STUDY PROTOCOL

The combined use of serum Neurotensin, and IL-8 as screening markers for colorectal adenomas and cancer. A prospective study.

Georgios Sgourakis MD, PhD, FACS, FRCS(Ed).

Consultant General Surgeon.

Barrow in Furness 16/02/2019

Table of Contents

STUDY SYNOPSIS

1.1 PRINCIPAL INVESTIGATOR/COLLABORATORS

#	Name	Affiliation	Role / Responsibility
1	**Mr. Georgios Sgourakis MD, PhD, FACS.**	**Consultant General Surgeon** Department of Surgery, University Hospitals of Morecambe Bay.	**Principal investigator/ Lead applicant:** Study design-concept/Trial co-statistician, Patient recruitment, endoscopies/blood samples and collection of data. Critical revision.
2	**Dr Rachael Rigby.**	**Senior Lecturer** Department: Biomedical and Life Sciences, University of Lancaster	**Collaborator:** Supervision of student, data analysis, reporting of data. Critical revision.
3	**Research Student (one year Master)**		**Named research staff:** ELISA measurements. Data Management (database).
4	**Miss Panna Patel, FRCS (Ed).**	**Consultant Colorectal Surgeon** Department of Surgery, University Hospitals of Morecambe Bay.	**Collaborator:** Patient recruitment, endoscopies/blood samples and collection of data.

5	**Two Research Nurses**	**0.25 FTE at both FGH and LTHTR sites.**	Obtain patient consent. Blood sample collection and transfer.
6	**Mr. Arnab Bhowmick, MB ChB, FRCS (Ed).**	**Consultant Colorectal Surgeon** Department of General Surgery, Royal Preston Hospital.	**Collaborator:** Patient recruitment, endoscopies, and collection of data. Critical revision

CV: See attachment

1.2 TITLE OF THE STUDY

The combined use of serum Neurotensin and IL-8 as screening markers for colorectal cancer and adenomas. A prospective study.

1.3 KEY WORDS

IL-8; Neurotensin; Colon cancer; Rectal cancer; Colorectal cancer Screening; Sensitivity and Specificity.

1.4 STUDY TYPE

Case control study

1.5 SAMPLE SIZE

To be analysed: **n =** 500

1.6 TRIAL DURATION

First patient in to last patient out: 12 months

Duration of the whole trial: 15 months

1.7 PARTICIPATING CENTERS

1. Department of Surgery, Furness General Hospital. University Hospitals of Morecambe Bay, UK,
2. Department of Surgery, Royal Preston Hospital, Lancashire Teaching Hospital Trust, UK,
3. Biomedical and Life Sciences, Lancaster University, UK.

1.8 THE NEED FOR A TRIAL

Colorectal cancer mortality can be lowered by both early diagnosis and cancer prevention.[1, 2] The objective of screening is to detect cancer at an early, curable stage. Individuals at average risk, those older than 50 years of age, with a negative family history and negative history of adenoma, colorectal cancer, or inflammatory bowel disease are among the highest yield group.

Recent literature proposes that after an initial negative colonoscopy (which is the current standard of screening not only in average risk but also in increased risk patients, should start at age 50 or earlier age with frequency depending on risk factors: e.g. personal/family history of polyps/cancer, inflammatory bowel disease, known genetic syndrome and prior colonoscopy findings), follow-up with rescreening after the age of 60 with annual guaiac fecal occult blood testing, annual fecal immunochemical testing, or computed tomographic colonography every five years provides approximately the same benefit in life-years with fewer complications and at a lower cost.[3] There is a lack of consensus concerning the use of CT colonography as a primary screening tool.

The number of CRC cases from colorectal cancer screening is still rather low. Sensitivity of guaiac tests for the detection of CRC/adenomas is 21.8%-46.3%.(4) Colonoscopy is the favoured screening method for individuals at average risk despite lower patient compliance with this modality.

A meta-analysis of 14 randomized controlled trials documented that although endoscopic surveillance was superior to stool testing in detecting advanced neoplasms, its benefit was eclipsed by a lower participation rate.[4]

Colonoscopy is the favored screening method for individuals at average risk despite lower patient compliance with this modality. Whilst colonoscopy is an outstanding tool for diagnosing potentially curable bowel cancer, too many patients are currently having an unnecessary procedure as the pick-up rate for bowel cancer is only 4%. Nevertheless, NCCN guideline panelists agree that any form of screening is better than none.[5]

An actual connection between the proposed screening markers and the detection of colorectal cancer and adenomas would have a significant impact on: a) target population compliance, b) re-definition of the cancer pathway for candidate patients by changing the indications for colonoscopy and c) the cost-effectiveness, vital for patients' survival and the sustainability of the NHS, by means of earlier cancer/adenoma diagnosis.

2. INTRODUCTION

Cancer research offers a better insight into regulatory mechanisms involved in cell proliferation in colorectal cancer, and might eventually guide us to new possibilities in terms of novel biomarkers that would facilitate early diagnosis.

