THE LANCET
Gastroenterology & Hepatology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: di Pietro M, Modolell I, O'Donovan M, et al. Use of Cytosponge as a triaging tool to upper gastrointestinal endoscopy during the COVID-19 pandemic. *Lancet Gastroenterol Hepatol* 2020; published online July 30. https://doi.org/10.1016/S2468-1253(20)30242-9.

Appendix

Table. Patients' characteristics and results of the Cytosponge™

Case	**Age (yrs)**	**Sex**	**Mellow Dysphagia score**	**Other symptoms**	**Clinical assessment of swallow**	**Cytosponge™ results**	**Clinical Follow up**
1	54	M	2	Weight loss	Incomplete	Glandular atypia Aberrant p53	Endoscopy/CT: Oesophageal Adenocarcinoma
2	66	M	1	Heartburn	Complete	Negative	Endoscopy: HH
3	69	M	2	Weight loss	Incomplete	No Glands No Atypia W/t p53	Endoscopy/CT: Oesophageal Adenocarcinoma
4	80	M	2	Weight loss	Incomplete	Glandular atypia Aberrant p53	Endoscopy/CT: Oesophageal Adenocarcinoma
5	67	F	1	Heartburn	Complete	Negative	Telephone f/up
6	65	M	1	Globus	Complete	TFF3 + No Atypia W/t p53	Endoscopy: LA A oesophagitis
7	55	M	1	Heartburn	Complete	TFF3 + No Atypia W/t p53	Endoscopy: gastritis, irregular Z-line with IM
8	75	M	1	No	Complete	Negative	Telephone f/up
9	55	M	2	Weight loss	Complete	Negative	Endoscopy: irregular Z-line with focal IM Oesophageal ulcer, no dysplasia
10	75	F	0	Chest pain	Complete	Negative	Telephone f/up
11	66	F	0	Chest pain Heartburn	Complete	Negative	Endoscopy: NAD
12	36	F	0	Epigastric pain Heartburn	Complete	Negative	Telephone f/up
13	52	M	1	Heartburn	Complete	TFF3 -	CT NAD

						Sq Atypia W/t p53	Endoscopy: LA A oesophagitis
14	66	F	0	Chest pain Heartburn	Complete	Negative	Telephone f/up
15	61	F	1	No	Complete	Glandular atypia W/t p53	Endoscopy/CT: Type III GOJ signet ring carcinoma
16	41	M	1	Chest pain	Complete	TFF3 + No Atypia W/t p53	Endoscopy: LA B oesophagitis Irregular Z-line with IM
17	41	M	1	Epigastric pain	Incomplete	No Glands No Atypia W/t p53	Endoscopy: NAD
18	64	F	1	Nausea Cough	Complete	Glandular atypia W/t p53	Endoscopy: HH
19	56	F	1	Heartburn	Complete	Negative (Candida observed)	Endoscopy: Oesophageal Candidiasis
20	64	M	1	Weight loss	Complete	Negative	Endoscopy: Irregular Z-line with focal LGD
21	50	F	1	Heartburn	Complete	TFF3 - Sq Atypia W/t p53	Endoscopy: NAD

Mellow dysphagia score: 0 = able to eat normal diet / no dysphagia; 1 = able to swallow some solid foods; 2 = able to swallow only semi solid foods; 3 = able to swallow liquids only; 4 = unable to swallow anything / total dysphagia

BE: Barrett's oesophagus; W/t: wild type; F/up: follow up; HH: hiatus hernia; NAD: no appreciable disease; Sq: squamous.

Figure 1. Representative cases of Cytosponge™ and endoscopy results. **A** and **B**. H&E and p53 staining, respectively, showed atypical cells and p53 over-expression. **C**. In the same patient as in A,B, endoscopy revealed a malignant stricture with short tongue of dysplastic Barrett's oesophagus at the 3 o'clock position. **D** and **E**. H&E and TFF3 staining, respectively, from a case with possible Barrett's oesophagus showed features of intestinal metaplasia with positive TFF3 staining. **F**. H&E from a case with glandular cardia type epithelium indicating the device had reached the stomach and with no other abnormal features.

