Biomarkers for Detection of Dysplastic Barrett's Oesophagus in Retrospective Capsule Sponge Samples (DysplasiaBAR)

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Lay Abstract

Oesophageal cancer is a disease with a very poor survival rate with fewer than 1 in 5 (<20%) patients surviving five years after diagnosis. This is primarily due to late stage diagnosis, resulting in poor response to available treatments.

However, there is a clear opportunity for early diagnosis in the most common subtype of the disease in Western countries (Oesophageal adenocarcinoma) with a pre-malignant condition called Barrett's oesophagus. Patients who are known to have Barrett's have an increased risk of developing cancer, though the risk is still relatively low with 1 in 300 patients with Barrett's diagnosed with a cancer per year.

A non-endoscopic cell collection (i.e. EndoSign) and biomarker test for Barrett's diagnosis and cancer risk has been offered by Cyted Ltd since 2020 as a minimally-invasive alternative to endoscopy for Barrett's surveillance. Cyted is currently developing new molecular biomarkers for detection of Barrett's and dysplasia/early cancer. In order to validate the selected biomarkers a cohort of patients known to be high risk due to previous capsule sponge testing who have had (or will have) a follow-up endoscopic biopsy will be asked to share their endoscopic pathology and prior diagnostic sample for analysis.

Protocol Summary

Full Study Title	Biomarkers for Detection of Dysplastic Barrett's Oesophagus in Retrospective Capsule Sponge Samples
Internal Reference	DysplasiaBAR
Trial Design	Retrospective real-world observational study
Inclusion Criteria	Cases: ● Age ≥ 18 ● Male or Female ● Previously diagnosed with Barrett's oesophagus ● Capsule sponge test performed after 1 June 2021 with a positive p53 and/or positive atypia biomarker result ● Endoscopic biopsy with pathology performed subsequent to capsule sponge Controls: ● Age ≥ 18 ● Male or Female ● Previously diagnosed with Barrett's oesophagus ● Capsule sponge test performed after 1 January 2022 with a negative p53 and negative atypia biomarker result ● Endoscopic biopsy with pathology performed subsequent to capsule sponge with non-dysplastic pathology observed
Exclusion Criteria	○ Age < 18 ○ Barrett's diagnosis unconfirmed ○ Capsule sponge biomarker test missing p53 and atypia results ○ Missing endoscopic pathology results ○ Patient deceased
Number of participants	Up to 150 patients identified with 125 consented to identify: ● 100 p53/atypia+ve capsule sponge cases with follow-up ● 25 p53/atypia-ve capsule sponge cases with a minimum 5 year follow-up without dysplasia
Sample size determination	No sample size calculations performed as this is a real-world evidence gathering study.
Project Duration	36 months

Objectives	***Primary*** Retrospectively validate methylation biomarkers selected for detecting p53 and/or atypia positive capsule sponge cases, and further optimise sensitivity of dysplasia detection biomarkers. ***Secondary*** ● Clinical validation of the performance characteristics (sensitivity, specificity) of the Barrett's oesophagus methylation test on capsule sponge samples with reference to known dysplasia. ● Molecular investigation of endoscopic biopsies for signals relating to dysplasia and future progression risk that would be validated in future capsule sponge testing.
Outcome measures	***Primary*** ● 90% concordance with performance of preliminary dysplasia detection model for accuracy measures. ***Secondary*** ● Rate of endoscopic diagnosis of dysplasia in biomarker positive capsule sponge cases. ● Diagnostic accuracy for Barrett's oesophagus methylation test for detecting cases with any grade of dysplasia. ● Observed markers that related to future progression risk.

