Joint Research Management Office (JRMO) Research Protocol for Research Studies

Full Title	Plasma biomarkers in stratifying patients referred via the lower gastro-intestinal (LGI) suspected cancer two-week wait (2WW) pathway
Short Title	New bio**M**arkers t**O** stra**TI**fy c**O**lorectal ca**N**cer referrals (**MOTION study**)
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IRAS Number ***Integrated Research Application System***	321809
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1. Table of Contents

2. Glossary

2WW	Two-week wait pathway for cancer diagnosis
PG	Progastrin
hPG80	Progastrin
CRC	Colorectal Cancer
STT	Straight to Test
GP	General Practitioner
FIT	Faecal immunochemical test
GI	Gastrointestinal
CRF	Case Report Forms
TE	Transposable Elements
NBRC	National Bowel Research Centre

3. Signature page

CI Agreement

The study, as detailed within this Research Protocol, will be conducted in accordance with the principles of Good Clinical Practice (GCP), the UK Policy Framework for Health and Social Care Research, and the Declaration of Helsinki and any other applicable regulations. I agree to take responsibility for the statistical analysis and oversight of this study.

CI Name: **Dr M A Thaha**

CI Signature:

Date: 04 JUNE 2025

4. Summary and synopsis

Short title	New bio**M**arkers t**O** stra**TI**fy c**O**lorectal ca**N**cer referrals (**MOTION study**)
Methodology	Prospective, observational, cohort study design with post assay result patient questionnaire
Objectives/aims	1. To determine whether plasma progastrin (PG) levels accurately predict cancer diagnosis in lower GI 2-week wait (2WW) referral patients undergoing standard investigations. The study will also aim to distinguish the absence of cancer by virtue of actual levels of plasma PG. 2. To determine the sensitivity and specificity of plasma PG levels in patients with colorectal cancer (CRC), and benign colonic polyps when investigated via a 2WW referral pathway. 3. To identify and evaluate transposable elements (TEs) in the plasma of patients referred via the lower GI suspected cancer 2WW pathway as potential predictive biomarkers for colorectal cancer diagnosis. 4. To undertake a post-study, patient questionnaire survey exploring the patient preferred characteristics and choices of 2WW CRC diagnostic test(s). Patient experience of a blood-based test will be explored in detail and compared against other commonly used but more invasive, diagnostic tests using a designed Likert scale questionnaire.
Number of participants	Progastrin assay - a total of 582 patients will be enrolled in the study based on an anticipated 18 weeks recruitment period or until the required recruitment number is achieved. TE assay - 100 (25 proven colorectal cancers and 75 with no cancers) patients from the overall cohort of 582 will be randomly selected for TEs identification and proof of principle validation .
Inclusion and exclusion criteria	**Inclusion criteria** • Adult, lower GI 2WW and or Straight to Test (STT) referral patients with suspected lower GI cancer. • Male and Female patients aged ≥18 years. • 2WW referral patients with no history of inflammatory bowel disease. • Performance status (ECOG 0-2; and 3 pending clinical assessment of fitness). • Patients with capacity to consent to the study. **Exclusion criteria** • Any patients referred outside of the 2WW and or STT referral pathways with suspected Lower GI

	cancer or those referred as an emergency with or suspected CRC. • Age < 18 years. • Patients not fit for standard investigations (e.g. not fit for gastroscopy, colonoscopy or CT colonography) in the 2WW pathway. • Patients with no capacity to consent or who declined consent for participation. • Patients with untreated solid organ cancers. • Patients with known inflammatory bowel disease. • Patients with documented familial type CRC.
Statistical methodology and analysis (if applicable)	The primary outcome will be the accuracy of plasma PG levels to predict a cancer diagnosis in a 2WW referral cohort. Positive and negative predictive values along with the relevant Receiver Operator Characteristic (ROC) curve will be calculated and plotted for patients with confirmed cancer and benign polyp diagnoses. Secondary outcome of the study is identification and proof of principle validation of novel TEs as potential biomarkers for colorectal cancer diagnosis. Tertiary outcome is to explore patient preferences and choices regarding diagnostic test(s) for CRC using an electronic patient questionnaire survey.
Study duration	One year

5. Introduction

At present, the standard practice for investigating bowel symptoms includes a stool based, faecal immunochemical test (FIT) before referral to the 2-week wait (2WW) pathway. The 2WW pathway was introduced into the health system to make a diagnosis of cancer rapidly and set timeline guidelines for the initiation of treatment. The 2WW pathway for colorectal cancer (CRC) in the United Kingdom is designed to ensure that patients referred with suspected cancer symptoms receive rapid assessment and diagnosis. The process typically begins when a general practitioner (GP) or another healthcare professional identifies red flag symptoms in a patient that raise suspicion of CRC. Common symptoms include unexplained weight loss, persistent abdominal pain, changes in bowel habits, and rectal bleeding. The GP then refers the patient to a specialist for further assessment through the rapid assessment 2WW pathway.

The 2WW referral and assessment pathway is bound by strict time guidelines provided by the Department of Health and Social Care. Upon receiving the referral, the specialist service has to complete the first assessment appointment within 2 weeks. All diagnostic tests initiated following the first assessment has to be completed within 6 weeks and patients who were diagnosed with CRC have to commence the first treatment within 31 days of a cancer diagnosis.

