BMJ Open

Accuracy of blood-based biomarkers for screening advanced pre-cancerous colorectal lesions: a protocol for systematic review and meta-analysis.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-060712
Article Type:	Protocol
Date Submitted by the Author:	31-Dec-2021
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Keywords:	GASTROENTEROLOGY, Gastrointestinal tumours < ONCOLOGY, Colorectal surgery < SURGERY

Accuracy of blood-based biomarkers for screening pre-cancerous colorectal lesions: a protocol for systematic review and meta-analysis.

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Word count: 3380 words

Keywords:

Colorectal neoplasms; Biomarkers, adenoma; Sensitivity and Specificity; Diagnostics; Bowel.

Ethics statement:

No ethical approval is required for this protocol as this will be a collation of previously published literature.

ABSTRACT

Introduction Colorectal cancer (CRC) is the third most diagnosed cancer and the second most common cause of cancer mortality worldwide. Most CRCs develop through either the adenoma-to-carcinoma or the serrated pathways, and therefore detection and removal of these pre-cursor lesions can prevent the development of cancer. Current screening programs can aid in the detection of CRC and adenomas; however, participation rates are suboptimal. Blood-based biomarkers may help to address these low participation rates in screening programs. Although blood-based biomarker tests show promise for cancer detection, limited attention has been placed on the sensitivity and specificity for detection of the pre-cursor lesions. The aim of this research is to conduct a systematic review and meta-analysis to evaluate the accuracy of blood-based biomarker tests in detecting advanced pre-cancerous lesions.

Methods and Analysis This protocol was informed by the PRISMA-P and results will be reported in line with the PRISMA guidelines. Literature searches will be conducted on PubMed, Embase and Web of Science. Two reviewers will conduct the searches, and independently screen them, according to title and abstract and then the full-text versions of those selected articles as well as the risk of bias via the QUADAS-2. The GRADE guidelines will be used to validate the certainty of evidence for recommendations based on the risk of bias findings. Meta-analysis will be conducted where appropriate on groups of studies with low heterogeneity.

Ethics and Dissemination No patient data will be included in our review and therefore ethics approval is not required. It is anticipated that the review will identify the most promising candidate biomarkers for clinical translation in the screening of advanced pre-cancerous lesions. The results will be published in a peer-reviewed journal.

STRENGTH AND LIMITATIONS OF THIS STUDY

- While many reviews have been published on blood biomarker test to detect colorectal cancer, our work will provide the first systematic review to evaluate the accuracy of blood-based biomarker tests in detecting pre-cancerous lesions;
- It is anticipated that the results of this systematic review and meta-analysis will identify candidate blood biomarkers for improving the non-invasive detection of pre-cancerous lesions;
- Due to the clinical and methodological diversity of different types of blood biomarkers studied for colorectal neoplasms, a limitation of our review could be heterogeneity.

INTRODUCTION

Colorectal cancer (CRC) is the third most diagnosed cancer and the second most common cause of cancer mortality worldwide[1]. In Australia, the age-standardised CRC incidence rate is approximately 50 per 100,000 people[2]. CRC screening programs (colonoscopy and/or faecal based screening measures) have demonstrated efficacy in reducing both CRC incidence and mortality[3 4]. CRC develops through the adenoma (or sessile serrated) carcinoma pathway, where pre-cancerous lesions such as advanced adenomas and sessile serrated lesions may progress to CRC[5 6]. The purpose of screening is to detect CRC at an early stage, enabling earlier interventions, which can lead to more efficacious treatment options and better patient outcomes including reduced morbidity and mortality. Furthermore, screening can also assist in the detection of pre-cancerous lesions, such as adenomas and advanced sessile serrated lesions that can be removed at colonoscopy, preventing approximately 80% of cancers[7 8]. While colonoscopy is used as the main form of screening in several countries[9], there are risks associated with the procedure such as bowel perforations (3.1/10,000 procedures) and major bleeding (14.6/10,000)[10]. Colonoscopy can also be expensive, and many

countries have limited capacity and resources for this procedure. Therefore, implementation of less-invasive strategies for screening for pre-cancerous lesions is needed.

