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A novel blood based triage test for colorectal cancer in primary care

Authors:

Cerys A Jenkins PhD[1], postdoctoral researcher

Freya E R Woods MPhys[1], research scientist

Susan Chandler MBChB[2], clinical lecturer

Kym Carter PhD[2,3], study manager

Rhys A Jenkins PhD[1], research scientist

Andrew Cunningham MBChB[2], clinical lecturer

Kayleigh Nelson PhD[3], qualitative researcher

Rachel Still MBChB[4], consultant clinical scientist

Jenna Walters[4], Healthcare Sciences Associate Practitioner R&D

Non Gwynn[4], Healthcare Sciences Associate Practitioner R&D

Wilson Cheah MBChB[5], core surgical trainee

Rachel Harford[6], research nurse

Claire O'Neill PhD[2,3], triallist

Julie Hepburn[7], lay representative

Ian Hill[7], lay representative

Heather Wilkes MBChB[8], general practitioner

Greg Fegan PhD[2,3], director

Peter R Dunstan PhD[1], head of department

Dean A Harris MBChB MD[5*], consultant surgeon

Affiliations and addresses:

[1]Physics Department, College of Science, Centre for NanoHealth, Swansea University, SA2 8PP

[2]Swansea University Medical School, Swansea University, SA2 8PP

[3]Swansea Trials Unit, Swansea University, SA2 8PP

[4]Department of Laboratory Medicine, of Medical Biochemistry, Swansea Bay University Health Board, SA6 6NL

[5]Department of Colorectal Surgery, Swansea Bay University Health Board, Morriston Hospital, SA6 6NL

[6]Research and Development Department Swansea Bay University Health Board, Institute of Life Science 2, Swansea University, SA2 8PP

[7]Public Involvement Community, Health and Care Research Wales, Cardiff, CF11 9AB

[8]Briton Ferry Primary Care Centre, Neath, SA11 2FP

[*]Corresponding author and request for reprints – Prof Dean A Harris

Email - Dean.A.Harris@wales.nhs.uk

ORCID: orcid.org/0000-0003-2673-8946

Telephone - 01792 285459 (secretary)

Address: Department of Colorectal Surgery, Swansea Bay University Health Board, Morriston Hospital, Swansea SA6 6NL

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Abstract

Background The majority of colorectal cancers (CRCs) are detected after symptomatic presentation to primary care. Given the shared symptoms of CRC and benign disorders it is challenging to manage this risk of missed diagnosis. Colonoscopy resources cannot keep pace with increasing demand. There is a pressing need for access to simple triage tools in primary care to help prioritise patients for referral.

Aim To evaluate the performance of a novel spectroscopy-based CRC blood test in primary care.

Design and setting Mixed methods pilot study of test performance and GP focus group discussions.

Method Urgent suspected cancer patients were recruited for the Raman spectroscopy (RS) test coupled to machine learning classification ('Raman-CRC') to identify CRC within the referred population. Qualitative focus group work evaluated the acceptability of the test in primary care by thematic analysis of focus group theorising.

Results 532 patients age over 50 referred on the USC pathway were recruited from 27 GP practices. Twenty nine patients (5%) were diagnosed with CRC. Raman-CRC identified CRC with sensitivity 95.7%, specificity 69.3% with Area Under Curve (AUC) of 0.80 as compared to colonoscopy as reference test (248 patients). Stage I/II cancers were detected with 78.6% sensitivity. Focus group themes underlined the convenience of a blood test for the patient and the test's value as a risk assessment tool in primary care.

Conclusions Our findings support this novel, non-invasive blood-based method to prioritise those patients most likely to have CRC. Raman-CRC may accelerate access to diagnosis with potential to improve cancer outcomes.