Study Procedures	• 100 patients who have undergone a capsule sponge (EndoSign or Cytosponge) procedure after 1 June 2021 and were prioritised for endoscopy due to a p53 and/or atypia positive result will be contacted to invite consent to be retrospectively included in this study. • 25 patients who have undergone a capsule sponge (EndoSign or Cytosponge) procedure after 1 June 2021 and were p53/atypia -ve and have subsequently had a surveillance endoscopy but have had 5 years without dysplasia will be retrospectively consented. • Consenting patients will be asked to allow Cyted to access (1) follow-up endoscopic pathology report, (2) use of capsule sponge sample for molecular analysis, and (3) access to endoscopic biopsy samples held in tissue bank. • Patient demographic information and endoscopic pathology will be collated pseudonymously alongside molecular results. • Existing diagnostic capsule sponge samples will be analysed using the NGS methylation approach by Cyted and the grade of dysplasia (low-grade, high-grade, intramucosal cancer) compared to selected biomarkers. • Endoscopic biopsy samples will be collected from two timepoint: the nearest follow-up post capsule sponge, and a previous endoscopic biopsy 2-5 years prior to the capsule sponge test and analysed using NGS methylation approaches to compare to capsule sponge. • Endoscopic biopsy slides for both timepoints will be digitised for future pathology if required.
Statistical Analysis	• Validation of the accuracy of the previously selected dysplasia biomarkers will be analysed against the gold standard endoscopic pathology. • Sensitivity of the existing Barrett's oesophagus methylation test will be reported against the endoscopy pathology. • General descriptive statistics on the real-world cohort and comparison with the population of Barrett's surveillance patients reported by Cyted. • Differential methylation analyses between the p53/atypia +ve and -ve groups, as well as between the two timepoints.

1 Introduction

1.1 Background & Aims

Oesophageal adenocarcinoma (OAC) has seen a rapid rise in incidence in the U.K.[1] since the 1990's with little improvement in the overall 5-year survival rate that continues to be less than 20%.[2] The pre-malignant tissue called Barrett's oesophagus provides an opportunity for early detection of OAC through monitoring of patients identified to have this condition.[3,4]

Barrett's identification and monitoring presents additional complexities to the determination of risk. Only 0.3% of patients with a non-dysplastic Barrett's diagnosis will progress to an early cancer each year.[5] Current clinical surveillance strategies for patients rely on regular endoscopic biopsies with histopathology, creating a significant burden on patients who are unlikely to develop cancer and specialists spend significant time and resources on each endoscopy. Despite this a Barrett's diagnosis is the best opportunity for early detection of this cancer.

Since 2020 patients at specific NHS sites in Scotland who were diagnosed with Barrett's have been offered an oesophageal capsule sponge test (i.e. Cytosponge™ or EndoSign®) for surveillance of previously non-dysplastic Barrett's.[6] Patients with a positive p53/atypia biomarker test on the capsule sponge are recommended an urgent endoscopic investigation to look for dysplasia or early cancer. Patients with a negative biomarker test are recommended ongoing surveillance by capsule sponge or alternating endoscopy.

The Research & Development team at Cyted Health Ltd has developed novel molecular biomarker targets for diagnosis of Barrett's and dysplasia using a genomic methylation sequencing based approach. This offers a quantitative and objective diagnostic tool that will decrease the burden on histopathology by prioritising patients for investigation.

1.2 Rationale

This study aims to validate the use of methylation targets in patients with Barrett's oesophagus that detect varying degrees of dysplasia or adenocarcinoma in patients previously identified as having biomarkers indicative of high-risk (i.e. p53 IHC and/or atypia) in capsule sponge surveillance. In addition, this study will identify new targets that directly correspond with a patient's endoscopic diagnosis of dysplasia or cancer in the capsule sponge cell samples.

2 Study Objectives & Outcomes

2.1 Primary Objectives

The primary objective for this study is to retrospectively validate methylation biomarkers selected for detecting p53 and/or atypia positive capsule sponge cases with respect to positive endoscopic follow-up for dysplasia, and further optimise sensitivity of dysplasia detection biomarkers.

2.2 Secondary Objectives

The capsule sponge cases will be included in retrospective validation of the performance characteristics (sensitivity, specificity) of the Barrett's oesophagus methylation test for detection of Barrett's in cases with existing dysplasia.

Longitudinal endoscopic biopsies will also address a secondary question around detection of molecular signals relating to dysplasia and future progression risk. This secondary aim will generate preliminary clinical data that would be validated in future capsule sponge testing.

2.3 Primary outcomes/measures

90% concordance with performance of preliminary dysplasia detection model in capsule sponge samples for accuracy measures.