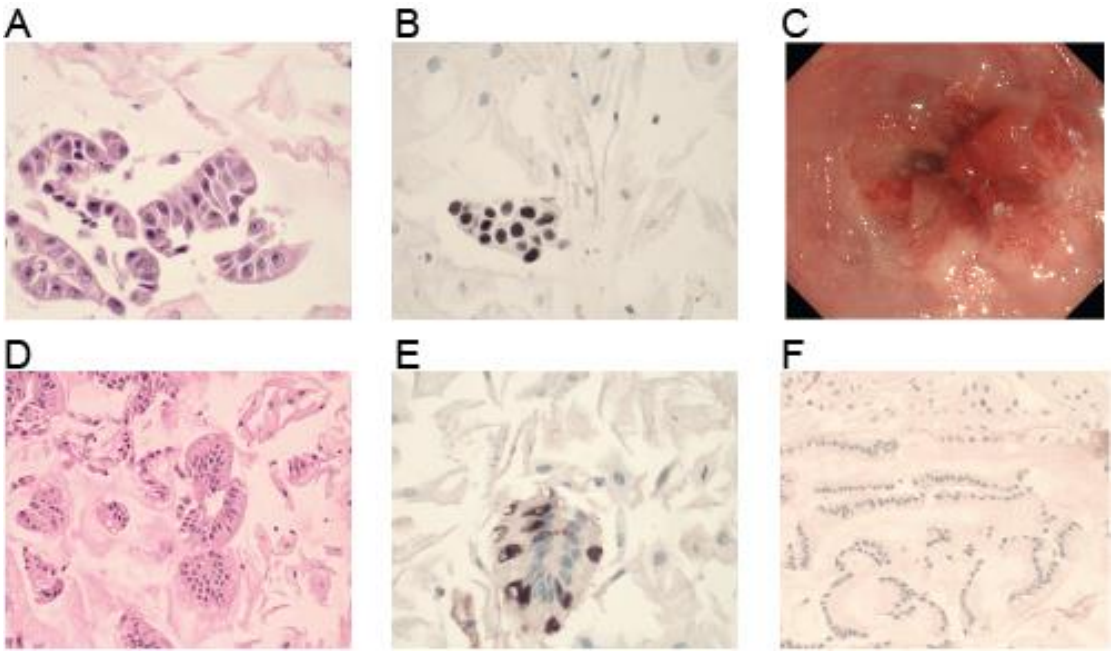
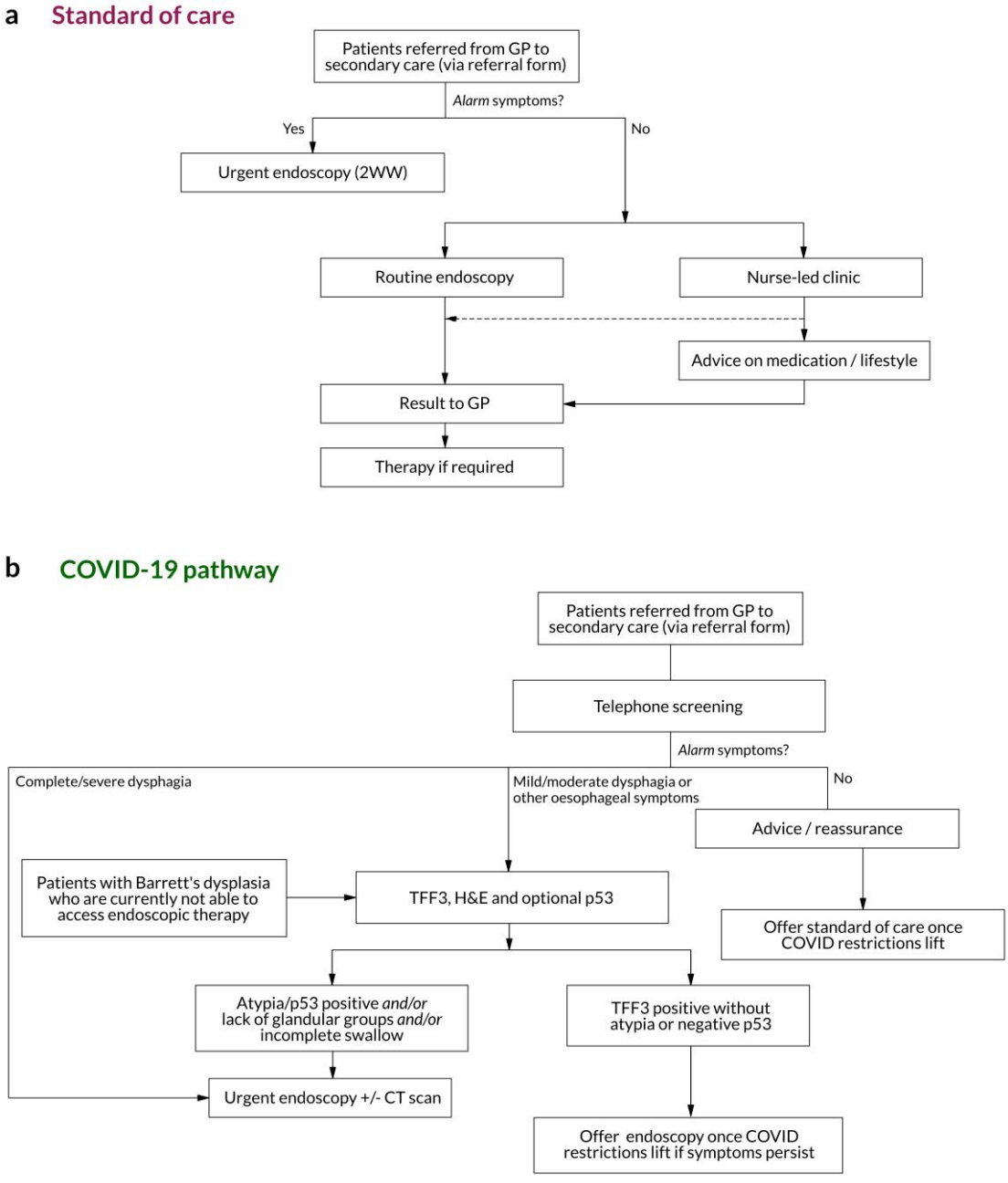