5.1. Background

The two-week wait pathway (2WW) is a bespoke pathway aimed at rapidly processing patients with suspected cancer symptoms to enable early diagnosis. There are plans to reduce the timelines stipulated within the current 2WW system which are symptoms based. The criteria for eligibility for a 2WW pathway referral were devised from a set of red flag symptoms circulated to all GPs. Recently the introduction of FIT testing has helped a lot in the process of stratification of patients presenting with red flag symptoms. These new implemented guidelines produce immense pressure on an already over-stretched system and may not be particularly accurate at diagnosing cancers in these sites. A systematic review from 2009 found that when using this symptom profile only 9.5% of patients will have a lower GI cancer[1].

It would be clinically beneficial to have a diagnostic screening test apart of FIT to predict which symptomatic patients have cancers and which don't. Such a screening test would greatly relieve the target pressure on the health system allowing the most urgent cases to be prioritized and reviewed rapidly. A reliable screening test would also create thresholds for specialist investigations more effectively reducing the overall costs without exposing patients to potential harms from invasive investigations and or a missed cancer.

Progastrin (PG), a circulating blood biomarker, has emerged as a promising tool in the field of cancer detection and monitoring. PG a precursor of gastrin, has been implicated in the pathogenesis of various gastrointestinal diseases, including CRC.

This is because the Wnt / b cadherin pathway coding for b cadherin is abnormal in many GI cancers[2]. A further target of this pathway is the gene encoding PG[3]. PG is converted to gastrin in normal healthy people (and is therefore undetectable) but can be detected in the plasma of many patients with both early and advanced GI malignancy[4]. A study of colorectal polyps and cancers demonstrated an elevated level of PG in cancer subjects compared with benign polyps[5]. Moreover, cancers going on to develop liver metastases produce higher PG levels in plasma[6]. The sensitivity varies between different cancer types but in upper GI and CRCs is 80-95%[7]. This is comparable to "missed" cancers at colonoscopy (3.5%)[8].

Beyond progastrin, transposable elements (TEs), mobile genetic elements that can disrupt gene function, have emerged as promising candidates for cancer biomarker development. Recent research has identified TEs associated with various cancers, including CRC[9]. However, the role of TEs in predicting CRC diagnosis in 2WW referral cohorts remains underexplored. Liquid biopsy approaches, such as the analysis of circulating free nucleic acids (cfDNA and cfRNA) in conjunction with machine learning algorithms, have shown promise in cancer detection[10]. These methods offer a less invasive alternative to traditional biopsies. Notably, recent studies, including those conducted by the Dr. Madapura's lab, have demonstrated that transposable element-derived RNA signatures can be superior predictors of diseases like pre-eclampsia and cancers[11]. While these findings are encouraging, further research is required to establish the sensitivity and specificity of liquid biopsy approaches for CRC diagnosis. Independent cohort studies encompassing diverse ethnic populations are essential to ensure the generalisability of these methods. Moreover, multi-omics approaches, integrating data from various molecular levels, could enhance the diagnostic accuracy of liquid biopsy assays.

Given that over 90% of patients referred to the lower GI cancer pathway will not have cancer, a screening test or tests to rule out cancers would reduce the burden on NHS targets and could be cost effective at excluding unnecessary tests. The most useful group to be tested and excluded from the pathway in the future would be those patients where the index of suspicion is low. These include those without a family history of CRC, ages between 18 and 55 (not yet eligible for CRC screening programme), no weight loss, good performance status of 0-1 and no history of inflammatory bowel disease.

Global CRC screening programs aim for a 65% participation rate, a target met in many European nations and reaching 74% in the USA. However, CRC screening lags behind breast and cervical cancer screening, which typically exceed 70% participation. A significant barrier to CRC screening is the low attendance rate for colonoscopy or CT colonography following abnormal faecal immunochemical tests (FIT) or faecal occult blood tests (FOBT), ranging from 10% to 30%. This low attendance is often attributed to negative experiences, word-of-mouth discussion, and sociocultural factors.

As emerging technologies like liquid biopsy, utilising biomarkers such as progastrin or transposable elements, offer potential for non-invasive CRC detection, understanding patient preferences for different screening modalities becomes crucial. These tests

vary in invasiveness, frequency, and risk profiles, and their effectiveness in altering risk profiles of CRC differs.

To optimise patient adherence and early detection, it is essential to identify the factors that influence patient choice among these diverse diagnostic options.

5.2. Rationale

For a patient, being placed on the 2WW pathway is stressful and uncertain. Timely and simple diagnostic tools to improve the accuracy and speed of diagnosis will also reduce patients' anxiety. The research hopes to determine whether the addition of a simple blood test to the pathway has the potential to improve diagnosis and patient experience. It also has the potential to deliver cost savings for the overburdened healthcare systems.

Several patients with direct experience of the 2WW pathway developed the research question and design together with the Chief and Co-Investigators. They provided this rationale, co-authored the research proposal, co-designed the study, will serve as members of the project management board, and will fully engage in dissemination and public engagement activities.

5.3. Risks / benefits

The risks of the study involve a single blood draw incorporated in the normal pathway visit. The risks are therefore low: patients may feel slightly faint and will be directed to remain seated for an appropriate amount of time to allow for monitoring.

There are no direct benefits to the study participants involved in this study. However, the knowledge gained from this study may have long term clinical impact on introducing a new blood test in screening suspected lower GI malignancy referrals in the population.