2.4 Secondary outcomes/measures

- Rate of endoscopic diagnosis of dysplasia in biomarker positive capsule sponge cases.
- Diagnostic accuracy for Barrett's oesophagus methylation test for detecting cases with any grade of dysplasia.
- Observed markers that are related to future progression risk.

3 Study Design

This is a retrospective study to identify patients in NHS Scotland with Barrett's oesophagus who received surveillance capsule sponge since June 2021 and were identified as high-risk, resulting in an urgent endoscopy to detect dysplasia or early adenocarcinoma.

- High-risk patients have been indicated by a p53+ve IHC result and/or atypia+ve result on a diagnostic capsule sponge test
- All high-risk patients who have had a follow-up endoscopy with a dysplasia or adenocarcinoma result will be included

An additional subset of low-risk patients who received a capsule sponge since June 2021 and have subsequently undergone a routine endoscopy with non-dysplastic diagnosis as part of Barrett's surveillance will be included as controls.

3.1 Data Collection

The primary clinical site will identify patients meeting the inclusion criteria within their existing database of Barrett's surveillance patients. Selected patients will be contacted by phone or mail to invite their participation in the study. Consent will be taken over the phone or by mail.

Patients who agree to enrol will allow their GI consultant to share their pseudonymized clinical results and tissue with researchers at Cyted. No other procedures or data collection will be required.

3.2 Study Setting

We will recruit up to 150 patients from NHS Scotland sites, with an aim to consent 100 +ve and 25- ve patients in total. This study recruitment period will last 6 months from the start of recruitment.

3.3 Study Duration

The study will run for 36 months. The primary objective will be addressed in the first 12 months as patients are consented. The secondary analyses, including the longitudinal analyses of endoscopic biopsy tissues will be addressed in the following 24 months.

3.4 End of study

The sponsor will inform the REC within 90 days of the 'end of trial' that the study has closed.

Following the submission of the end of trial notification to the REC, the sponsor should ensure that the end of trial report is submitted within 12 months of this notification.

In circumstances of early termination of the trial or temporary halt by the sponsor, the sponsor will notify the REC within 15 days of the decision and a detailed, written explanation for the termination/halt will be given.

4 Study Procedures & Schedule of Assessments

4.1 Patient Recruitment

The primary clinical site will identify patients meeting the inclusion criteria within their existing database of Barrett's surveillance patients. Selected patients will be contacted by phone or mail to invite their participation in the study. Consent will be taken over the phone or by mail.

Patients who agree to enrol will allow their GI consultant to share their pseudonymized clinical results and tissue with researchers at Cyted. No other procedures or data collection will be required.

For this study we aim for a total of 100 cases with biomarker +ve and 25 -ve results with follow-up endoscopic histopathology.

4.1.1 Eligibility

4.1.1.1 Inclusion Criteria

Cases:

- Age ≥ 18
- Male or Female
- Previously diagnosed with Barrett's oesophagus
- Capsule sponge test performed after 1 June 2021 with a positive p53 and/or positive atypia biomarker result
- Endoscopic biopsy with pathology performed subsequent to capsule sponge

Controls:

- Age ≥ 18
- Male or Female
- Previously diagnosed with Barrett's oesophagus
- Capsule sponge test performed after 1 January 2022 with a negative p53 and negative atypia biomarker result
- Endoscopic biopsy with pathology performed subsequent to capsule sponge with non-dysplastic pathology observed

4.1.1.2 Exclusion Criteria

- Age < 18
- Barrett's oesphagus diagnosis unconfirmed
- Capsule sponge biomarker test missing p53 and atypia results
- Missing endoscopic pathology results subsequent to capsule sponge
- Patient deceased

4.2 Subject Withdrawal

4.2.1 General Withdrawal Criteria

Participants are free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment. Patients will be aware that their samples and data will continue to be used in research after withdrawal and be analysed and data retained in the project.

4.2.2 Data Collection and follow-up for withdrawn subjects

Participants are free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment. Patients will be aware that their samples and data will continue to be used and analysed in research after withdrawal and data retained in the project.