Keywords Colorectal cancer; two-week wait; pilot study; qualitative; Raman spectroscopy; primary health care

How this fits in

Current colorectal cancer referral pathways are resource intensive with a low conversion rate. There is currently a lack of effective triage tests for suspected colorectal cancer in primary care. The Raman-CRC blood test is highly sensitive for all-stage (95.7%) and stage I/II (78.6%) colorectal cancer detection. GP focus groups agreed that the test would help increase early stage cancer detection in primary care.

Introduction

Colorectal cancer (CRC) is the second largest cause of cancer related deaths worldwide.[1] The majority (54%) of CRC in the UK is diagnosed through primary care consultation and referral.[2] Patients satisfying strict clinical referral criteria can be referred from primary care on the Urgent Suspected Cancer (USC), or Two Week Wait (2WW) pathway for further investigations and treatment within a 62 day target.[3]

The strict referral criteria fail to take into account GP's 'gut instinct' for serious pathology and have a low cancer conversion rate as the USC symptom profile is based on a minimum positive predictive value (PPV) for cancer of just 3%. Despite demand for colonoscopy doubling over the last five years[4] the current USC pathway has failed to detect CRC earlier nor changes the outcomes of CRC,[5] with the UK having one of the poorest CRC survival records in Europe.

Increasing numbers of patients with lower GI symptoms are presenting to primary care. With the limitations of endoscopy resource there is a need for simple triage tests available to GPs to help risk manage presenting patients, particularly given the rapid increase in early age of onset CRC.

Faecal immunochemical testing (FIT) for faecal haemoglobin (*f*-Hb) was introduced in 2017 for low risk symptom triage in primary care (NICE DG30)[6] with evidence growing for its use in high-risk symptoms meeting NICE NG12 criteria. The recent NICE FIT study reported test sensitivity of 90.9% and specificity of 83.5% at cut-off of 10µg/g *f*-Hb.[7] FIT may not be the ideal triage tool for primary care use given its low return rate[8,9] (just 62% of patients in the NICE-FIT study) its lack of cancer specificity and its lack of approval for rectal bleeding,[10] the commonest presenting symptom.

There is much recent interest in the use of artificial intelligence (AI) to identify cancer more efficiently in primary care, such as the 'C the Signs' and 'Pinpoint-AI' applications. We have developed a simple blood test which uses Raman spectroscopy (RS) to measure cancer-related molecular species (proteins, nucleic acids, lipids) in serum to produce a cancer-specific 'biochemical fingerprint'. An AI-algorithm analyses the spectral output and classifies the patient into either high or low likelihood of colorectal cancer. This test could help GPs to identify and prioritise suspected cancer patients for further investigation as a referral decision support tool.[11]

Here, we present results of a pilot application of the Raman-CRC model to detection of CRC in cohort of patients meeting USC referral criteria from a primary care setting. This study

presents a mixed methods approach considering the utility of a Raman-CRC blood test to streamline the referral pathway for suspected cancer patients and explores its potential to translate into a clinical setting.

Methods

Study design

A prospective cohort pilot study evaluating the performance of Raman-CRC in primary care to triage need for referral and diagnostic testing for CRC as outlined by The Detecting Cancer Early Setting Partnership.[12]. This work was performed as a phase 2 evaluation of clinical test performance (analytic validity in intended setting) in accordance with the CanTest framework.[13] Results of Raman-CRC were compared to final patient diagnosis after USC investigations to determine sensitivity and specificity in an enriched symptomatic primary care population.

The study was conducted within Swansea Bay University Health Board primary care practices and managed by Swansea Trials Unit. Patient demographics, current USC pathway timelines and final diagnosis were obtained from electronic patient records and recorded in a REDCap database.[14] Interval cancers were captured up to 9 months after diagnosis. Results were reported according to QUADAS-2 standards.

A nested qualitative study was performed and reported according to the consolidated criteria for reporting qualitative research (COREQ) checklist. This involved semi-structured focus group discussions with GP practices[15] to explore attitudes towards the current USC pathway and the potential uses of Raman-CRC in primary care (Figure 1).