Potential benefits of the study are, principally:

- more effective treatment due to more accurate and timely diagnosis.
- alleviation of patients' stress through more accurate and timely diagnosis.
- shorter time to treatment or discharge.
- avoidance of colonoscopy-related complications by reducing the overall number of colonoscopies used in a 2WW pathway.
- cost savings through avoidance of unnecessary tests.
- non-invasive nature of blood-based biomarkers

6. Study objectives

6.1. Primary objective

The primary objective of this study is to test whether plasma PG levels accurately predict the diagnosis of CRC and or polyps in a group of 2WW patients undergoing the standard investigations. The study will also aim to distinguish the absence of cancer by virtue of actual levels of plasma PG. To determine the sensitivity and specificity of plasma PG levels in patients with colorectal cancer (CRC), and benign colonic polyps when investigated via a 2WW referral pathway

6.2. Secondary objective

Identify and evaluate transposable elements (TEs) in the plasma of patients referred via the lower GI suspected cancer 2WW pathway as potential predictive biomarkers for colorectal cancer diagnosis.

6.3. Tertiary objective

To undertake a post-study, patient questionnaire survey exploring the patient preferred characteristics and choices of 2WW CRC diagnostic test(s). Patient experience of a blood-based test will be explored in detail and compared against other commonly used but more invasive, diagnostic tests using a designed Likert scale questionnaire.

6.4. Primary endpoint

The accuracy of plasma PG levels to predict a cancer diagnosis in a 2WW referral cohort. Positive and negative predictive values along with the relevant Receiver Operator Characteristic (ROC) curve will be calculated and plotted for patients with confirmed cancer and benign polyp diagnoses.

6.5. Secondary endpoint

Identification and proof of principle validation of novel TEs as potential biomarkers for colorectal cancer diagnosis.

6.6. Tertiary endpoint

Exploration patient preferences and choices regarding diagnostic test(s) for CRC using an electronic patient questionnaire survey.

7. Study population

All adult patients who are referred by the GPs to the Barts Health NHS trust under the 2WW referral pathway and the STT pathway would be potentially eligible. We are planning to recruit patients initially only from the Royal London Hospital site and there is potential to expand recruitment to other hospital sites of Barts Health NHS Trust, if necessary. The cancer performance team maintains a running list of all 2WW and STT referrals and these lists will be perused by the study team on a weekly basis to screen for eligible participants. Patients who meet the eligibility criteria (see section 7.1) on initial screening will be sent the study information (PIS) and consent form by post. Further verbal information will be provided during the first clinical appointment (virtual appointment or face to face appointment in the hospital) usually within 1 week of referral and consent to approach sought by the clinical team. The research team will follow-up by contacting patients on their preferred phone number, will answer all relevant questions and will help complete the informed consent form to participate in the study. The study team will re-confirm the informed consent during the subject's normal hospital visit for their 2WW diagnostic test (e.g., colonoscopy, CT Colon) and will draw 20 mL of blood sample for the study. This will occur either in the outpatient department, in the endoscopy department or in the radiology department depending on the reason for and site of their visit. Participants will not be asked to make any additional visits for the study purposes.

Patients will complete the standard diagnostic tests as deemed clinically necessary in the 2WW pathway and decided by the treating clinicians and no change in the standard pathway will be required for participating in the study. The study team will obtain the relevant clinical information and contact details by perusing the patient's electronic health records at the end of the pathway and this information will include the outcomes of the 2WW investigations, particularly the final diagnosis of a cancer, polyp or normal.

Patients will be asked to fill in a post-test questionnaire exploring patient preferences and other quality attributes of potential screening tests after completing their 2WW or STT investigations. The study team will collect this feedback through a e-questionnaire distributed via SurveyMonkey™. The link for the survey will be emailed and texted to the patient's phone number. After 2 weeks a telephone or email remainder will be sent by the research team to those patients who have not completed the questionnaire. A second contact will be made after another 2-5 days, if the questionnaire has still not been returned

7.1. Inclusion criteria

The following inclusion criteria will be used for the study.

- Adult, lower GI 2WW and or Straight to Test (STT) referral patients with suspected lower GI cancer.
- Male and Female patients aged ≥18 years.
- 2WW referral patients with no history of inflammatory bowel disease.
- Performance status (ECOG 0-2; and 3 pending clinical assessment of fitness).
- Patients with capacity to consent to the study.

7.2. Exclusion criteria

The study will use the following exclusion criteria.

- Any patients referred outside of the 2WW and or STT referral pathways with suspected Lower GI cancer or those referred as an emergency with or suspected CRC.
- Age < 18 years.
- Patients not fit for standard investigations (e.g. not fit for gastroscopy, colonoscopy or CT colonography) in the 2WW pathway.
- Patients with no capacity to consent or who declined consent for participation.
- Patients with untreated solid organ cancers.
- Patients with known inflammatory bowel disease.
- Patients with documented familial type CRC.

Trust approved language line services will be used to aid oral communications for those patients with a lesser command of English and who would need additional support.

8. Study design

This will be a prospective, observational, cohort study and will involve all eligible patients who are referred by the GPs to the Barts Health NHS Trust with the suspicion of CRC under the 2WW pathway or STT pathway. This is a single center study where the patients will be recruited from Barts Health NHS Trust, initially only from the Royal London Hospital site. The study will also involve a questionnaire survey at the end of the clinical pathway and will explore patients preferences about screening tests for CRC using a post-test e-questionnaire distributed via SurveyMonkey™.