The faecal occult blood test, in particular the one using immunochemical test (FIT) technology, which detects the level of human haemoglobin (Hb) in the stool, has been shown to have benefit in the early detection and prevention of CRC[10-12]. Most organised CRC screening programs around the world use FIT, mainly focusing on people at average risk (i.e., no family history[23] and/or no previous pre-cancerous lesions[13-15]). As outlined in a recent review[13], FIT appears to maintain both high sensitivity (range 55-100%) and specificity (range 77-97%) across 12 previous studies in the detection of CRC[14,24]. While FIT has high accuracy for CRC detection, it can only detect up to 40% of advanced adenomas[15] and only 16% of advanced sessile serrated lesions[16 17] (depending on the positivity threshold applied and the number of samples collected). Even though FIT has good sensitivity for detecting CRC, the participation rate in FIT CRC screening programs is low, mainly due to faecal aversion and other issues leading to low acceptability in consumers[18]. An earlier study reported 78% of those surveyed preferring blood-based tests over faecal tests[18]. Furthermore, 83% of consumers would also prefer to have blood-based tests over colonoscopy, indicating the high acceptance rates of blood sampling over current screening options [19]. This highlights the need for blood-based biomarkers, which may improve participation in CRC screening, as well as potentially increasing sensitivity for detection of pre-cancerous colonic lesions.

Blood based biomarker tests can target the various changes occurring along the advanced adenoma to carcinoma pathway, contributing to aberrant protein, metabolic and immune functions[20 21]. Following these early initiating events, hyperproliferation of the colorectal epithelial cells can lead to the formation of polyps, which if left in place, can become adenomas and ultimately become invasive cancer. An alternate pathway (proposed more recently) is that of the alternative serrated pathway (15-30% of CRC)[22], where the precursor lesion is the sessile serrated lesion. A useful diagnostic blood biomarker should be sensitive and specific for detecting early neoplastic transformation as well as for CRC, and have clinical accuracy to allow for optimal detection of CRC and pre-cancerous lesions[23 24]. To date, there are no reviews investigating the accuracy of blood-based biomarkers for detection of advanced colonic adenomas and/or sessile serrated lesions. This project aims to investigate the sensitivity and specificity of blood-based biomarkers for the detection of advanced colonic adenomas and advanced sessile serrated lesions.

Objectives

1. To evaluate the accuracy (sensitivity and specificity) of blood-based biomarkers for detection of important pre-cancerous lesions, namely advanced colorectal adenomas and advanced sessile serrated lesions.
2. To determine if the accuracy of blood-based biomarkers is influenced by clinicopathological features of pre-cancerous lesions.

METHODS AND ANALYSIS

The protocol for this review was based on Cochrane guidelines[25 26], PRISMA guidelines[27 28] and other reviews already conducted in this area[29-31]. Registration was submitted to PROSPERO (International Prospective Register of Systematic Reviews), an international database for systematic reviews prospectively registered by the Centre for Reviews and Dissemination of the University of York (https://www.crd.york.ac.uk/prospero).

Patient and public involvement

The development of the research question and outcomes measures has been informed by patients' priorities, experience and preferences through regular contact with consumer groups. Patients will not be involved in the analysis and data collection of the systematic review and meta-analysis.

Eligibility criteria

Population

People over the age of 18 of either sex with a diagnosis of advanced adenomas and/or sessile serrated lesions based on colonoscopy findings, who have also had any blood-based biomarker test, will be included in the study. Advanced adenoma features are defined as advanced are polyp size ≥ 10mm, villous features, or high-grade dysplasia, whereas advanced sessile serrated lesions include those with dysplastic changes[32], and/or size ≥ 10mm[5]. Studies investigating high-risk patients (e.g., familial risk and hereditary syndromes) will be excluded, as this does not represent the average population risk for development of colorectal adenomas or sessile serrated lesions. Furthermore, only studies published after 2006 will be included given the increase in the number of studies from this period and the changes in technology to accurately detect blood-based biomarkers.