Neurotensin (NT) mediates its effects by binding to three neurotensin receptors NTSR-1, -2, and -3: the first two are G-protein coupled receptors, whereas the third belongs to the sortilin receptor superfamily. [6-8]

NT via NTSR-1 initiates a multiplicity of intracellular signaling pathways in colon cancer cells and in non-transformed NCM460 colonocytes, as well as the epidermal growth factor receptor (EGFR) and downstream mitogen-activated protein kinases (MAPK) via the metalloproteinase-dependent cleavage of pro- TGF alpha.[9] NT-mediated activation of MAPK signaling and the downstream transcription factor activator protein 1 (AP1) also occurs rapidly in human colorectal cell lines.[10] Moreover, transactivation of the IL-8 promoter is triggered by NT-mediated AP1 activation [10, 11] while NT also stimulates Ras pathway and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) -dependent secretion of IL-8.[6, 12]

Intense transcription of the NTSR-1 gene via TCF/Lef- and nuclear β-catenin-dependent pathways can explain NTSR-1 overexpression in colon cancer.[13] Higher NT levels are also obtained from colon cancers compared to normal colon tissues, and in vivo evidence establishes that NT/NTSR-1 signaling is fundamental for tumor growth.[14]

Gui et al [15] found that NTSR1 expression was considerably increased in both adenomas and adenocarcinomas compared with histologically normal epithelium and in adenocarcinomas compared with adenomas.

Given that colorectal cancer is the second leading cause of cancer-related death in Europe and the third in the USA, a new non-invasive screening modality with the potential for high population compliance at a lower cost than the current screening system and with the capacity for early detection of

colorectal cancer and precursor adenomatous polyps is of paramount importance.[16, 17]

This pilot study aims to investigate whether specific pattern of serum neurotensin/NTSR1/IL-8 values can detect patients with adenomas or colorectal cancer and determine the feasibility of these agents being used as a screening tool.

2.1 EVIDENCE

2.1.1 Serum neurotensin/IL-8 values in colorectal cancer

Serum neurotensin/IL-8 values in the diagnosis of colorectal cancer have been reported for first time in literature by our own group.[18, 19]

This pilot study aimed to determine the feasibility of serum neurotensin/IL-8 values being used as a screening tool for colorectal cancer. Fifty-six patients and 15 healthy controls were assigned to seven groups according to their disease entity based on theater records and histology report. Blood samples for neurotensin and IL-8 were measured using an enzyme-linked immunosorbent assay.

There were no differences in the clinical and biochemical parameters of patients and controls. Group ($p= 0.003$) and age ($p= 0.059$: marginally significant) were independent predictors of neurotensin plasma values. Neurotensin ($p= 0.004$) and IL-8 ($p= 0.029$) differed between healthy and colorectal cancer patients. Neurotensin values differentiated the control group from all remaining groups.

The value of plasma neurotensin ≤54.47 pg/ml at enrollment selected by ROC curves demonstrated a sensitivity of 77%, specificity of 90%, and an estimate of area under ROC curve (accuracy) of 85% in predicting colorectal cancer.

At enrollment, the value of plasma IL-8 ≥8.83 pg/ml had a sensitivity of 85%, specificity 80%, and an estimate of area under ROC curve (accuracy) of 81% in predicting colorectal cancer.

Our results depicted that IL-8 should be used complementary to neurotensin due to its lower specificity. None of the colorectal cancer patients displayed a combination of high neurotensin and low IL-8 values (beyond cutoffs). Unpredictably, 3 out of 13 patients in the colorectal cancer group had neurotensin values above the cut-off value defined by the ROC curve. Notwithstanding, the IL-8 values of the same patients were high and well above the cut-off value, as our analysis anticipated for this group. Two other colorectal cancer group patients unexpectedly had low IL-8 values, but they also had low neurotensin values, as our analysis expected for this group. None of the colorectal cancer group patients displayed a combination of high neurotensin and low IL-8 values (which would be in contrast with our analysis).

Finally, one healthy control had a low neurotensin value as well as a low IL-8 value.

This pilot study[18] implicated that a blood neurotensin/IL-8 system may be used as a screening tool for colorectal cancer.

The addition of NTSR1 was of paramount importance. Neurotensin and its receptors stimulate cancer cell growth[6], mainly through binding to NTSR1, which is overexpressed in many cancer cell lines.[20]

According to our model individuals with low Neurotensin values (according to the defined cutoff) should be suspected for bearing colorectal cancer needing further assessment with colonoscopy. In this patient population having low

Neurotensin plasma values there is no need to search for IL-8 values. As a second step, individuals with high Neurotensin values and high IL-8 values should also undergo colonoscopy. Accordingly, IL-8 values should be considered as complementary to Neurotensin measurement and not as a standalone screening marker.