8.1 Progastrin assay

For the PG assay part of the study, approximately 582 consecutive 2WW/STT patients who meet the inclusion criteria will be consented to participate and will have a 'time 0' 20ml of venous blood sample collected. The patients will then undergo the standard clinical investigations that are deemed appropriate by the treating clinicians according to existing hospital protocols. The final (62-day or later for breaches) diagnosis of a colorectal cancer or not will be correlated to the plasma PG levels to determine the predictive usefulness of PG test as a potential biomarker in lower GI, 2WW/STT pathways. The collected blood samples will be processed according to the sample processing protocols.

8.2 Transposable elements assay

In order to fulfil the secondary outcome of the study, we will randomly select a total of 100 samples (25 samples from patients with confirmed CRC and 75 non-cancer

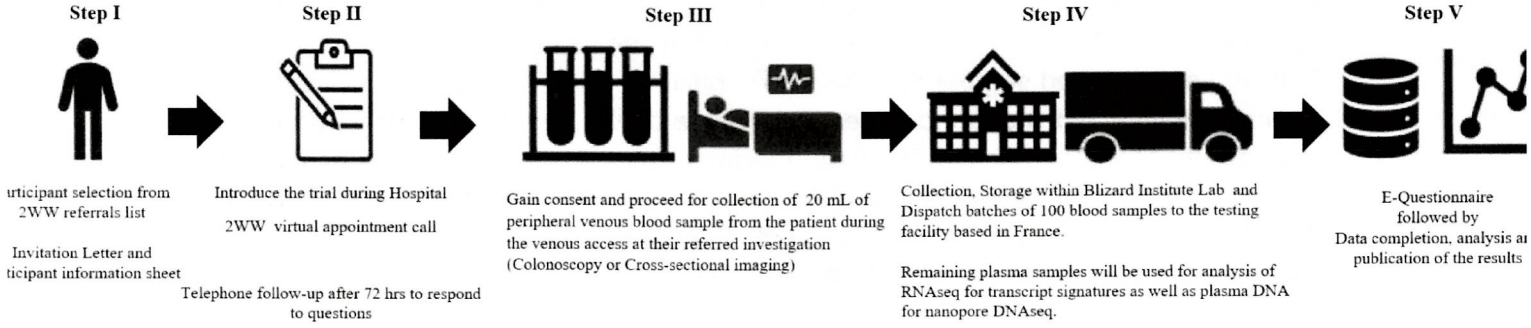
(normal) samples) out of the original cohort of 582 participants. The plasma from this sub-population of 100 patients will be subjected to RNAseq analysis looking for transcript signatures to identify potential transposable elements. Also Plasma DNA will be used for nanopore DNAseq (provides sequence variants and epigenetic modifications of DNA). The TEs identified in this project to predict CRC will be also validated by testing it on publicly available additional cfRNA cohorts (http://111.198.139.65/cfomics/source) for CRC prediction.

The study will be a single centre, pilot, cohort observational study performed within the setting of the NHS. Indemnity will be provided by Queen Mary University of London. However, there is no harm that can be foreseen from enrolment into this study as the only intervention is a blood draw done during routine intravenous cannulation for the standard clinical care. There is no IP opportunity from the PG assay study. The PG blood test is a commercially available assay. However, the use of TE as a biomarker for colorectal is a relatively new field and the investigators will explore the possibilities for IP. No samples from this study will be stored by Progastrin Manufacturing, Biodena Care, Cap Sigma-Zac Euromedicine II, 1682 Rue de la Vasière, 34790 Grabels, France. Any remaining plasma samples after the PG assay will be returned to the study team and will be stored at QMUL National Bowel Research Centre (NBRC), Blizard Institute and used by the research team at QMUL, for future research studies. Any future studies will have Ethics Committee permission. Patient clinical data will be securely destroyed at the end of the research studies. All staff undertaking future studies will abide by the Data Protection Act 1998 with any medical information relating to you being kept confidential.

9. Study procedures.

National Bowel Research Centre
leading diagnostic & therapeutic innovation for digestive diseases
Queen Mary University of London
Barts Health NHS Trust
MOTION study

A pilot feasibility study to determine the predictive value of plasma biomarkers in stratifying patients referred via the lower GI suspected cancer (two-week wait) pathway



Study procedures are displayed below in the flow-chart.

Enrolment to the study is anticipated to take four to five months or until 582 participants are recruited with subsequent analysis available when a histological diagnosis of cancer is confirmed or refuted after appropriate investigation on the pathway. Analysis will then take a further two to three months. Patients will be numbered according to a random number generation to maintain confidentiality and anonymity. Samples will be labelled only with the appropriate trial number. Informed consent will be recorded in written form and a copy uploaded to the patients electronic health records (EHR).

Personal data pertaining to this study will be accessed for six months after the study closes and data kept for independent review for 5 years. This will be under the personal supervision of the principal investigator and kept in a secure lockable office and on Trust password-protected computers. Participants and investigators will not receive payment for this study. The study is funded by a research grant from LAPResearch UK (registered charity number: 1130523), a charitable trust.

Patients will be screened for suitability and recruited to the study within the 2WW clinic. Upon the receipt of informed consent, patients' medical history and a blood sample will be taken. This includes initials, date of birth, sex, BMI, ethnicity, height, and weight, to be recorded on the CRF. The blood sample will be transported to and processed to extract the plasma and stored in the laboratories at the Blizard Institute based at the NBRC, 1st Floor, Abernethy Building. Batches of 100 fully anonymised plasma samples will be sent to Progastrin Manufacturing, Biodena Care, Cap Sigma-Zac Euromedicine II, 1682 Rue de la Vasière, 34790 Grabels, France for the plasma progastrin assay. The remaining unused plasma will be stored in Blizard Institute, Whitechapel, London lab and will be used for RNAseq analysis for transcript signatures to identify potential transposable elements and nanopore DNAseq to provide sequence variants and epigenetic modifications of DNA. The latter assay's will be conducted at the Blizard Institute laboratories.

