Protocol

R&D Project: Biomarker improvement and development in non-endoscopic samples

Research reference numbers

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Research summary

Full Study Title	Biomarker development to improve diagnostic accuracy for Barrett's oesophagus, oesophageal cancer and other oesophageal diseases in non-endoscopic samples
Internal Reference	R&D Project: Biomarker improvement and development in non-endoscopic samples
Design	Internal process improvement research
Research Samples	General: • Male and Female • Aged 18 and above • Reported through Cyted's pathology platform minimum 3 months prior to access
Planned Sample Size	Molecular analysis: Sample size calculations dependent on specific indication Image-based AI: All available diagnostic images
Project Duration	36 months
Objective	Our primary objectives are: to improve our reporting of patient disease risk through development of new biomarkers; and extend our existing artificial intelligence models to improve accuracy. This will ensure that our pathology service stays relevant and up-to-date with current disease knowledge.

Project Summary

Background: Incidence of the cancer type oesophageal adenocarcinoma (OAC) has increased 6-fold since the 1990s. Clinical guidelines have focussed on minimising endoscopy referrals unless patients have "alarm symptoms" suggestive of cancer, however these symptoms occur when a patient is likely to have an advanced cancer which results in the poor survival rates for this disease.

A major risk factor for this cancer is chronic heartburn caused by reflux, and it's estimated that between 6-10% of patients with reflux may have the precursor lesion called Barrett's oesophagus. Most patients with Barrett's are never diagnosed, in part because a large proportion of the population will experience chronic heartburn but will not have an endoscopy.

A non-endoscopic cell collection (i.e. Cytosponge™) and TFF3 test for Barrett's diagnosis has been offered by Cyted Ltd since 2020 as an non-invasive alternative to endoscopy for screening reflux patients for Barrett's oesophagus and provide an early cancer risk diagnostic.

Improvements: In the past two years Cyted has provided more than 10,000 TFF3 tests with pathology to NHS Trusts around England and Scotland. These tests are provided for two indications currently: to screen high-risk individuals with reflux for Barrett's oesophagus based on a single slide-based stain (i.e. TFF3); and to evaluate patients with Barrett's for indications of cancer (i.e. slide-based P53 stain or cellular atypia). The planned research would enable continued improvements to our non-endoscopic test services, including increasing the accuracy of the available tests for Barrett's oesophagus and early oesophageal adenocarcinoma.

All samples that are submitted to Cyted for diagnosis are anonymised upon receipt, and all subsequent data generated has only that anonymous identifier. We would aim to use these diagnostic samples for the following:

- Improvements to our current biomarker tests through identifying additional biomarkers (i.e. molecular, immunohistochemistry) that can increase the sensitivity and specificity of existing tests for Barrett's and cancer.
- Identifying biomarkers used in other testing that provide additional information for diseases that are currently entirely dependent on specialised pathology information such as EoE.
- Adding existing digital slide pathology images to our current AI pipeline to improve the accuracy of existing models which rely on large amounts of specialised image data for which no public data sources exist.

The current non-endoscopic oesophageal cell collection device with slide-based pathology has improved access to screening for oesophageal conditions and helped decrease endoscopy backlogs. Cyted would like to continue to improve these tests for patients by ensuring that diagnostic tests are not at risk of single point of failure due to batch issues, or supply disruptions (i.e. TFF3 antibody). Additionally, we would like to identify new biomarkers that could be more accurate or require less pathologist time for low-risk cases.

Study Procedures

Researchers at Cyted Ltd will undertake a database search of our internal laboratory information management system based to identify appropriate samples based on the specific condition of interest. These samples are anonymized upon receipt at the laboratory with minimal clinical information such as gender and current age recorded. Search results will provide only the necessary information associated with the anonymous identifier, such as the diagnostic result of a TFF3 test or clinical details such as pre-existing Barrett's oesophagus.

The primary search focus will be on samples where pathologists reported TFF3 positive cases for Barrett's, and P53 positive samples that were reported as possible cancer. Based on internal sample statistics we will select samples from three possible cohorts:

- Case only from Barrett's surveillance. This will provide a cohort for biomarker thresholding based on sample-specific characteristics.
- Case-control from reflux screening. About 10% of these cases will be TFF3 positive, this will provide a validation cohort for sensitivity/specificity comparisons with TFF3.
- Case-control from TFF3 positive for probable cancer. Currently only 5% of our cases have been P53 or atypia positive, indicating higher risk for cancer. These will need to be considered for a split cohort of discovery and validation for any biomarker improvements.

General inclusion criteria

- Patient aged 18 and over
- Male or female
- Final pathology report submitted a minimum of 3 months prior to search

Study Methods

The non-endoscopic sponge sample collection devices used by Cyted (i.e. Cytosponge^{TM}) collects cells the entire length of the oesophagus from the stomach to the mouth. This results in a mix of cells from normal healthy gastric and squamous tissues to Barrett's columnar cells if present. We will be focusing on looking for different molecular and image-based signals for these diverse tissue types.

Molecular methods

A targeted panel of genomic methylation sequencing will be performed on selected samples to assess:

- Methylation signal sufficiency for Barrett's and gastric columnar cells. The targeted panel will have been previously selected based on the methylation signals found in relatively pure tissues (i.e. squamous versus Barrett's). We will use these targets to evaluate sponge samples for the different cell types.
- Assess the sensitivity and specificity of a Barrett's-specific target list in reflux patients who have been diagnosed TFF3+ and TFF3-.
- Evaluate differential methylation signals in samples that were diagnosed as high cancer risk (P53/atypia+) versus low cancer risk to identify additional biomarkers.
- Develop a methylation range for normal, healthy squamous tissues that can be used to compare to Oesophageal squamous cell cancer (OSCC) in future.

Image-based AI methods

We have developed in-house AI on H&E pathology images that is intended to help us stratify samples for evaluation by pathologists. This is possible through an exhaustive process of manual image annotation, followed by retraining the existing AI models. In general AI works best when provided large amounts of unbiased data. We would use the existing digital slide diagnostic archive to generate new models to:

- Better characterize the cellular mixture through population-based estimates of cell type ratios in the different cohort types (i.e. reflux screening, Barrett's surveillance).
- Increase the overall accuracy of our internal quality control (QC) models by including larger numbers of training slides.
- Tune the molecular signals processing based on the cell type ratios identified.
- Begin to characterize other meaningful information including immune cell infiltration or changes to squamous cells that we currently lack the necessary data to visualize.

Objectives and Primary measures

This study's primary objective is to improve our available biomarker tests to ensure continued high-quality diagnostic information is provided to patients. This will be achieved by identifying additional biomarkers that can be used in addition to, or as an alternative to the current TFF3/P53/atypia slide-based stains.

Primary Objective	Improve our diagnostic services to ensure we continue to provide good diagnostic information by identifying new biomarkers for Barrett's and early cancer specifically and improving our image-based AI models.
Primary Measures	Sensitivity and specificity of any new biomarker compared to the current TFF3 and P53/atypia tests. Increased accuracy of image-based AI models.
Secondary Measures	General improvements to our internal laboratory processes for sample testing and reporting to pathologists. Identification of biomarkers for other oesophageal conditions including EoE or OSCC.