Participants

Eligible participants were aged 50 or over and had presented to their GPs with high-risk symptoms raising suspicion of CRC as per NICE guidelines (NG12).[3]

Blood sample preparation

Fasted venous blood samples (Vacutainer SST tubes BD, USA) were centrifuged, aliquoted and stored at -80 °C before batch analysis.

Statistical analysis

Sample size planning

The study was designed to estimate test performance of the Raman-CRC model in a population with a cancer prevalence representative of its intended application. A sample size of at least 75-100 patients is required as an independent blinded test set.[16] A model sample set of 300 patients is utilised to surpass this minimum sample requirement. To provide a definitive sample size for precise determination of the performance of the analysis model we assumed a 5% prevalence would require 691 recruited participants based on a specificity of 90% with absolute precision of 0.1.[17,18]

Raman spectroscopy

Serum samples were analysed using previously reported high throughput (HT) Raman methodology with modifications.[11] Serum samples were thawed prior to analysis, liquid serum samples (200 µl) were placed into the HT platform and analysed with a 785 nm laser using a Raman microscope (InVia Renishaw, UK).

Raman-CRC machine learning model

All Raman spectra underwent data pre-processing including wavenumber calibration, data binning, smoothing, background subtraction and normalisation.[19,20] A random forest based machine learning model showed optimal performance and a diagnostic model was developed using a retrospective cohort of 300 patients with known clinical outcomes of CRC (histologically confirmed) or non-cancer control (normal colonoscopy) in a 50:50 split.[21] The Raman-CRC model was internally cross-validated using a repeated 5-fold cross validation of training data to produce a preliminary AUC and sensitivity and specificity values within R.

Prospective clinical validation study

35 GP practices within SBUHB were invited to take part in the study from 2017-2019 of which 27 practices participated (77%) with additional recruitment from secondary care after referral. Nine patients declined study participation leaving 595 patients that provided blood samples at time of consent (98.5% compliance). (Figure S1).

Analytic researchers were blinded to final diagnosis. The average cancer probability for all spectra from each patient was then aggregated to produce an overall predicted cancer

probability. Any patient with a probability of greater than or equal to 0.5 was classified as CRC, and less than 0.5 non-cancer.

Reference standard

The resultant decision for each patient produced by Raman-CRC was compared to final diagnosis as confirmed following colonoscopy or CT colonography with histological verification. Patients who did not undergo reference standard tests or had data missing from diagnostic results were excluded. Colonoscopy was the primary reference standard. The results were analysed per investigation and reported separated as CT colonography has reduced ability to detect small polyps and flat cancers.[22,23] Patients who were investigated with flexible sigmoidoscopy were excluded.

Primary care interviews

Semi structured focus groups were carried out at primary care practices in South Wales. Scenarios were presented during the focus groups to explore attitudes toward test application for different clinical situations with data on RS performance based on a previous pilot results (sensitivity 85.7%, specificity 68%, Table S1, Box S1-2). The focus groups were conducted face to face at GP practice sites (one via video conferencing) by DAH and were audio recorded and transcribed verbatim before analysis.

Qualitative analysis

Following checked transcription NVivo software (Version 12, QSR International Pty Ltd.) was used to code and analyse the transcripts. 3 researchers (one male, two female) independently coded the interviews to identify potential themes and the independent analyses were merged into a final coding scheme (Table S2).[24] Subthemes were generated based on consensus.

Results

Development of the Raman-CRC diagnostic model

300 patients were age- and sex-matched using propensity score matching to develop the Raman-CRC blood test model (Table S3). The Raman CRC model showed an area under the curve (AUC) value of 0.842 for a typical fold where an AUC between 0.8 and 0.9 is considered excellent.[25] The model achieved a sensitivity of 70.5% and specificity of 76.8% when trained

on 150 known cancers (52.7% (79/150) Stage I/II, and 47.3% (72/150) Stage III/IV) and 150 controls (Figure S2).