The plasma progastrin levels will be sent back to the study team at Queen Mary University of London based at the NBRC, 1st Floor, Abernethy Building by the Progastrin Manufacturing team, Biodena Care Cap Sigma-Zac Euromedicine II, 1682 Rue de la Vasière 34790 Grabels, France and will be added to the study database. Participants are free to withdraw from the study at any time and will be provided with reassurance that this will not affect their care in any way.

Before enrolment into the study, participants will be fully informed of the nature of the study and all relevant aspects of study procedures. It is the investigators' responsibility to explain the nature of the study, its purpose and associated procedures, the expected duration and the potential benefits and risks of participation to each subject prior to their entry into the study (i.e. before examinations and procedures associated with selection for the study are performed).

Each prospective participant will be provided with the Patient Information Sheet and Informed Consent Forms that are to be compiled as one integrated document. Each subject will be given a minimum of 24 – 48 hours to study the provided information and to ask questions and will be informed about their right to withdraw from the study at any time without any disadvantage and without having to provide reasons for their decision. Once the essential study information has been provided and the Investigator

is assured that each individual patient understands the implications of participating in the study, the eligible patients who pass the screening evaluation will be recruited. Consent Forms shall be signed and dated by the appropriate parties. A notation that written informed consent has been obtained will be made on the and a record made in their clinical notes. Completed Consent Forms will be retained by the Investigator, a copy entered into the clinical notes, and a copy will be provided by the Investigator to the patient. The Informed Consent Form is included as part of the submission to the Ethics Committee.

The informed consent form and any other written information provided to subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol which necessitates a change to the content of the subject information and/or the written informed consent form. The investigators will inform the subject of changes in a timely manner and will ask the subject to confirm to continue their participation in the study by their signature on the revised informed consent form. Any revisions to the written informed consent form, and written information will receive main REC approval in advance of use. Patients will be free to withdraw at any time without affecting their healthcare or legal rights.

Participants will be informed that their data are held on file, that these data may be viewed by the Sponsor and by external auditors on behalf of either the sponsor or regulatory agencies. They will similarly be informed that this data and a report of the study will be submitted to the Sponsor and may also be submitted to government agencies and perhaps for publication, but that they will only be identified in such reports by their study identification number. The investigators undertake to hold all personal information in confidence and in compliance with the Data Protection Act 2018 and Caldicott committee.

A study specific database will be created on the REDCap (QMUL approved) and will be used for all study purposes. The access to the study REDCap database will be strictly limited to the core study team (CI, PI and RF) and will be password protected.

Trial termination is guided by: 1) complete data collection (primary & secondary outcomes) for minimum of 582 participants, ensuring sufficient data to address research questions; 2) mandated follow-up periods for specific trials; 3) post-recruitment data analysis for robust findings; 4) regulatory/ethical committee requirements; and 5) resource constraints for efficient allocation.

The end of the study will represent recruitment complete analysis of both plasma progastrin and TE of 582 plasma samples.

10. Statistical considerations

Our study aims to evaluate the potential utility of PG levels as a novel biomarker to predict CRC diagnosis in patients referred via the 2WW and STT suspected cancer

referral pathways. The study population will be drawn from the Barts Health NHS Trust catchment area. Barts Health NHS Trust have five different sites of which three sites (Royal London Hospital, Whipps Cross Hospital and Newham University Hospital) receive 2WW/STT referrals. Approximately, some 7,030 patients are referred via the 2WW/STT from primary care to the secondary care with the suspicion of lower gastrointestinal (GI) cancers every year.

The current feasibility study aims to recruit the study participants from the Royal London Hospital site of Barts Health NHS Trust, and can be expanded to other sites within the trust if recruitment becomes challenging within the stipulated recruitment timeframe of the study. The department at the Royal London Hospital receives around 50 new two week wait referrals every week (200 new patients per month), giving approximately 2,400 (34% of the total BHT referrals) potential patients to be screened for the study. Roughly, 9.5% of 2WW referral patients will have a colorectal cancer confirmed as per our local data. Anticipating a minimum recruitment period of 18 weeks, we will recruit 582 eligible patients from the screened 2WW/STT population at the Royal London Hospital. This sample size, was adjusted for a 30% screening failure rate and is expected to yield approximately 55 CRC cases and 527 control subjects from the study population to test the hypothesis of PG as a potential biomarker for CRC diagnosis in 2WW/STT patients.

The secondary outcome of identification and proof of principle validation of circulating TE as a potential novel biomarker for CRC is exploratory and hence does not require formal power calculation. Instead, for this exploratory study we will select a sub-set of 100 (25 proven CRC and 75 non-cancer (normal) patient samples from the 582 study cohort for RNAseq and DNAseq analyses.