Intervention

This review will consider studies that evaluate diagnostic accuracy (sensitivity and specificity) of blood-based biomarkers for detection of advanced adenomas and/or advanced sessile serrated lesions. The blood-biomarker study must apply wet-lab based methods (e.g., by testing a biological mechanism that is thought to be dysregulated in cancer) in blood samples collected from patients. Further, sufficient detailed methodology about the technique used for testing, sample preparation methods and analytical technique used is required in order to assess the reliability and the validity of each blood-based biomarker.

Comparison

The studies must compare the diagnostic accuracy of the blood-biomarkers from individuals with colonic pre-cancerous lesions alongside 1) individuals with no evidence of colonic neoplastic disease, and/or 2) individuals with CRC.

Outcomes

The main outcomes to be evaluated are:

i) Accuracy: the sensitivity and specificity of a blood-based biomarker test to detect advanced pre-cancerous lesions(s);
ii) How the accuracy of the test to detect advanced adenomas/ sessile serrated lesions compares to its ability to detect CRC.

Additional outcomes may include:

a) Whether the blood test can detect adenomas/sessile serrated lesions in certain places of the colon (e.g., distal vs proximal);

b) The association between the blood test results and pathology of the pre-cancerous lesions;

c) The assessment of lifestyle factors influencing the accuracy of the blood test; and,

d) Whether there have been investigations into the cause of false positive blood test results in participants without adenomas, sessile serrated lesions or CRC.

Measures of effect: Sensitivity will be presented on a 0 (least sensitive) to 1 (most sensitive) scale on Forest plots produced from the analysis. Concurrent analysis of sensitivity/specificity will be presented on a ROC/AUC curve for the combination of all the tests.

Studies

Given initial searches so far, it is anticipated that the level of evidence for this review is most likely to be based on observational (and some experimental studies), including cohort, case-control, and cross-sectional designs. The inclusion and exclusion criteria for this review are summarised in Table 1.

Table 1: Summary of inclusion and exclusion criteria

Inclusion criteria	
Population	Adult patients of both sexes with a diagnosis of colonic advanced adenomas and/or advanced sessile serrated lesions based on colonoscopy outcomes as a part of screening, diagnostic or surveillance, and who have also had blood collected prior to polypectomy for biomarker analysis.
Intervention/Exposure	Blood-based biomarker methodology is explained in detail, including the nature of the biomarker (e.g. DNA, miRNA), quantitation technique, preparation method, accuracy for patients with colonic adenomas/sessile serrated lesions.
Comparison	Blood-based biomarker studies in non-cancer controls and/or those with colorectal cancers.
Study type	Quantitative observational and experimental studies.
Exclusion criteria	
Population	Those without a diagnosis of advanced colonic adenomas/sessile serrated lesions based on colonoscopy (i.e. otherwise healthy controls or those with CRC). Those where colonoscopy has been completed for familial risk conditions.
Intervention/Exposure	Non blood-based biomarkers (e.g., faecal biomarkers).
Study type	Studies not in English, published prior to 2006, review articles, articles investigating blood-based biomarkers with *in-vitro* or animal models. Those not including a measure of test sensitivity.

Information sources

Information sources will be restricted to publications in English and articles published after 2006. Specific search strategies using medical subjective heading (MESH) will be utilized where appropriate. The following databases will be used for the literature search: PubMed, Embase (OVID interface) and Web of Science. Authors who have published conference abstracts of work not yet published in peer reviewed journals as a full text original research article will be contacted to identify relevant unpublished literature. Grey literature will be included in the review via a Google Scholar search.

Search strategy

The search will be conducted by two authors (RG, TL) and informed by subject-specific expertise (ES, JW, MW, GY). The keyword search strategy was developed for PubMed and identified appropriate MESH keywords to ensure completeness of the search. If MESH search terms do not add any further hits, these will be removed and only keywords used to improve the precision of the search approach. Different search terms may be used to reflect differences across the three databases. The initial PubMed search strategy is included in the supplementary material (Supplementary Table 1).