Prospective validation study

The study captured a wide variance of diagnoses within the 532 eligible primary care patients including non-cancer conditions, pre-cancerous polyps and other cancer types (Table S4). 29 patients (5%) were diagnosed with CRC. Patient ages were comparable between groups with a male predominance in the CRC group (Table S5). Minimal differences in symptom frequency or routine blood results (haemoglobin, ferritin) between cancers and non-cancer diagnoses was observed, highlighting the lack of diagnostic specificity of currently used clinical features.

After patient exclusions 405 patients remained with CRC or non-CRC diagnosis based on colonoscopy or CT colonography. Compared to gold standard colonoscopy the model showed a sensitivity of 95.7% and a specificity of 69.3% (figure 2 and table 1).

Test performance by colonoscopy and CT colonogram combined found sensitivity of 89.7% and specificity 65.7%. The Raman blood test detected early stage CRC (UICC stage I/II) with 78.6% sensitivity and III/IV with 100% sensitivity (Table 2) comparing favourably with FIT data.

Acceptability of a Raman blood test in primary care

A nested qualitative study was conducted through focus group discussion across six primary care practices and included 24 GPs. The mean meeting duration was 45 minutes (range 35-55 minutes) and followed a semi-structured question format. Four key themes were identified from the discussions: perceptions of the current referral pathway, utility of Raman-CRC as a triage tool, utility of Raman-CRC as a diagnostic tool, and GP acceptability of Raman-CRC (summarised with quotes in Table S7).

Perceptions of the current referral pathway

GPs agreed that they carefully considered appropriateness of USC referrals and were conscious of current capacity problems within secondary care. GPs also felt under pressure to get patients seen within USC pathway timeframes. They highlighted patients often experiencing long waits for 'urgent' referrals and as such would try to "*shoehorn"* (GP 2,

practice 5) patients into the USC pathway to fulfil their duty of care in a timely manner. Whilst most GPs thought the referral criteria were very rigid making it difficult to refer patients outside of the criteria but for whom the GP had clinical concerns.

Utility of Raman-CRC as a triage tool

GPs welcomed the proposed Raman-CRC test to help triage patients being referred and make more appropriate referral decisions. They highlighted that the test might reduce the number of unnecessary referrals and that test may be preferable for some patients particularly when compared to faecal based tests. It was thought the test would go some way to help GPs remove barriers to earlier diagnoses by using the test results as evidence to refer patients (as a 'rule-in' test rather than a 'rule-out' test).

GPs also highlighted other potential uses for the test and all agreed that it would be most useful in helping to provide an evidence base for, and enabling better management of, patients who had symptoms which did not meet the USC referral criteria. The test would be an acceptable method to reassure patients and reduce their anxiety.

Utility of Raman-CRC as a diagnostic tool

GPs highlighted that the test has potential as a diagnostic tool in populations where invasive testing is not appropriate e.g. frail patients potentially providing a diagnosis without invasive diagnostic procedures causing harm or distress to patients. GPs on the whole felt comfortable using it as a screening tool and iterated they would be comfortable providing the results to patients.

GP acceptability of Raman-CRC

To have the confidence to use Raman-CRC routinely in primary care all agreed it needed to be adopted into local or national guidelines. However, GPs agreed that if the test were available and within the guidance then it would be well utilised. *"if a Raman blood test was available then I would do it, and I think you would find every GP would."* (GP 1, practice 3).

Discussion

Summary

We report the first prospective proof of concept study to analyse blood serum with label-free Raman spectroscopy combined with machine learning as a disruptive new technology for transforming the current suspected cancer pathway for CRC. It shows early evidence that Raman-CRC has sufficient test performance for future utility in primary care as a 'rule in' triage test. This could be of great value in detecting CRC in younger symptomatic patients in primary care, in whom cancer incidence is rising, and to streamline the referral pathway for diagnostic investigations.