Under GDPR, patients consented to new patient information will be made aware of limitations in their rights to withdraw their identifiable data at a later date. For participants who lose the capacity to consent, their data and samples will continue to be retained in the study. No new data or samples will be taken. Their data will continue to be analysed.

4.3 Consent Procedures

All subjects for this study will be provided with a patient information sheet and a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the appropriate ethics committee or institutional review board for the study. The formal consent of a subject, using the approved consent form, must be obtained before a subject's data is shared with investigators. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

The clinical team will retain overall responsibility for obtaining informed consent of participants at their site. They must ensure that any person delegated responsibility to participate in the Informed Consent process is duly authorised, trained and competent to participate according to the ethically-approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki. The taking of consent can be delegated to research nurses (or in appropriate circumstances, practice nurse), who have undertaken informed consent training.

Participants will agree to their personal identifiable data (PID) being held by Cyted Ltd and the hospital research team for the purposes of study. Consent will also be obtained for the use of clinical data and images, and human tissues samples in future research.

A person is assumed to have the mental capacity to decide unless it is shown to be absent. Mental capacity is considered to be lacking if, in a specific circumstance, a person is unable to make a decision for him or herself because of impairment or a disturbance in the functioning of their mind or brain.

A capable person will:

- Understand the purpose and nature of the research.
- Understand what the research involves, its benefits (or lack of benefits), risks and burdens.
- Understand the alternatives to taking part.
- Be able to retain the information long enough to make an effective decision.
- Be capable of making this decision at the time it needs to be made.

It is the responsibility of the Investigator, or a person delegated by the Investigator to obtain written informed consent from each subject prior to participation in the trial, following adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study.

Where a participant can consent but later becomes incapacitated, the management of these participants must also be stipulated in the protocol; in all such cases the original consent given endures the loss of capacity, providing that the trial has not significantly altered (there may be clinical justification under such circumstances for cessation of any further clinical intervention while data collection for follow-up purposes continues).

4.4 Data Collection

4.4.1.1 Patient Data

General demographic information and histopathology results will be recorded by the site in a spreadsheet for each participant. Including:

- Pseudonymised patient identifier
- Capsule sponge sample pot identifier (unique identifier)
- Date of capsule sponge procedure
- Sex
- Date of birth
- Smoking status (if available)
- Date of endoscopic investigation post capsule sponge
- Follow-up histopathology results for dysplastic assessment (e.g. non-dysplastic, low-grade, high-grade, adenocarcinoma)
- Date of endoscopic investigation 2-5 years prior to capsule sponge
- 2-5 years prior histopathology results for dysplastic assessment (e.g. non-dysplastic, low-grade, high-grade, adenocarcinoma)

4.5 Processing and Storage of samples

4.5.1 Tissue blocks (capsule sponge/endoscopic biopsy)

All FFPE blocks will be cut and scrolls used to extract genomic DNA (gDNA) for methylation analysis.

1. Extracted DNA

Any unused DNA will be disposed of within 6 months of trial end.

2. FFPE Blocks

The capsule sponge FFPE blocks and slides of the cells used for diagnosis are kept for a minimum of 30 years as per industry guidelines for diagnostic samples. Blocks and slides are stored locally at the Cyted pathology laboratory at room temperature, long term per industry guidelines. They are held by Cyted and are not part of a tissue bank.

Endoscopic biopsy tissue blocks will be returned to tissue bank after scrolls have been taken.

4.5.1.1 Destruction

The capsule sponge slides and blocks are retained for 30 years per industry standard. For sample destruction a disposal list is generated, and the samples listed are manually checked. Blocks or extracted DNA in Eppendorf tubes are placed into a clinical waste bag which then is placed inside a hard yellow clinical waste "WIVA" bin and the lid is clipped on. These bins are collected and destroyed by an accredited specialist clinical waste service company on a regular basis. The disposal list is completed and then scanned, and the scan is stored indefinitely on the laboratory document control system.

5 Statistics and Data Analysis

5.1 Sample size determination

Sample size is based on a real-world assessment of the biomarker positive cases that have been detected since 2021 using capsule sponge testing and the likely rate (~10%) of positive cases that continue to be detected in current Barrett's surveillance testing.