Two authors (RG, TL) under the guidance of the other authors (ES, JW, MW, GY) will search all information sources to identify suitable studies and then independently screen the full-text articles of all eligible studies. Information extracted (using a specified extraction template) will be completed by RG, with accuracy checked by TL. There will be no blinding by author, research group and/or institution in the included studies.

Data management

The complete record for each eligible study (including citation, abstract and other identifiable information) will be imported into Endnote 20 (Clarivate Analytics). The full text version for all included studies will be obtained and store in Endnote 20. Screening will be carried out utilizing a predetermined template to reflect the above stated inclusion and exclusion criteria.

Selection process

The study selection process aims to reflect the best practice guidelines outlined in the Cochrane handbook[31]. Initial screening aims to only include studies aligning with the inclusion criteria readily identifiable from the title and abstract. The reason for exclusion for each study will be recorded. Where eligibility is unclear, the reviewers will obtain and review the full text of the article, using the predetermined screening template to ascertain eligibility for inclusion. Studies identified as unclear will be checked to determine the eligibility of the study.

Data collection process

Data will be extracted from studies by two independent reviewers (RG, MW) using a standardised extraction form, based on the Cochrane data extraction template[25] as a guide and entered into a Microsoft Excel spreadsheet. The data extracted will include study population (including age, gender, country, reason for colonoscopy), type of blood-based biomarker (e.g. methylated DNA), test method (e.g. serum vs plasma; analysis technique such as digital droplet PCR), type of study, number of participants, pathology details (e.g., type of pre-cancerous lesion, stage of CRC, how patients were classified as 'healthy' or without cancer), and outcomes of significance to the review objectives (i.e., the sensitivity/specificity of the blood-based biomarker for colonic adenomas/sessile lesions as well as CRC and non-neoplastic controls). Data extraction domains will involve: 1) article details (author, title, country of origin), 2) population, 3) methods, and 4) results. A standardised extraction sheet will be tested for completeness on a subset of studies, after which any relevant updates will be made prior to full extraction of all eligible studies. The extracted data will be reviewed for accuracy by TL and any discrepancies discussed and resolved as a group.

Data items

A summary of data items to be extracted from included studies is outlined in Table 2. Where data is not identifiable or unclear, attempts will be made to contact the corresponding author for clarification. On attempts to contact the author being unsuccessful within a set timeframe and the clarification potentially having an impact on the eligibility, the study will be deemed ineligible based on ambiguity. If there is evidence of overlapping samples, where the same cohort appears to have been used for multiple studies, the authors will be contacted to confirm eligibility. Where other types of biomarkers (e.g. tissue-based biomarkers) or blood-based biomarkers used for other advanced cancers are used, only the blood-based biomarkers specific to colonic advanced pre-cancerous lesions (and CRC) will be used for extraction.

Table 2: Summary of items to be extracted from eligible studies using the standardised extraction form

Information area	Data extracted
Background	Authors
	Year of publication
	Name of the blood-based biomarker
	Blood-based biomarker type (e.g., cfDNA, miRNA etc.)
Methodology	Specimen type (e.g., serum vs plasma)
	Study type
	Number of participants included
	Cohort included (e.g. no neoplasia detected, advanced adenoma, CRC)
	Technique used for blood-biomarker assay (e.g. qPCR)
Results	Accuracy of blood-based biomarker (sensitivity, specificity) for advanced adenoma/sessile serrated lesion
	Accuracy of blood-based biomarker (sensitivity, specificity) for CRC