The diagnostic performance of a blood neurotensin/IL-8 system for colorectal cancer proved to be satisfactory. The American Cancer Society, for instance, conducted a systematic review of the literature assessing PSA performance. [21] A pooled analysis showed the estimated sensitivity of a PSA cutoff of 4.0 ng/mL as 21% in detecting prostate cancer and 51% in detecting high-grade cancers (Gleason score ≥8) while the corresponding sensitivity using a cutoff of 3.0 g/mL was higher: 32% and 68%, respectively.

We consider the fact that not a single colorectal cancer patient in our study displayed a combination of high neurotensin and low IL-8 plasma values to be the most important finding.

Martin et al [22] conducted a thorough analysis to explore the functional significance of SORT1/NTSR1 heterodimers in HT29 cells. SORT1 shifted the neurotensin dose–response curve to the right whilst increasing maximal stimulation for ERK phosphorylation two-fold. The authors proposed that interaction between NTS1 and SORT1 produced a new means of cell activation that could prove essential for the growth effect of neurotensin. Whether this mechanism can justify the high neurotensin values (above the cut-off value) in three of our colorectal cancer group patients is uncertain.

Low IL-8 values of two colorectal cancer patients were possibly due to the use of statins. The mechanism of anti-inflammatory activity of statins became

clear after finding that simvastatin or fluvastatin blocks the synthesis of IL-6 and IL-8 in isolated blood monocytes that had been stimulated by C-reactive protein or lipopolysaccharide.[23, 24] Sakoda et al. [25] showed that the dose-dependent effect of simvastatin diminished IL-1a-induced IL-6 and IL-8 production in human epithelial cells.

2.1.2 Involvement of neurotensin in cancer growth

In an excellent review, Carraway et al[6] describe the pathways that seem to play a role in NT-induced cancer cell growth. NT interacts with NT receptor (NTSR) to activate a set of protein kinase C (PKC) isotypes that may specify which paths the signal undertakes. This additionally relies on the level of expression for the effectors implicated. Path 1: cell surface shedding of EGF-like ligands via the action of metalloprotease(s) activates EGFR, which employs a variety of kinase cascades made known to support nuclear transcription and cell cycle control. The effect of PKC could be both causal (via path 1) and permissive (via path 2). Path 2: direct activation of Raf-1 by PKC can also take place independent of EGFR. Path 3: by stimulating store-operated Ca2+-channels, NT triggers phospholipase A2 (PLA2) and diacylglyserol (DAG) lipase to release arachidonic acid, which is changed to lipoxygenase (LOX) products. This additionally enhances the activation of growth-regulatory PKCs. Path 4: NT can raise cAMP levels in cancer cells, which is considered to involve a superactivation of adenylyl cyclase (AC). This result necessitates a background of Gs activity, seemingly from another G protein-coupled receptor (GPCR) coupled to Gs. Commonly, the result is growth inhibition via PKA-mediated effects on Raf-1. However, in cases where B-Raf levels are high, growth augmentation occurs via effects on

mitogen-activated protein (Mek).

Even though NTS1 expression might amplify gradually through the inflammation-carcinogenesis-loss of barrier and enhancement sequence, Carraway et al[6] hypothesize that NT signaling is augmented mainly by upregulation of its signaling partners [EGFR, ERK (extracellular signal-regulated kinase), Akt (Protein kinase B) and LOX] and possibly by an increased release of NT in response to fat. The sequence of signaling partner expression undeniably differs for various cancers.

Muller et al [26] propose that neurotensin stimulates phosphorylation of ERK and Akt is mediated by diverse pathways: in HCT116 cells, neurotensin-induced DNA synthesis and phosphorylation of ERK is mediated mainly by PKC separately to EGFR transactivation. In addition, neurotensin provokes phosphorylation of Akt via activation of metalloproteinases, succeeding detachment of ligands that activate the EGFR.

Maoret et al [14] found that NT stimulated growth of five different human cancer lines (SW480, SW620, HT29, HCT116, C1.19A) shown to express NTSR1, whereas it had no effect on caco-2 cells shown to lack NTSR1. In SW480 cells, NT enhanced proliferation by two- to three-fold and increased colony formation by approximately 50%.

Gui et al [15] showed that NTSR1 expression enhancement is gradual through neoplastic transformation of colonic epithelium. In the normal colon, NTSR1 expression was principally confined to basal cells in the deep portion of the crypts, a compartment known to include the self-renewing epithelial stem cell population.[27] Quite the opposite, NTSR1 expression was weak to absent in superficial epithelial cells, suggesting that NTSR1 may be a factor in

supporting the growth of self-renewing cells, and that downregulation of the receptor may be necessary during terminal differentiation in the normal colonic epithelium. In addition, NTSR1 expression was generally higher in the deep advancing margins and invasion sites than in the rest of the tumor. Souaze et al [28] claim that the upregulation of NTSR1 in colon cancer is mainly assigned to activation of the Wnt/APC signaling pathway. Modifications in Wnt/APC take place frequently at the early premalignant stage, and are seen as critical to the progression of colon cancer. Activation of the NTSR1 gene was achieved by agents that triggered b-catenin buildup, as well as a drop in endogenous NTSR1 when the tumor suppressor gene APC was restored to cancer cells.