Analysis of focus group transcripts revealed overwhelming support for the blood test and highlighted the need for a primary care-based companion test to triage primary care referrals. GP attitudes were positive towards adoption and clinical utility for a blood-based test for CRC in primary care. The projected reduction in patient anxiety was positively received. Test performance was considered acceptable even at this proof of concept stage and would be used to influence referral behaviour if routinely available.

Strengths and limitations

This prospective test evaluation was conducted to strict guidelines with blinding of analysts to final diagnosis. Reference standard was restricted to colonoscopy as gold standard. It is recognised that the cancer event rate was small (yet representative of the USC population) which could have made the sensitivity of the Raman-CRC test appear higher. Raman-CRC specificity is currently inferior to that of FIT (69.3% vs 83.5%) but still exceeds the NICE NG12 criteria specificity of just 35%[26] as a significant advance over the symptom-based referral route alone (Table 3). FIT was not available locally at the time of the study as a comparison group, but is to be included in follow-on studies.

Comparison with existing literature

Raman-CRC showed superior overall sensitivity for CRC (95.7% vs 90.9%) compared to recently published FIT performance at a cut-off of ≥10 µg/g of *f*-Hb (table 3).[7,26]. The Raman-CRC test has a sensitivity for early stage I/II CRC of 78.6%. Sensitivity by stage for FIT is poorly reported in general, although Niedermaier reported pooled sensitivity of 79-82% (70-87%, 95% confidence interval) for stage III/IV cancers and 73-80% (65-84%) for stage I/II cancers with just 40% sensitivity for T1 cancers (Table 5).[27] Data from multi-cancer circulating DNA blood tests suggest even worse performance for early stage CRC (67% sensitivity stage I/II)[282]

which may not be the solution to achieve the NHS Long Term Plan of detecting 75% of cancers at stage I/II by 2028.[29]

Implications for research and practice

The International Cancer Benchmarking Partnership (ICBP) has reported the UK as having the lowest survival rates for colorectal cancer, in part through differences in diagnostic pathways and referral timelines.[30,31] More accurate and acceptable tests such as Raman-CRC could improve this situation as the test could be applied at first primary care consultation to avoid missed opportunities for earlier detection, particularly for early onset CRC. It may also encourage earlier help-seeking behaviour of hard-to-reach patient groups given its familarity as 'just a blood test'.

It is recognised that this proof-of-concept model will require collection of larger datasets to train more advanced models for validation. An improved AI algorithm trained on greater numbers of cancers with inclusion of high risk adenomas and patient metadata (demographics, symptoms, routinely available blood test results) is in development with potential for superior test performance and to enhance specificity rates. A larger cohort study evaluating Raman-CRC in combination with FIT (CRaFT study, IRAS 254366) will further develop the current AI model and measure individual and combined test accuracy with FIT. The NICE-FIT study highlighted the need for a further test alongside FIT to reduce the false positives and false negatives.[7] CRaFT will also capture symptomatic patients' experiences and attitudes with Raman and FIT. Future work is planned to conduct a health economic cost effectiveness analysis, impact analysis in terms of earlier detection and use of downstream resources and qualitative patient and clinician test acceptability.

Other emerging technologies include circulating tumour (ct)DNA in plasma. Although showing promising sensitivity and specificity in advanced cancers, these technologies are not yet validated in target clinical populations with low cancer prevalence and may not be cost-effective for NHS use.[32] Raman-CRC has discernible advantages through being a rapid, reproducible, high throughput technology whose cost per test is minimised by its reagent-free approach. It also shows early promise in multi-cancer detection in a community Rapid Diagnostic Clinic setting. [33]

In conclusion Raman-CRC has shown potential to become an acceptable decision support tool in primary care for symptomatic patients at risk of underlying CRC. The test is applicable to all relevant symptoms and could help upgrade patients with low-risk symptoms (including rectal bleeding) onto the USC pathway towards earlier detection. A positive test would circumvent the traditional referral route by dovetailing with a 'Straight To Test' pathway (Figure 3).[34] We now plan to validate and expand our proof-of-concept findings through both the CRaFT study and further large-scale primary care trials.

