>• EoE undergoing endoscopy</li> </ul>



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)

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

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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 <sup>2</sup> )			
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

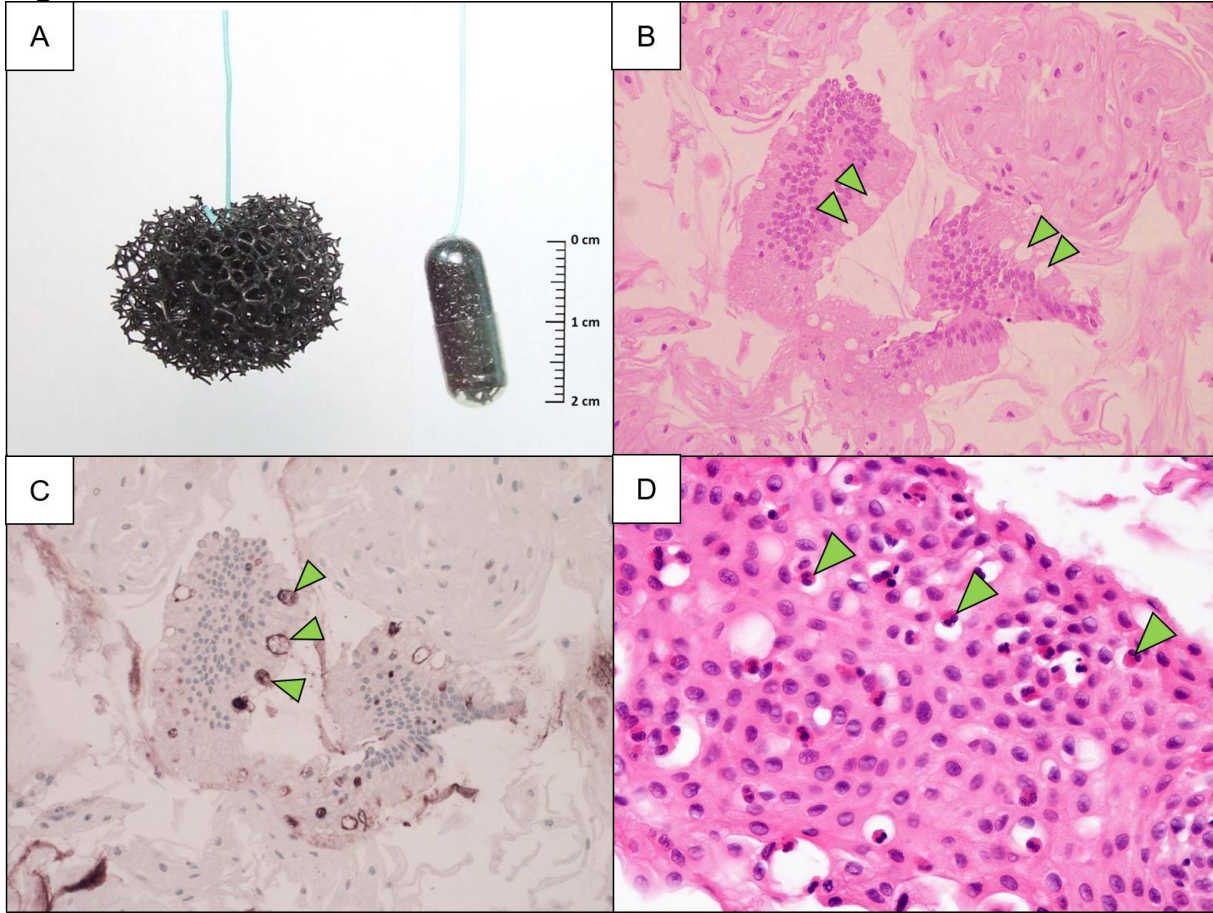
576 \* The proportion (%) of male and female patients refers to the number of participants  
577 from each group (first row), not the total participant number