2.1.3 Neurotensin-Mediated Interleukin-8

The signaling pathways and transcription factors regulating IL-8 expression have been described in different cell types in response to various stimuli.[29] Resembling other physiologic agents, neurotensin takes effect through Ca2+/PKC, ERK activation and the ubiquitous AP-1 and NF-kB transcription factors to provoke IL-8 gene expression in HCT116 cells. Most studies have revealed that NF-kB plays the principal role in IL-8 regulation. Wang et al. found that both ERK-dependent AP-1 and ERK-independent NF-kB activation was necessary for neurotensin-mediated IL-8 mRNA and secretion in HCT116 cells.[30]

2.1.4 Low levels of neurotensin

Low plasma neurotensin values in colorectal cancer group can be partially explained by the internalization of the peptide. A model emerges providing G protein-coupled receptors (GPCR) endocytosis, implicated in a number of

intracellular signaling mechanisms like the MAP kinases pathway.[31] When internalized, the ligand–receptor complex is targeted to early endosomes, and the receptor is then either recycled to the cell surface or addressed to lysosomes for degradation. Neurotensin receptors [NTSR1, NTSR2, and Sortilin1 (SORT1] competently internalize the peptide. The involvement of accessory proteins like dynamin, amphiphysin and intersectin in the internalization of NTSR1 has been studied in different cell expression systems.[32] Interestingly, overexpression of intersectin, a scaffold protein, inhibits the efficiency of NTSR1 sequestration in COS-7 cells but not in HEK293 cells, suggesting cell-type specific pathways of NT endocytosis via NTSR1.[32] Neurotensin receptors can act individually, or in combination in certain tissues or organs. A complex formed between the NTSR1 and the SORT1 is able to internalize NT in HT29 cells.[33] Metabolic clearance of the peptide in addition to inactivation at sites of action is probable, given the low levels of intact NT mirrored by high levels of NT fragments in the systemic circulation. [34, 35] According to these data, a future study on tissue expression vs. circulating NT level is justified.

3. STUDY DESIGN ASPECTS

We consider sending Participant Information Sheet (PIS) about the present study to patients due to have colonoscopy via regular mail along with their appointment letter. Research Nurses are already available on both sides will inform and consent patients for blood sampling. We consider let all participants know about the analysis of results of this study. A letter will be

sent with the main findings and the potential implications for the future of the bowel cancer screening.

Blood sample will be Centrifuged and serum will be kept frozen at -80^{0}C in both hospital sites until it will be transferred to the Biomedical and Life Sciences, Lancaster University for ELISA measurement.

Each Participant will be explained the objectives of the study, will sign the consent form for participation and will be cannulated for colonoscopy (the vast majority of patients undergoing colonoscopy wish to have sedation/analgesia) and blood will be drawn from the cannula before the administration of the sedation/analgesia for their colonoscopy.

Two of the investigators (Miss Panna Patel and Mr. Arnab Bhowmick will be involved in participants colonoscopy.

Participant data or samples will be identified by the unique study number.

Participants will not be referred to a separate research team and the arrangements for identification and referral will be kept strictly to the participating trusts. The aforementioned information will be explained clearly in the letter of invitation and/ or written information for participants. In the occasional case that we will recruit prisoners, their GP will receive all the necessary information.

The initial approach to potential participants should be made by a member of the healthcare team responsible for patients' colonoscopy as it is normally done in the endoscopy unit. The research Nurse will be present as well in order to inform and consent patient for the taking of the blood sample.

The identification of potential participants will not involve reviewing or screening the identifiable personal information. Research will entail the taking

participant blood, measurement of biomarkers and statistical analysis to document their predictive value. There will not be any direct or indirect clinical implication to participant health care management.

Blood samples sent to the Faculty of Health and Medicine in Lancaster University will be identified by the Hospital Number and the NHS Number. Data will be inserted in Hospital (FGH, RPH) based computer database. Resources for participants' data will be GP referrals to Hospital outpatient clinics and Hospital Electronic Patient Records. Responsible for the aforementioned arrangements will be the principal investigator: Georgios Sgourakis, Consultant General Surgeon.

Data storage will be in hospital and university-based computers. Data base transfers from the two participating trusts to the University of Lancaster and vice-versa will be performed with military encryption USB sticks provided by the trusts.

Research data after the study has ended will be stored in the network of University Hospitals of Morecambe Bay. Confidential information (including personal data) will be destroyed and disposed of securely once it is no longer required, after periods of retention have expired, or in cases where destruction is required for legal or ethical reasons, in accordance with the Lancaster University’s Information Handling Policy. Sensitive paper documents will be shredded, and electronic data will be securely erased. We will seek assistance from our IT Services for advice on the secure disposal of electronic data.