Additional Information

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Clinical investigators

Department of Colorectal Surgery, Swansea Bay University Health Board: Prof J Beynon, Prof U Khot, Mr M Davies, Mr MD Evans, Mr TV Chandrasekaran, Mr GW Taylor, Mr S Ather.

General practitioners (site principal investigators):

Richard Tristram, Clydach Primary Care Centre, Swansea; Heather Potter, Skewen Medical Centre, Neath; Emma Manson, Uplands Surgery, Mumbles, Swansea; David Martin Jones, Mumbles Medical Centre, Swansea; Stephen Hailey, Pennard Surgery, Swansea; Kirstie Truman, Gower Medical Practice, Swansea; Julien Bell, Grove Medical Centre, Swansea; Lynne Dowding, Kings Road Surgery, Swansea; Matthew Seager, Sketty and Killay Medical Centre, Swansea; Kirstie Truman, Mark Davies (retired), West Cross/St. Thomas' Surgery, Swansea; Tim Evans, Fforestfach Medical Centre, Swansea;

Owen Powell, Fforestfach Medical Centre (Powell practice), Swansea; Ceri Todd, High Street Surgery, Swansea; Rebecca Jones, Dulais Valley Primary Care Centre, Neath; Alistair Bennett, Dyfed Road Surgery, Neath; Steve Harrowing, Vale of Neath

practice, Glynneath; Anjula Mehta, Cymmer and Cwmavon Health Centre; Richard Beynon, Llansamlet Surgery, Swansea; Richard Thomas, Kingsway Surgery, Swansea; Sherard Lemaitre, Oak Tree Surgery, Bridgend; Daniel Tacagni, Strawberry Place Surgery, Swansea; Russell Clark, Llys Meddyg, Sway Rd, Morriston; Emma Rees, Pontardawe Health Centre,

Swansea; Griff Hopkin, Gowerton Medical Practice, Swansea; Duncan Williams, Amman Tawe Partnership; Alison Lilley, Castle Surgery, Neath; Mark Goodwin, Glyncorrwg Afan Valley Group Practice; Amrita Amin, St. Helen's Medical Centre, Swansea; Dhamayanthi Vigneswaran, Cheriton Medical Centre, Swansea; Maria Cronje, North Cornelly Surgery, North Cornelly; Clare Perman, Cwmfelin Medical Centre, Neath

Author Contributions

CAJ, FW, SC provided study data, completed the literature review, data analysis and drafted the manuscript. RJ, AC, KN, WC provided study data and/or contributed to the interpretation of results. KT, DAH, PRD, JH, IH, CON, RS, NG, JW, GF have made substantial contributions to the conception and design of the work and subsequent protocol revisions; CAJ, DAH, SC, RJ, KT, FW,AC, KN, RS, JW, NG, RH, GF, HW, WC, CON, JH, IH and PRD drafted the manuscript and/or provided critical revision; approved the version submitted for publication; agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

This study received a favourable ethical opinion by Wales REC6 (14/WA/0028). Written informed consent was obtained from all patient participants in the study and all focus group participants before interviews. The study was performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable

Data availability

The datasets generated and/or analysed during the current study are available within the article and supplementary information files are available from the authors upon reasonable request.

Competing Interests

PRD, DAH and CAJ declare that they are all co-founders and managing directors of CanSense Ltd, an incorporated cancer diagnosis spin-out company from Swansea University (UK company no: 11367637). All other authors declare no competing interests.