10.1. Sample size

Approximate Annual Referral Volume for RLH ≈ 2400 patients per year
Recruitment Period:18 weeks (4-5 months)
Eligible Patients:
18 weeks * (2400 patients/year / 52 weeks) ≈ 831 eligible patients
Screen Failure:
30% screen failure rate
Potentially 70% of eligible patients enter the study: 831 * 0.7 ≈ 582 patients

Case and Control Allocation:
Based on cancer office for LGI approximately 9.5% of lower GI patients have a cancer hence:

Cases: 9.5% of 582 ≈ 55 patients with LGI cancer
Controls: (100-9.5)% of 582 ≈ 527 patients without LGI cancer - assuming no other cancers are detected

Sensitivity and Specificity of PG:
The sensitivity of the PG test is estimated to be between 70% and 87% at a cutoff of 0.2 pM. The specificity of the test at this cutoff is 97.5%.

Statistical Power and Significance Level:
To ensure adequate statistical power, we aim to achieve a power of 0.8 and a significance level of 0.05.

Receiver Operating Characteristic (ROC) Curve Analysis:
A power calculation was performed using the pROC package in R. The following parameters were used:
Number of cases (GI cancer): 55
Number of controls (no cancer): 527
Area under the curve (AUC): 0.6118569
Power = 0.8

To address the secondary outcome of the study a random number of 100 samples out of 582 number of samples (25 LGI cancer proven samples and 75 non-cancer (normal) samples) will be selected and tested for TEs in order to identify and validate novel biomarkers as potential tests for colorectal cancer diagnosis

10.2. Method of analysis

Descriptive Analysis:
Basic demographic characteristics of the study population will be summarized using descriptive statistics such as mean, median, standard deviation for continuous variables, and frequencies or percentages for categorical variables.

Primary Outcome Analysis:
Positive and negative predictive values (PPV and NPV) of plasma PG levels in predicting a diagnosis of cancer will be calculated.
Receiver Operating Characteristic (ROC) curve analysis will be conducted to assess the discriminatory ability of plasma PG levels for cancer diagnosis. This includes calculating the area under the ROC curve (AUC).
Sensitivity, specificity, accuracy, and likelihood ratios will also be calculated to evaluate the diagnostic performance of plasma PG levels.

Secondary Outcome Analysis:

Subgroup Analysis:
Subgroup analysis may be performed to assess whether the predictive ability of plasma PG levels varies across different patient subgroups (e.g., age, sex, referral pathway).

Statistical Testing:

Depending on the nature of the data and research questions, appropriate statistical tests may be conducted. For example, t-tests or Mann-Whitney U tests for continuous variables and chi-square tests or Fisher's exact tests for categorical variables.

Survival Analysis (if applicable):
If assessing time-to-event outcomes (e.g., time until cancer diagnosis), survival analysis techniques such as Kaplan-Meier curves and Cox proportional hazards models may be utilized.

Multivariate Analysis (if applicable):
If relevant covariates are identified, multivariate regression analysis may be conducted to assess the independent association between plasma PG levels and cancer diagnosis while adjusting for potential confounders.

Sensitivity Analysis:
Sensitivity analysis may be performed to assess the robustness of the results to variations in assumptions or analytical methods.

Qualitative Analysis (if applicable):
If qualitative data are collected (e.g., patient experiences or perspectives), thematic analysis or other appropriate qualitative methods may be employed to extract themes and insights.

Ethical Considerations:

Throughout the analysis, ethical considerations regarding patient confidentiality, consent, and privacy will be strictly adhered to, ensuring compliance with ethical guidelines and regulations.

11. Ethics

REC and HRA approval will be sought from
Recruitment will take place in 2WW and STT (virtual and face-to-face) outpatient clinics at Barts Health NHS Trust. Informed consent will be sought through a conversation, printed information, and a consent form.
Confidentiality
The study will be conducted in accordance with the principles of the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).
The Protocol, questionnaires, Consent Forms and Patient Information Sheet will be submitted to a National Research Ethics Service (NRES) approved Research Ethics Committee before patients are recruited and patients are enrolled. The Investigators will receive all the documentation needed for submitting the present Protocol to the Ethics Committee. No study activities will be initiated until the written approval of that Committee is received. A copy of the respective approval letters will be transmitted to

the Sponsor before starting the study. If approval is suspended or terminated by the Ethics Committee, the Investigator will notify the Sponsor immediately.
It is the responsibility of the Chief Investigator to report study progress to the Ethics Committee as required or at intervals not greater than one year. The Chief Investigator will be responsible for reporting any serious adverse events to the Ethics Committee as soon as possible and in any event within 72 hours.

11.1. Annual Safety Reporting

All day-to-day management of the study will be the responsibility of Dr MA Thaha (CI). The co-investigators, especially the Research Fellow (Dr V Butnari) will also be involved in the day-to-day operations of the study. The study team will meet every two months to discuss, the study progress and the findings. These meetings will review and ensure all the study materials are up-to-date, and all the team members are fully trained to undertake the research activities (GCP trained). Formal minutes will be kept for these meetings. Due to the low-risk nature of the study, there will be no data monitoring and ethics committee (DMEC). The trial may be prematurely discontinued due to a lack of recruitment, and the CI will advise on whether to continue or discontinue the study and make a recommendation to the sponsor. There will be no formal stopping rules based on the intervention outcomes.

12. Public Involvement

Patients worked with the Chief Investigator, Dr MA Thaha, to design and develop the research as well as this application and will be involved in the delivery and dissemination. PPIE members are equal partners in the research.
Dr Eldrid Herrington, a 2WW patient, is a named contact on this application and is joined by four members of the After Bowel Cancer PPIE group who represent some of the diverse patient populations in east London in terms of ethnicity, gender, SEC, age, religion, sexuality, as well as nationalities and languages spoken. These PPIE representatives will join the project events.