3.1 CONTROL(S) / COMPARATOR(S)

All individuals fulfilling the inclusion criteria will be enrolled. After the refinement of participants by the exclusion criteria blood sample will be drawn for Neurotensin and IL-8. Following the report of colonoscopy and the histology individuals will be assigned in three groups: a) group A - cancer patients, b) group B – adenoma (polyp) patients and c) group C – No pathology/normal colonoscopy. Two primary analyses will be conducted to define the cut-off plasma values for Neurotensin and IL-8 for a) diagnosing cancer (group A versus group C) and b) diagnosing adenomas (group B versus group C). A secondary analysis will be conducted comparing the performance of the Neurotensin/IL-8 system towards the 2-weeks referral and FOB test positive patients for the diagnosis of colorectal cancer and adenomas.

The reason for using participants without bowel pathology is because we need to define the “normal range” of Neurotensin and IL-8 serum values.

3.2 INCLUSION / EXCLUSION CRITERIA

3.2.1 Inclusion criteria:

~~Individuals over 50 years of age referred for colonoscopy under any indication.~~

Over 60 years of age,

Change in bowel habit to looser/more frequent stools,

+/- PR bleeding AND

2 positive FIT/FOB tests.

3.2.1 Exclusion criteria:

a) Need for emergency surgery,

b) Presence of Inflammatory bowel disease,

c) Known history of inherited colorectal cancer,

d) History of cancer in another primary site,

e) Presence of liver metastases (Neurotensin is metabolized in the liver),

f) Negative previous colonoscopy for cancer,

g) Haemolysis in serum samples,

h) Informed consent not signed/withdrawal and

i) Persons who will not have the capacity to decide for themselves, who are unable to represent their own interests or are particularly susceptible to coercion.

Information concerning performance status, use of statins, steroids or any anti-inflammatory / immunosuppressive medications will also be collected.

3.3 OUTCOME MEASURES

The primary endpoints are firstly the cut-off of the serum Neurotensin and IL-8 values selected by the ROC curves and the demonstrated estimate of area under ROC curve (accuracy) in predicting colorectal cancer and colorectal adenomas.

3.4 METHODS AGAINST BIAS

We will apply population controls matched by gender, age, BMI and ethnic background.

3.5 PROPOSED SAMPLE SIZE / POWER CALCULATIONS

Assuming that the AUC of the new test is commensurate with the pilot study (AUC=0.85), we calculate the number of samples required to obtain a 95%

confidence interval of size 0.1. We assume that the ROC curve follows a binormal distribution, and that the prevalence of adenoma/cancer is between 20-30% in the study participants. We obtain that we require between 400 (30% prevalence) and 553 (20% prevalence) participants. We therefore target an enrollment of 500 patients. We intend to perform an interim analysis after 50% of the patients have been included and evaluated.

3.6 FEASIBILITY OF RECRUITMENT

The collaborative departments (Surgical Department and Gastroenterology department) of Royal Preston Hospital and the respective departments from Furness General Hospital screen approximately 18-20 cases per week. Assuming a participation rate of 50%, 500 patients can be recruited during a 12 months' period.

4. STATISTICAL ANALYSIS

4.1 Confirmatory analysis of primary endpoints

In order to compare the low to high risk groups, the propensity score will be used that is the probability of a case being in a particular group based on a given set of covariates (e.g., age, gender, BMI and ethnic background). Generally, will be calculated using logistic regression with group (Treatment /Control) as dependent and covariates as independent variables. In order to deal with missing data in covariates, we will apply multiple imputation by either Separate creation of propensity scores from the matching or we will aggregate cases to get mean (median) propensity score.

H0: p1=p2 is tested against the alternative H1: p1 ≠p2 using the exact Fisher tests.

A receiver operating characteristic (ROC) curve will be used to summarize the performance of the two-class classifiers (for Neurotensin and IL-8) for cancer and adenomas across the range of possible thresholds.

4.2 Exploratory analysis of secondary endpoints

Adenoma frequency and other qualitative characteristics: H0: p1=p2 is tested against the alternative H1: p1 ≠p2.

The diagnostic performance of the proposed biomarker system of Neurotensin and IL-8, will be assessed against the current screening system with 2-weeks referral/FOB test by using propensity scoring in binary logistic regression.

4.3 Descriptive analysis

For qualitative characteristics we compute absolute, relative frequencies, and odds ratios. For quantitative variables we compute median, minimum and maximum, lower and upper quartile and graph them in box plots, mean and standard deviation are computed if appropriate.

Comparisons of quantitative variables between cases and controls using t-tests or Mann-Whitney tests as appropriate.

4.4 Subgroup analyses

All analyses mentioned above are also performed stratified by gender, BMI and ethnic background respectively.