5.2 Analysis methods

- General descriptive statistics will be used to summarise the patient cohort.
- Proportions of dysplasia will be tested against the observed biomarker results using appropriate categorical tests (i.e. Fisher exact test, Chi-square)
- Overall concordance between the methylation states for predicted dysplasia and observed dysplasia will be calculated
- Sensitivity of the existing Barrett's oesophagus methylation test will be reported against the endoscopy pathology.
- Differential methylation analyses between the p53/atypia +ve and -ve groups, as well as between the two timepoints will be described using fold change between groups.

6 Data protection and confidentiality

All investigators and study site staff will comply with the requirements of GDPR and the UK Data Protection Act 2018 with regards to the collection, storage, processing, and disclosure of personal information and will uphold the Act's core principles. Permission for the transfer, storage, and use of person identifiable data (PID) in the study will be provided by consenting participants.

Participants will consent to Cyted Ltd and hospital research sites receiving, transferring, storing, and using their personal identifiable data for the purposes of this study. All electronic transfers of person-identifiable data will meet industry and NHS-mandated standards including encryption to at least AES 256. Participants will be aware that Cyted Ltd use third party storage services.

Data will be retrievable on databases in a linked anonymised manner in which the participant's identifying information is replaced by an unrelated code. PID will be stored in a logically- separated system with limited access to central team individuals for quality control, audit, analysis, and communications. All paper based PID will be securely handled and maintained in controlled access locations and following local NHS policies and procedures for information security.

Participants will be aware that their PID may be accessed by external regulatory bodies for the purposes of assessing legal compliance and meeting relevant regulatory obligations. They will be made aware that external health care professionals may access data for the purpose of second opinion on clinically relevant findings. PID will be stored for 10 years following completion of the study.

6.1 Ethical and Regulatory Considerations

The Chief Investigator will ensure that the protocol and supporting participant-facing documentation receive HRA Approval, including being presented to a relevant Research Ethics Committee for approval.

Following ethical review, research will only take place once appropriate HRA approvals are in place. The Chief Investigator will also:

- Notify the REC of the end of the study.
- Notify the REC of reasons for premature termination if required.
- Submit to the REC a final report including any publications/abstracts.

6.1.1 Regulatory compliance

The Study will not commence without the necessary regulatory and organisational approvals being in place. The Study will have appropriate ethical approvals and site confirmation of capacity and capability in place for partner sites.

6.1.2 Protocol compliance

Any accidental protocol deviations will be adequately documented. All deviations from the protocol which are found to frequently recur will require immediate action and could potentially be classified as a serious breach.

Notification of serious breaches to GCP and/or the protocol: A "serious breach" is a breach which is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the Study
- the scientific value of the Study

The Sponsor will be notified immediately of any case where the above definition applies during the study conduct phase.

6.2 Amendments

Study amendments will be prepared by the study team for submission according to REC/HRA requirements.

7 Patient Public Involvement

We work with the charities Guts UK and Heartburn UK to develop focus groups with patients to communicate the study protocols, discuss any specific concerns and update/alter protocols and documents accordingly.

8 Dissemination policy

8.1 Discoverability of dataset

Researchers will be able to find information via the ISRCTN Registry (https://www.isrctn.com) once the REC has been approved.

8.2 IP considerations

All IP resulting from the study data will be managed in line with the Non-Interventional Study Agreement.

9 Insurance and indemnity arrangements

Cyted Health Ltd and partner sites will ensure that appropriate insurance and indemnity arrangements are in place for the study.

10 Appendices

1. Patient Study Information Sheet
2. Patient Consent Form

11 Key References

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4. Tan, W. K., di Pietro, M. & Fitzgerald, R. C. Past, present and future of Barrett's oesophagus. *European Journal of Surgical Oncology* **43**, 1148–1160 (2017).

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6. Chien, S. *et al.* Oesophageal cell collection device and biomarker testing to identify high-risk Barrett's patients requiring endoscopic investigation. *British Journal of Surgery* **111**, (2024).