We believe that the level and quality of the partnership between PPIE members and clinical research staff is a model of involvement and engagement and hope to co-author publications and deliver public talks about this strength, as a means of encouraging an ecosystem of best practice in QMUL, Barts Health, and beyond.

13. Data handling and record keeping

13.1. Data management

The use and control of all data will comply at all times with the requirements of the Data Protection Act. All data management procedures will be detailed and referred to as the Data Management Plan.

In summary; the data collected during the trial will be a combination of clinical data – 2WW referral symptoms, age, gender, history of cancers and other GI diseases (e.g. inflammatory bowel disease), investigation-related including any diagnosis of cancers), progastrin assay results and final outcome from the 2WW referral episode. Most of the data will be recorded straight on CRF's and routine data that can be verified with the medical notes. All data will be entered and checked by a designated researcher. The database will be designed and built by the research team on REDCap data system and will be uploaded onto a dedicated folder on the secure virtualised environment at the Barts Cancer Centre (BCC). The BCC environment requires dual factor authentication to access the portal and the folders where the data are stored are only accessible to the appropriate members of the study team.
The Case Report Forms (CRFs) will be solely for the use of researchers to facilitate data collection as per protocol before it is transcribed onto the REDCap database. The CRF documents contain the Participant ID, study name, site number/ID, researcher name, (where appropriate), CRF document name and other relevant information on each page and space to record appropriate signatures.
In accordance with its current Records Retention Schedule, research data will be retained by the sponsor, Queen Mary University of London for 5 years after the research has ended. Access to stored records is strictly controlled.

For trials involving Barts Health NHS Trust patients, undertaken by Trust staff, or sponsored by BH or QMUL, the approved repository for long-term storage of local records is the Trust Modern Records Centre.

13.2. Source Data

The study team will work closely with staff at the participating site(s) to ensure accurate (complete, valid and reliable) collection of data. Appropriate data validation will be incorporated into the eCRF. The site PI is responsible for ensuring that all data queries are resolved. Ongoing data entry, validation at adherence to the trial protocol at sites will be closely monitored by the study team and any concerns will be raised to the CI. A data management plan will cover all aspects of managing the data such as, the CRF design, the data management system for data collected, data entry, data handling processes including data checking, query management and cleaning, data transfer, quality control procedures, processes for interim and final data extractions, the procedures for freezing and locking the databases.

13.3. Confidentiality

The CRFs will be anonymised and study data will not be linked to individual patients other than by those authorised to have access and not in any publication of results.
The CRF to be used for the study consists of pages that contain within the header or footer the protocol number, subject initials, subject number and other relevant information. It is composed of an introductory section for the selection and inclusion of participants in the study and a section for the assessment period. Contained within

the treatment period section are the forms for registration of possible adverse events and for any suspension of the study.

All CRFs will be completed using a ball-point pen with black ink. All unused CRFs for dropouts must be retained and will be made available to the Sponsor for inspection at any site visit.

All requested information must be entered on the CRFs. If an item is not available or is not applicable, this fact should be indicated; there should be no blank spaces.

Corrections should be made by striking through the incorrect entry with a single line and by entering the correct information adjacent to it. The correction must be initialled and dated by an Investigator or a designated qualified individual. Each set of completed CRFs must be reviewed, signed and dated by an Investigator.

The use and control of all data will comply at all times with the requirements of the Data Protection Act 2018. The PI of the study will register with the local Data Protection Officer. All data management procedures will be detailed and referred to as the Data Management Plan.

The database will be designed and built by the research team on QMUL approved REDCap data system within the Barts Cancer Centre virtual environment. All study data will be uploaded onto a dedicated folder on the secure virtualised environment at the Barts Cancer Centre (BCC) which holds the REDCap system. Appropriate CRFs will be prepared for the collection of the data requested by the protocol. All response variables will be entered into the database by the data management personnel.

13.4. Record retention and archiving

In accordance with the UK Policy Framework for Health and Social Care Research, study participant records will be kept for 5 years after the study has been completed. All records will be stored within the Barts Health Trust Corporate Records Centre. When 5 years after the conclusion of the study has elapsed, all of the study documentation will be shredded and destroyed.

14. Laboratories

A 20 mL blood sample will be obtained from each participant. This sample will be processed in the Blizard Institute laboratory based at the NBRC, 1st Floor, Abernethy Building, 2 Newark Street, Whitechapel, London E1 2AT. Plasma will be extracted from the whole blood sample and stored at -80^{0}C. Batches of 100 fully anonymised (identified only by study numbers) plasma samples (4 mL/patient) will be sent to the France for PG assay in their laboratory (Progastrin Manufacturing, Biodena Care, Cap Sigma-Zac Euromedicine II, 1682 Rue de la Vasière, 34790 Grabels, France). The remaining plasma will be stored in Blizard Institute, Whitechapel, London lab and used for RNAseq analysis for transcript signatures to identify potential transposable elements. Also Plasma DNA will be used for nanopore DNAseq (provides sequence variants and epigenetic modifications of DNA) within Blizard Institute (Madapura's) Lab. Plasma samples will be stored in 1.5 ml tubes as aliquots. 0.2-0.5 ml of plasma samples will be used for the isolation of RNA and 0.2-0.5 ml for DNA followed by high-

throughput sequencing. Sequencing-ready library preparations will be performed within Madapura's Lab.