Figure 2. The standard clinical care pathway (a) is compared with an alternative pathway (b), whereby patients can be identified via a telephone clinic for Cytosponge™, which allows triaging them for urgent endoscopy vs conservative management with elective endoscopy when possible.



Additional text

This was a prospective cross-sectional pilot study at a single tertiary care institution (Cambridge University Hospital, NHS Foundation Trust). The study was approved by the Cambridgeshire Research Ethics Committee (HBREC.20202.18). Individuals referred via the 2 week-wait service for urgent endoscopy underwent a screening telephone call to evaluate their suitability for Cytosponge™. The Mellow dysphagia score was use to assess eligibility for Cytosponge™ as follows: 0 = able to eat normal diet / no dysphagia; 1 = able to swallow some solid foods; 2 = able to swallow only semi solid foods; 3 = able to swallow liquids only; 4 = unable to swallow anything / total dysphagia. Patients with severe dysphagia (Mellow score ≥3) or unable to swallow a capsule were recommended for direct access endoscopy. Those with mild or moderate dysphagia (Mellow score 1-2) or other alarm oesophageal or extra-oesophageal symptoms were offered an urgent Cytosponge™ test. 24 hours prior to the appointment a further phone call ensured that patients did not refer symptoms in keeping with COVID-19 and there was no contact with individuals with confirmed or suspected Coronavirus infection. The procedures took place an hour apart to minimise any contact with other patients in the waiting room. All patients provided individual informed consent prior to the procedure. The procedure was performed in the endoscopy recovery room, which had Flakt air handling unit, which ensures at least 15 air changes per hour. The nurse performing the procedure wore level 2 PPE (FFP3 mask, surgical cap, visor, long-sleeved gown and gloves). The nurse was 2 metres apart from the patient during the procedure except for the few seconds required to administer and retrieve the device.

Study procedures

The Cytosponge™ consists of a CE marked 3cm diameter, polyester, medical grade foam sphere on a string, compressed within a vegetarian capsule (Medtronic, Sunnyvale, CA, USA). The capsule is swallowed whilst holding onto the string. After 7 minutes the gelatin capsule

dissolves allowing the foam sphere to expand. Using the string the foam sphere is pulled from the stomach to the oesophagus and mouth thus collecting cells from along its path. The nurse administering the Cytosponge™ (ID, CP) noted whether the device was thought to have reached the stomach based on the tension in the thread on retrieval, this information was used together with symptomatology and Cytosponge™ cytopathology to assess whether a stricture was likely.

Following retrieval of the device samples were placed into Surepath preservative (BD) and processed to an FFPE block in Addenbrooke's Tissue Bank. FFPE 4 µm sections were stained with Haematoxylin and Eosin (H&E), TFF3 immunohistochemical staining (in-house clone) and TP53 (DO-7 Leica Biosystems) as previously described.[1]

The slides were evaluated by an experienced upper GI pathologist (MOD) and consensus agreement from two to three pathologists was used in any case of uncertainty. If no glandular cells were present on the sample, this was deemed a low-confidence result as the device may not have reached the stomach and hence distal pathology could be missed.

Follow up

The Cytosponge™ result was used to triage for further investigations. Patients with high-risk features on the Cytosponge™ (atypia or aberrant p53 staining) or low confidence result (incomplete swallow and/or lack of glandular group) were referred for urgent endoscopy. Patients with high confidence results and no risk features had their procedure deferred until local restrictions on the endoscopy service lifted.

Data collection and processing

Personal information was collected on NHS Trust information systems and stored to AES256 and other NHS information governance standards in compliance with the Data Protection Act (2018). Patients gave consent to use clinical details for research purposes. This was a pilot study to assess whether Cytosponge™ is safe to identify patient with high-risk clinical features in this study cohort. Therefore, there was no pre-specified sample size.

Author contributions

RCF and MdP conceived the project and wrote the manuscript, MdP, ID, CP, MOD, IM and NDP performed the study procedures; CP and MdP collected the data; MdP analysed the data; all authors approved the final text.

References

1 Ross-Innes CS, Chettouh H, Achilleos A, et al. Risk stratification of Barrett's oesophagus using a non-endoscopic sampling method coupled with a biomarker panel: a cohort study. *Lancet Gastroenterol Hepatol* 2017; **2:** 23–31.