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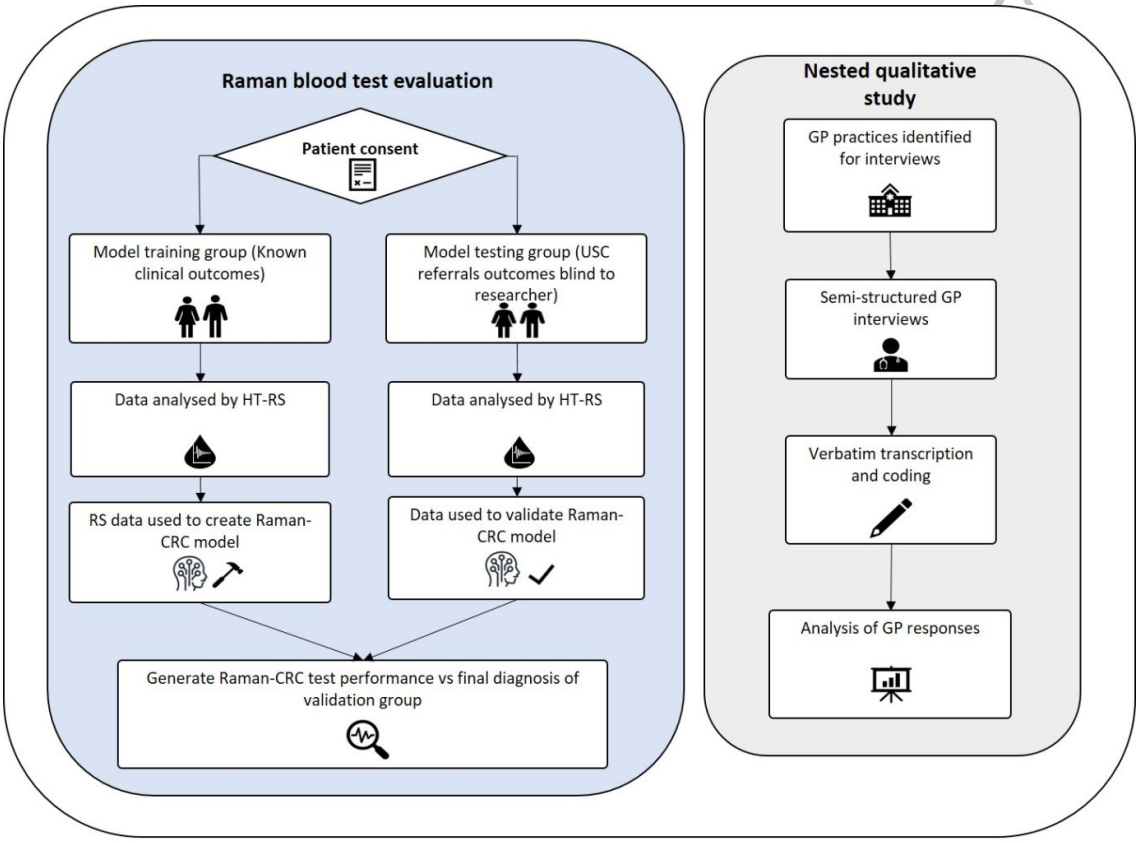
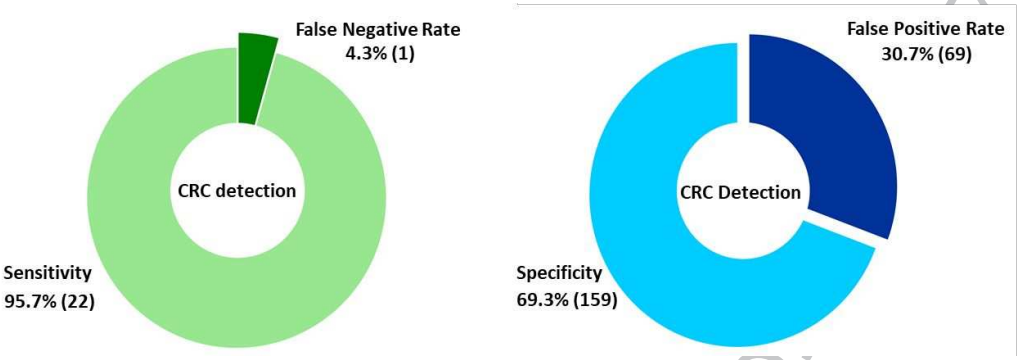
Figure legends