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

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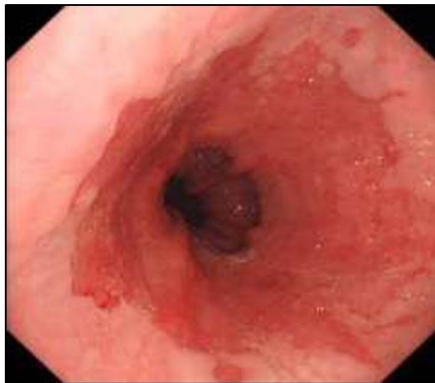
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**Figure 1**



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**Grade 1**



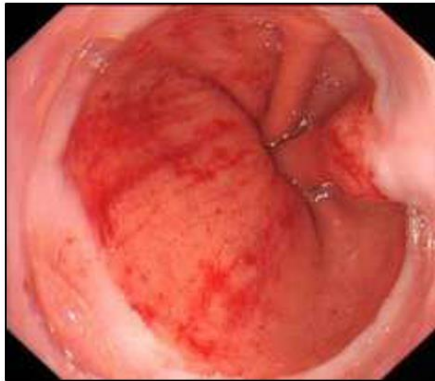
**Mild abrasions:** 1 or more abrasions < 5 mm in length with no visible oozing vessel

**Grade 2**



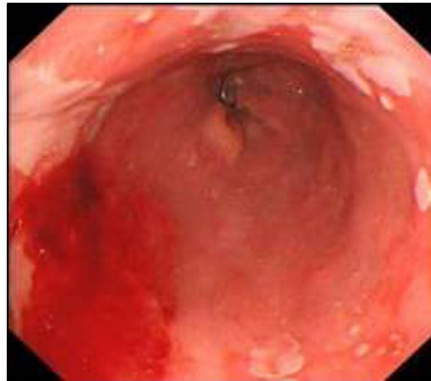
**Mild abrasions:** 1 or more abrasions ≥ 5 mm with no visible oozing vessel

**Grade 3**



**Moderate abrasions:** 1 or more abrasions with no active bleeding but minimal ooze form vessel without compromising mucosal views during endoscopy

**Grade 4**



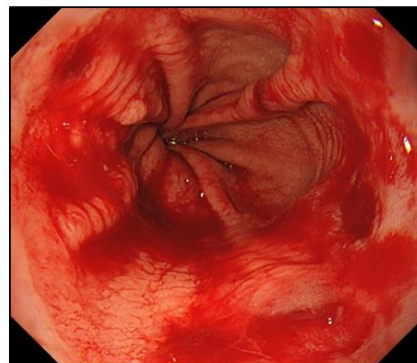
**Severe abrasions:** 1 or more abrasions with no active bleeding but with some oozing of blood which compromises mucosal views during endoscopy

**Grade 5**

**Complicated abrasions:** one or more abrasions resulting in active bleeding and requiring endoscopic or surgical intervention (not shown)

Mucosal defect after standard Barrett's esophagus surveillance biopsy protocol:

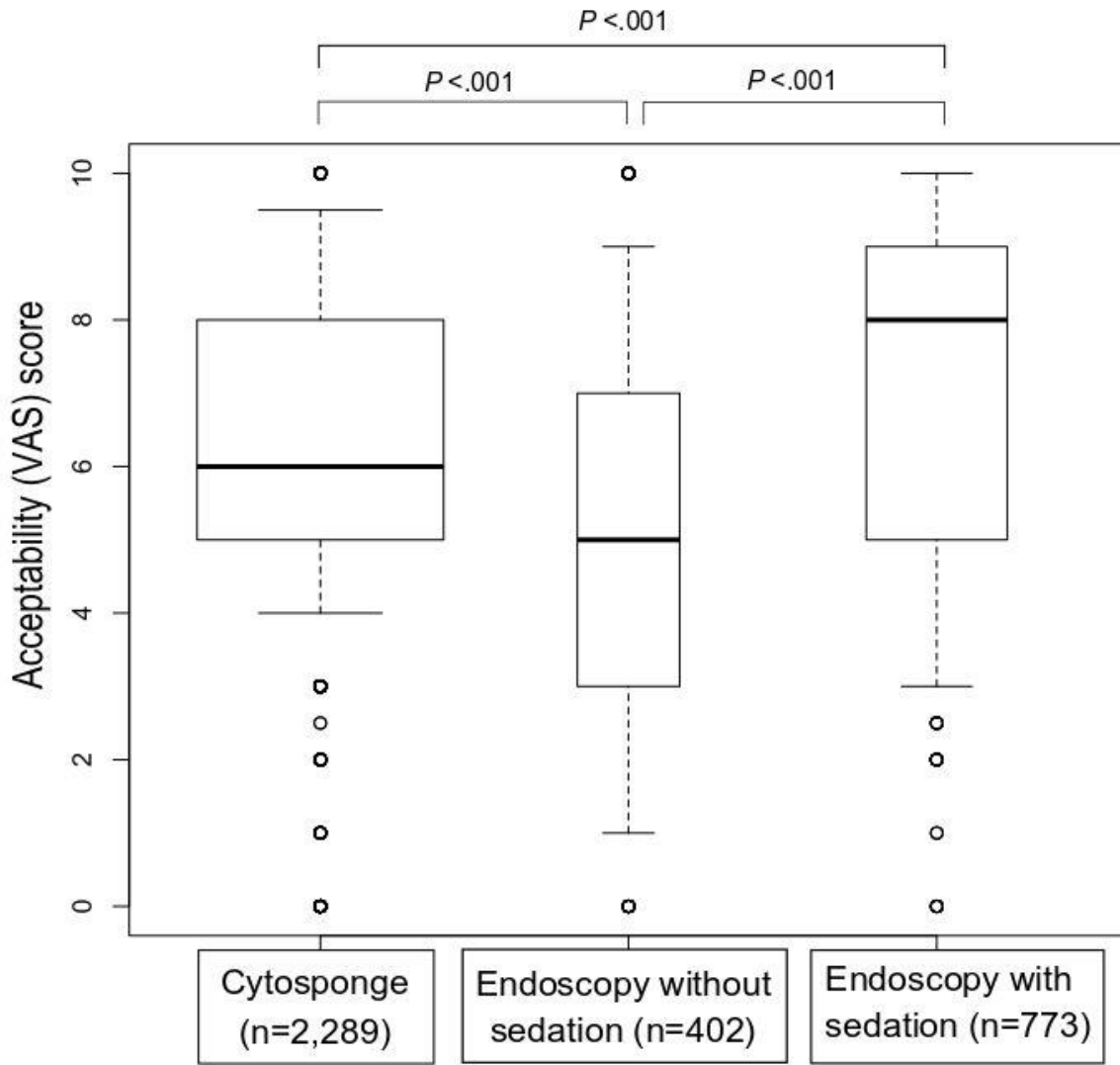
**Seattle protocol**



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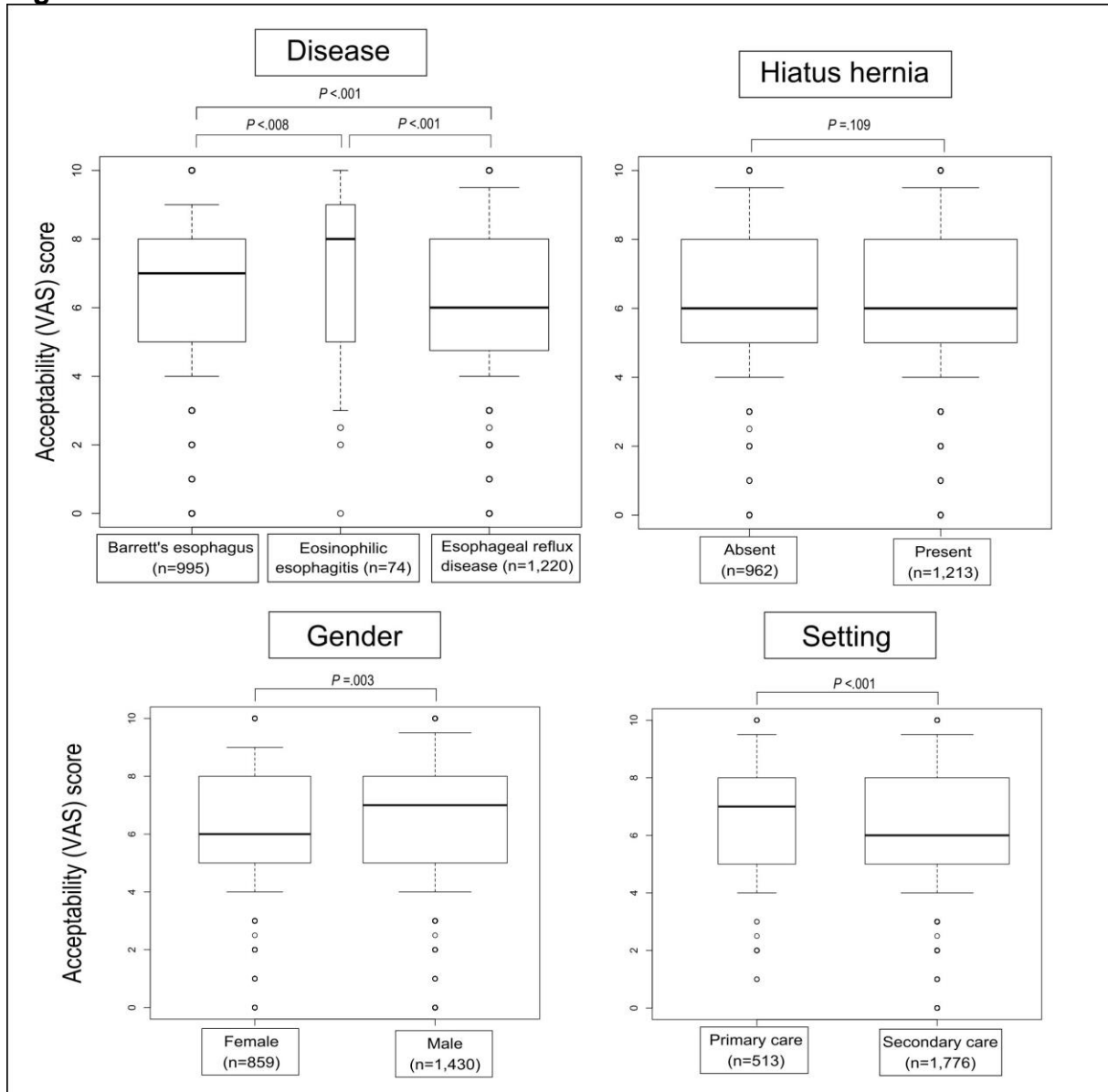
**Figure 2**

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**Figure 4**



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

Factor	OR	95% CI	P value
<b>Gender</b>			
Female (n=932)	1.00	-	-
Male (n=1,486)	1.08	0.61-1.90	<i>P</i> =0.79
<b>Study setting</b>			
Primary care (n=518)	1.00	-	-
Secondary care (n=1,900)	5.13	1.48-17.79	<b><i>P</i>&lt;0.01</b>
<b>Body mass index*</b>			
Normal BMI (n=447)	1.00	-	-
Overweight (n=854)	1.02	0.52-2.03	<i>P</i> =0.94
Obese (n=739)	1.75	0.91-3.36	<i>P</i> =0.09
<b>Indication</b>			
BE + EoE (n=987+102)	1.00	-	-
GERD (n=1,329)	0.63	0.35-1.14	<i>P</i> =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
<b>Total</b>		<b>12</b>

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