4.5 Interim analysis

An interim analysis with regard to the primary endpoint will be performed after inclusion and evaluation of 250 persons. If H0 can be rejected at this stage the study will be stopped. All persons included in the study up to the decision to stop will be evaluated and considered for a secondary analysis. If H0 cannot be rejected at this stage, a re-design is possible because of the adaptive strategy.

5. ETHICAL CONSIDERATIONS

5.1 Consent

We strongly support the concept that for participants to be enrolled in the study will have the capacity to decide for themselves. The present research study will not involve participants who are unable to represent their own interests or are particularly susceptible to coercion. The aforementioned information will be highlighted to the research Nurses that they are going to consent patients for taking the blood samples. During the consent process participants will clearly be explained the purpose and nature of the research, its potential benefits or no benefits and risks and their right to withdraw consent at any time. We will make it clear that It is up to them to decide whether or not to take part. If they agree, will be asked to sign a consent form. Participants will be free to withdraw at any time without giving a reason. It will be clearly communicated that a decision to withdraw at any time, or a decision not to take part, will not affect the care they will receive.

5.2 Confidentiality

All participants will be reassured for keeping confidentiality according to the "Caldicott Principles" set out an ethical framework for use of identifiable data. Participant's data will be inserted and analysed under the hospital identification Number or NHS number.

Participants will be reassured that any information about themselves that leaves the hospital will have their name and address removed so that they cannot be recognised.

However, GPs may be informed of their participation in this study, but they may indicate their choice on the consent form.

If any problems arising from the processing of identifiable data and/or samples we will immediately let them know for a shared decision.

Confidentiality to be broken if participants or others are at serious risk on rare occasions.

5.3 Risks, burdens and benefits

The procedure of drawing blood will be discussed with potential participants.The risks involved in drawing blood from a vein may include, but are not limited to, momentary discomfort at the site of the blood draw, possible bruising, redness, and swelling around the site, bleeding at the site, feeling of light-headedness when the blood is drawn, and rarely, an infection at the site of the blood. All participants will be reassured that drawing blood is a common daily hospital procedure entailing minimal risks and that all the necessary measures for dealing with side-effects are in place in the endoscopy setting (IV fluids, monitoring, and medications).

5.4 Conflict of interest

There are no conflicts of interests as a researcher which could potentially conflict with my duties as a health care professional.

5.5 What will happen at the end of our study?

After our study has ended and If the outcomes of our study are promising for detecting colorectal adenomas and cancer, an effort will be to conduct a multicentre study. The results will be fed back to participants according to their wish.

5.6 Use of tissue samples in future research

At the end of the study any surplus serum from your sample can either be gifted for use in other, different, research projects, subject to ethical approval, or disposed of as clinical waste in accordance with the relevant material of the Human Tissue Act 2004.

6. STUDY MANAGEMENT

6.1 MAJOR PARTICIPANTS

#	Name	Affiliation	Role / Responsibility
1	Mr. Georgios Sgourakis	Consultant General Surgeon	Principal investigator/

	MD, PhD, FACS.	Department of Surgery, University Hospitals of Morecambe Bay.	**Lead applicant:** Study design-concept/Trial co-statistician, Patient recruitment, endoscopies/blood samples and collection of data. Critical revision.
2	**Dr Rachael Rigby.**	**Senior Lecturer** Department: Biomedical and Life Sciences, University of Lancaster	**Collaborator:** Supervision of student, data analysis, reporting of data. Critical revision.
3	**Research Student (one year Master)**		**Named research staff:** ELISA measurements. Data Management (database).
4	**Miss Panna Patel, FRCS (Ed).**	**Consultant Colorectal Surgeon** Department of Surgery, University Hospitals of Morecambe Bay.	**Collaborator:** Patient recruitment, endoscopies/blood samples and collection of data.
6	**Mr. Arnab Bhowmick,**	**Consultant Colorectal Surgeon**	**Collaborator:** Patient recruitment, endoscopies,

	MB ChB, FRCS (Ed).	Department of General Surgery, Royal Preston Hospital.	and collection of data. Critical revision

6.2 TRIAL-SUPPORTING FACILITIES

Two Research Nurses at both FGH and LTHTR sites: Will obtain patient consent responsible for blood sample collection and transfer to pathology laboratory for process and storing.