After the completion of the current study, the residual plasma will be stored in the Blizard Institute laboratories at the NBRC, 1st Floor, Abernethy Building. The present study will analyse and store only blood plasma, with the remaining blood components being discarded. As blood plasma is not being classified as human tissue, this study does not require a tissue bank to store all the samples. As such, the study is exempt from Human Tissue Authority (HTA) approval and licensing requirements.

14.1. Central and local laboratories

- **Processing of whole blood sample to extract and store the plasma** - Blizard Institute laboratory based at the NBRC, 1st Floor, Abernethy Building, 2 Newark Street, Whitechapel, London E1 2AT.
- **Progastrin Assay** – Progastrin Manufacturing, Biodena Care, Cap Sigma-Zac Euromedicine II, 1682 Rue de la Vasière, 34790 Grabels, France
- **TE Assay** – Blizard Institute (Madapura's) Lab.

14.2. Sample preparation and collection

Whole blood (20 mL) obtained from the participant will be brought to the NBRC laboratory where it will be cold centrifuged at $+4^0$ C at 1,300 g using a refrigerated centrifuge and plasma extracted. All samples will be anonymised and given a unique study number. The anonymised plasma sample will be stored in -80^0 C refrigerator. Batches of 100 samples containing 4 ml of plasma from each participant will be sent by registered courier services to the laboratory based in France (Progastrin Manufacturing, Biodena Care, Cap Sigma-Zac Euromedicine II, 1682 Rue de la Vasière, 34790 Grabels, France) for progastrin assay. After PG assay, any remaining plasma samples will be returned by registered courier to the study team at QMUL for storage at the Blizard laboratories based at the NBRC, 1st Floor, Abernethy Building. The plasma samples stored in Blizard Institute, Whitechapel, London lab will be used for RNAseq analysis for transcript signatures to identify potential transposable elements. Also Plasma DNA will be used for nanopore DNAseq (provides sequence variants and epigenetic modifications of DNA) within Madapura's Lab. Plasma samples will be stored in 1.5 ml tubes as aliquots. 0.2-0.5 ml of plasma samples will be used for the isolation of RNA and 0.2-0.5 ml for DNA followed by high-throughput sequencing. Sequencing-ready library preparations will be performed within Madapura's Lab

14.3. Laboratory procedures

20 mL samples of blood from each patient will be transferred to the laboratory within seven hours of phlebotomy and cold-centrifuged and stored at -80°C within Blizard Institute Laboratory based at the NBRC, 1st Floor, Abernethy Building. Batches of 100 samples will be sent to France for plasma PG assay. Samples will be anonymised to trial numbers to protect patient confidentiality and anonymity. hPG80 (PG) is measured in plasma sample by ELISA testing technique. The remaining plasma unused in PG assay will be stored in Blizard Institute, Whitechapel, London lab and used for analysis of RNAseq for transcript signatures to identify potential transposable elements. Also Plasma DNA will be used for nanopore DNAseq (provides sequence variants and epigenetic modifications of DNA) within Madapura's Lab. Plasma samples will be stored in 1.5 ml tubes as aliquots. 0.2-0.5 ml of plasma samples will be used for the isolation of RNA and 0.2-0.5 ml for DNA followed by high-throughput sequencing. Sequencing-ready library preparations will be performed within Madapura's Lab at the Blizard Institute.

14.4. Sample storage and transfer

Labelled vials with plasma will be stored at -20°C to -80°C in a designated ultra-low temperature freezer.

15. Safety reporting

Patients will have a single blood draw only, which constitutes one isolated intervention. Therefore, due to the nature and design of this study, safety reporting of adverse events is unlikely to occur.

16. Monitoring and auditing

The Sponsor or delegate retains the right to audit any study, study site or central facility. In addition, any part of the study may be audited by the funders where applicable.

17. Study committees

Due to the low-risk nature of the study, there will be no data monitoring and ethics committee (DMEC). A study / programme management group will convene on a two-monthly basis. Its members will be the CI, the PI, the co-investigator(s), the study manager, the statistician, and any PPIE representatives.

18. Finance and funding

This study (Worktribe project ID: 10042913) is funded by a research grant from LAPResearch UK (registered charity number 1130523): £186,581.57. All governance issues and appropriate study behaviour according to good clinical practice will be the responsibility of the principal investigator. Funding has been granted based on appropriate ethical approval. The views expressed in this publication are those of the author(s) and not necessarily those of the LAPResearch UK, or the company (Progastrin Manufacturing, Biodena Care, Cap Sigma-Zac Euromedicine II, 1682 Rue de la Vasière, 34790 Grabels, France) who runs the PG assay.

19. Insurance and indemnity

The insurance that Queen Mary has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

20. Dissemination of research findings

Internal findings will be presented within Barts Health and Queen Mary in appropriate fora, for example in MDTs and research symposiums – whether those findings are positive or negative/null. Positive research findings will drive discussions about any extended study or alteration to the 2WW pathway (new interventions / procedures).

We will present our findings to the funder / sponsor, for dissemination in Plain English in their literature / website.

The following publication of findings in academic journals are envisaged at this stage:

- Analysis and evaluation of PG tests as an indicator for CRC in the context of 2WW clinics (to include null or negative findings, if relevant);
- Identification and validation of new TE;
- PPIE best practice in respect of patient pathways, with this study as an exemplar.

The sponsors and funding will be acknowledged in all published and publicly available findings and presentations.

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