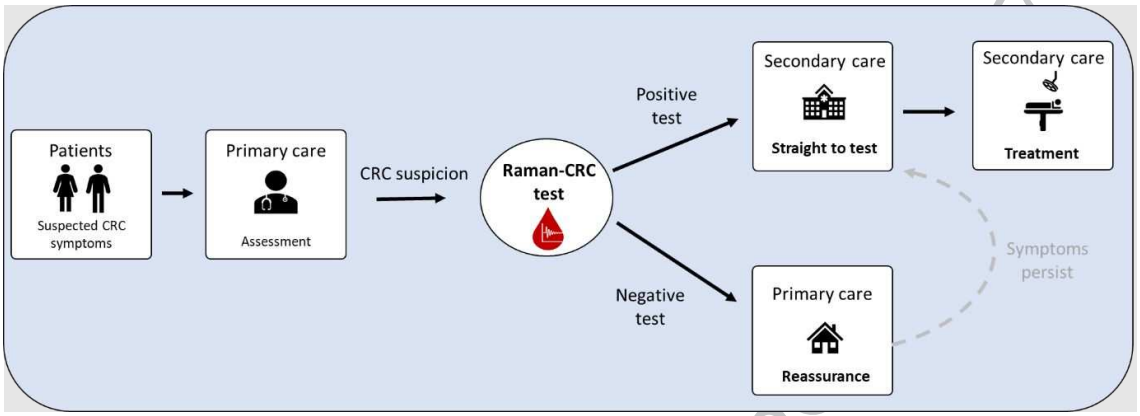
Figure 1: Study design. The mixed methods prospective clinical validation study incorporated a retrospective cohort analysis to build the Raman-model, the prospective study for clinical validation and a nested qualitative study for investigating attitudes of the test in primary care.

Figure 2: Disease prediction for the prospective validation cohort from secondary care USC referral patients. Overall sensitivity, specificity, false negative rate and false positive rate for the Raman-CRC model on a colonoscopy per-patient basis following blind analysis

Figure 3: Proposed new clinical pathway incorporating Raman-CRC testing as a triage tool in primary care. Symptomatic patients with a negative Raman-CRC test are reassured in primary care, relieving pressure on secondary care diagnostic services. The pathway could lead to earlier diagnosis and a reduction in time to treatment when a positive test is combined with a straight to test pathway.







	N, total	Sensitivity	Specificity
Colonoscopy	248	95.7 (78.1-99.9%)	69.3 (63.8-76.1%)
Colonoscopy and CTC	405	89.7 (72.7-97.8%)	65.7 (60.7-70.5%)

Table 1: Disease prediction for the prospective validation cohort from secondary care USC referral patients. Test sensitivity and specificity (95% confidence intervals according to initial diagnostic test.

	Sensitivity for UICC Guidance Stages			
Stage	I	II	III	IV
Raman-CRC	50% (n=4)	90% (n=10)	100% (n=12)	100% (n=3)
FIT[41]	73% (65%–79%)	80% (74%–84%)	82% (77%–87%)	79% (70%–86%)

Table 2. Raman-CRC model performance for different cancer stages vs. FIT.

	NICE NG12 pathway [9,48]	FIT (threshold 10ug/g) [9]	Raman-CRC
Sensitivity	93	90.9	95.7
Specificity	35	83.5	69.3
AUC	n/a	0.93	0.8

Table 3. Comparison of Raman-CRC test performance with NG12 pathway and FIT