7. FINANCIAL SUMMARY

Item	**Total funding period 12 months (£)**	**Costs**
Kits for Neurotensin, IL-8	• 6 x human neurotensin ELISA (CSB-E09144h): 2BScientific, 6 x ELISA (enough for 488 samples, allowing for optimization, £ 490 each= **£ 2,940**), quote # O-VM-18091101, Cusabio • IL-8 from Peprotech https://www.peprotech.com/human-il-8-standard-tmb-elisa-	**Total cost: £ 4,000**

	development-kit 2 kits **£ 410, while Cusabio offers at £ 520**	
One-year Masters by research student	Fees are £ 4,260 per annum	**Total cost: £ 4,260**
Case payment (centrifugation at 3000 rpm for 10 mins, and store at -20°C / -80 °C until assayed. ELISA measurements.	• The total costs for processing 200 blood samples and storage at -20 or -80 is £3,573 • Additional lab costs amounting to £1500	**Total cost: £ 9,600**
Research Nurses at both FGH and LTHTR sites.	Top scale band 5 nurse x 0.25 FTE =18,395 x 0.25 x2	
Biostatistics	Statistical consultancy rates range from £85 to £120 UK pounds per hour. For a total of 20 hours from £ 1700 to £ 2400	**Total cost: from £ 1,700 £ to £ 2,400**
Charges for shipping, delivery		**Total cost: £ 200**

TOTAL		**£ 30,000**

8. References

1 Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, Dash C, Giardiello FM, Glick S, Levin TR, Pickhardt P, Rex DK, Thorson A, Winawer SJ, American Cancer Society Colorectal Cancer Advisory G, Force USM-ST, American College of Radiology Colon Cancer C: Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the american cancer society, the us multi-society task force on colorectal cancer, and the american college of radiology. CA Cancer J Clin 2008;58:130-160.

2 Screening for colorectal cancer: U.S. Preventive services task force recommendation statement. Ann Intern Med 2008;149:627-637.

3 Knudsen AB, Hur C, Gazelle GS, Schrag D, McFarland EG, Kuntz KM: Rescreening of persons with a negative colonoscopy result: Results from a microsimulation model. Ann Intern Med;157:611-620.

4 Hassan C, Giorgi Rossi P, Camilloni L, Rex DK, Jimenez-Cendales B, Ferroni E, Borgia P, Zullo A, Guasticchi G: Meta-analysis: Adherence to colorectal cancer screening and the detection rate for advanced neoplasia, according to the type of screening test. Aliment Pharmacol Ther;36:929-940.

5 National Comprehensive Cancer Network, Colorectal cancer screening (Version 2.2013), Accessed 16/12/2013, http://www.nccn.org/professionals/physician_gls/gl_steering_committee.asp:

6 Carraway RE, Plona AM: Involvement of neurotensin in cancer growth: Evidence, mechanisms and development of diagnostic tools. Peptides 2006;27:2445-2460.

7 Bugni JM, Rabadi LA, Jubbal K, Karagiannides I, Lawson G, Pothoulakis C: The neurotensin receptor-1 promotes tumor development in a sporadic but not an inflammation-associated mouse model of colon cancer. Int J Cancer;130:1798-1805.

8 Vincent JP: Neurotensin receptors: Binding properties, transduction pathways, and structure. Cell Mol Neurobiol 1995;15:501-512.

9 Zhao D, Zhan Y, Koon HW, Zeng H, Keates S, Moyer MP, Pothoulakis C: Metalloproteinase-dependent transforming growth factor-alpha release mediates neurotensin-stimulated map kinase activation in human colonic epithelial cells. J Biol Chem 2004;279:43547-43554.

10 Ehlers RA, Zhang Y, Hellmich MR, Evers BM: Neurotensin-mediated activation of mapk pathways and ap-1 binding in the human pancreatic cancer cell line, mia paca-2. Biochem Biophys Res Commun 2000;269:704-708.

11 Ehlers RA, 2nd, Bonnor RM, Wang X, Hellmich MR, Evers BM: Signal transduction mechanisms in neurotensin-mediated cellular regulation. Surgery 1998;124:239-246; discussion 246-237.

12 Zhao D, Keates AC, Kuhnt-Moore S, Moyer MP, Kelly CP, Pothoulakis C: Signal transduction pathways mediating neurotensin-stimulated interleukin-8 expression in human colonocytes. J Biol Chem 2001;276:44464-44471.

13 Bossard C, Souaze F, Jarry A, Bezieau S, Mosnier JF, Forgez P, Laboisse CL: Over-expression of neurotensin high-affinity receptor 1 (nts1) in relation with its ligand neurotensin (nt) and nuclear beta-catenin in inflammatory bowel disease-related oncogenesis. Peptides 2007;28:2030-2035.

14 Maoret JJ, Anini Y, Rouyer-Fessard C, Gully D, Laburthe M: Neurotensin and a non-peptide neurotensin receptor antagonist control human colon cancer cell growth in cell culture and in cells xenografted into nude mice. Int J Cancer 1999;80:448-454.

15 Gui X, Guzman G, Dobner PR, Kadkol SS: Increased neurotensin receptor-1 expression during progression of colonic adenocarcinoma. Peptides 2008;29:1609-1615.

16 Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O: Randomised study of screening for colorectal cancer with faecal-occult-blood test. Lancet 1996;348:1467-1471.

17 Ahlquist DA, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Pierceall WE, Thibodeau SN, Shuber AP: Colorectal cancer screening by detection of altered human DNA in stool: Feasibility of a multitarget assay panel. Gastroenterology 2000;119:1219-1227.

18 Sgourakis G, Papapanagiotou A, Kontovounisios C, Karamouzis MV, Dedemadi G, Goumas C, Karaliotas C, Papavassiliou AG: The combined use of serum neurotensin and il-8 as screening markers for colorectal cancer. Tumour Biol;35:5993-6002.

19 Sgourakis G, Papapanagiotou A, Kontovounisios C, Karamouzis MV, Lanitis S, Konstantinou C, Karaliotas C, Papavassiliou AG: The value of plasma neurotensin and cytokine measurement for the detection of bowel ischaemia in clinically doubtful cases: A prospective study. Exp Biol Med (Maywood);238:874-880.

20 Myers RM, Shearman JW, Kitching MO, Ramos-Montoya A, Neal DE, Ley SV: Cancer, chemistry, and the cell: Molecules that interact with the neurotensin receptors. ACS Chem Biol 2009;4:503-525.

21 Wolf AM, Wender RC, Etzioni RB, Thompson IM, D'Amico AV, Volk RJ, Brooks DD, Dash C, Guessous I, Andrews K, DeSantis C, Smith RA: American cancer society guideline for the early detection of prostate cancer: Update 2010. CA Cancer J Clin;60:70-98.

22 Martin S, Navarro V, Vincent JP, Mazella J: Neurotensin receptor-1 and -3 complex modulates the cellular signaling of neurotensin in the ht29 cell line. Gastroenterology 2002;123:1135-1143.

23 Mantuano E, Santi S, Filippi C, Manca-Rizza G, Paoletti S, Consani C, Giovannini L, Tramonti G, Carpi A, Panichi V: Simvastatin and fluvastatin reduce interleukin-6 and interleukin-8 lipopolysaccharide (lps) stimulated production by isolated human monocytes from chronic kidney disease patients. Biomed Pharmacother 2007;61:360-365.

24 Malicki S, Winiarski M, Matlok M, Kostarczyk W, Guzdek A, Konturek PC: Il-6 and il-8 responses of colorectal cancer in vivo and in vitro cancer cells subjected to simvastatin. J Physiol Pharmacol 2009;60:141-146.

25 Sakoda K, Yamamoto M, Negishi Y, Liao JK, Node K, Izumi Y: Simvastatin decreases il-6 and il-8 production in epithelial cells. J Dent Res 2006;85:520-523.

26 Muller KM, Tveteraas IH, Aasrum M, Odegard J, Dawood M, Dajani O, Christoffersen T, Sandnes DL: Role of protein kinase c and epidermal growth factor receptor signalling in growth stimulation by neurotensin in colon carcinoma cells. BMC Cancer;11:421.

27 Potten CS, Booth C, Pritchard DM: The intestinal epithelial stem cell: The mucosal governor. Int J Exp Pathol 1997;78:219-243.

28 Souaze F, Viardot-Foucault V, Roullet N, Toy-Miou-Leong M, Gompel A, Bruyneel E, Comperat E, Faux MC, Mareel M, Rostene W, Flejou JF, Gespach C, Forgez P: Neurotensin receptor 1 gene activation by the tcf/beta-catenin pathway is an early event in human colonic adenomas. Carcinogenesis 2006;27:708-716.

29 van Eeden SF, Terashima T: Interleukin 8 (il-8) and the release of leukocytes from the bone marrow. Leuk Lymphoma 2000;37:259-271.

30 Wang X, Wang Q, Ives KL, Evers BM: Curcumin inhibits neurotensin-mediated interleukin-8 production and migration of hct116 human colon cancer cells. Clin Cancer Res 2006;12:5346-5355.

31 Gully D, Labeeuw B, Boigegrain R, Oury-Donat F, Bachy A, Poncelet M, Steinberg R, Suaud-Chagny MF, Santucci V, Vita N, Pecceu F, Labbe-Jullie C, Kitabgi P, Soubrie P, Le Fur G, Maffrand JP: Biochemical and pharmacological activities of sr 142948a, a new potent neurotensin receptor antagonist. J Pharmacol Exp Ther 1997;280:802-812.

32 Mazella J, Botto JM, Guillemare E, Coppola T, Sarret P, Vincent JP: Structure, functional expression, and cerebral localization of the levocabastine-sensitive neurotensin/neuromedin n receptor from mouse brain. J Neurosci 1996;16:5613-5620.

33 Mazella J, Vincent JP: Functional roles of the nts2 and nts3 receptors. Peptides 2006;27:2469-2475.

34 Leeman SE, Carraway RE: Neurotensin: Discovery, isolation, characterization, synthesis and possible physiological roles. Ann N Y Acad Sci 1982;400:1-16.

35 Kitabgi P: Inactivation of neurotensin and neuromedin n by zn metallopeptidases. Peptides 2006;27:2515-2522.