CfDNA: circulating cell free DNA, CRC: colorectal cancer

Risk of bias

A formal risk of bias assessment will be conducted via the QUADAS-2 tool, as recommended in the Cochrane Handbook[25], which is used for evaluating potential bias (quality appraisal) of studies assessing diagnostic test accuracy. The following domains will be assessed: i) patient selection, ii) type of blood biomarker, iii) reference standard and iv) flow of patients through the study and timing of the index test(s) and reference standard ("flow and timing"). The tool will be completed according to four phases: 1) state the review question; 2) develop review specific guidance; 3) review the published flow diagram for the primary study or construct a flow diagram if none is reported; and, 4) judgement of bias and applicability. Each domain will then be assessed in terms of the risk of bias and the first three domains are also assessed for applicability. To help reach a judgement on the risk of bias, signalling questions will be included, such as "were the participants representative of the general population of those with advanced colorectal lesion?" and "was there an acceptable reference standard referred to?". These identify aspects of study design related to the potential for bias and aim to help reviewers make risk of bias judgements.

Data synthesis

Studies will be synthesised using a best evidence synthesis. Meta-analysis of diagnostic test accuracy can be carried out; however more sophisticated methods may be required to simultaneously analyse outcome measures (i.e., sensitivity and specificity). Methods such as bivariate model and hierarchical summary receiver operating characteristic (HSROC) model may be carried out.

In order to be considered for meta-analysis, the outcomes and the methodology of eligible studies must maintain homogeneity. For example, they need to have used the same type of test with the same type of comparators (i.e., a DNA methylation test for adenomas vs non-cancer controls). The process for meta-analysis will be more clear pending data extraction, based on assumptions of homogeneity remaining true. The remainder of this section is based on such an assumption of homogeneity in outcomes and methodology of the studies; however, some changes may be needed following data extraction.

The heterogeneity of the eligible studies will be assessed according to outcome categories, such as:

1. the sensitivity of the test to detect pre-cancerous lesion (advanced adenoma and /or sessile serrated lesion);
2. the blood test methodology has been clearly described;

3. the blood test considers the anatomical status (i.e. distal vs proximal) of the pre-cancerous lesion (advanced adenoma/sessile serrated lesion);
4. the association between the blood test results and pathological or histological features of the pre-cancerous lesion (advanced adenoma/sessile serrated lesion);
5. the association between the blood test results and CRC status;
6. the assessment of factors influencing the accuracy of the blood test;
7. the quality of the studies included according to the QUADAS-2 tool.

Where homogeneity is sufficient between groups within these categories, then inclusion within meta-analysis either as a large group or several subgroups will be determined by all authors. If the eligible studies are clearly homogenous, then each reviewer will place studies into appropriate sub-groups for analysis. This process will be done by each reviewer independently to determine which factors will allow the best and most accurate comparisons to be made. Relevant sub-groupings are likely to be made based on the methodological factors listed in Table 2 above. For example, sub-groupings according to whether the patient group(s) have different classifications of colonic adenoma(s)/sessile serrated lesion(s) would be considered, as appropriate for the eligible studies. If suitable, reviewers can include studies in more than one sub-group (i.e., different genomic analysis, different technique), however, in this instance the subgroups will not be used in the same meta-analysis. Where reviewers agree on what is to be grouped for each meta-analysis, this process will be carried out. If there is not broad consensus on the groups, further discussion will take place. If the reviewers disagree, the authors as a group will determine suitability of meta-analysis or meta-synthesis.

On a meta-analysis being deemed appropriate by the reviewers, a statistical test of heterogeneity will be carried out, providing an I^2 value in the heterogeneity of the sample[38].The I^2 value will be reported as a percentage and interpreted as suggested in the Cochrane Handbook for Systematic Reviews[31]. Significance in the measure of heterogeneity as calculated by the chi squared test, will be set at $p \leq 0.10$. In the event significance was reported, the I^2 statistic will be then explored to define the magnitude of heterogeneity about the finding, where 0-40, 30-60, 50-90 and 75+ are suggestive of low, moderate, substantial, and considerable heterogeneity, respectively[31]. In the instance of statistical heterogeneity, leave-one-out sensitivity analyses may be performed; however, groups considered to exceed the minimal value for heterogeneity will be ineligible for meta-analysis and hence considered for meta-synthesis instead.

Comparisons in some of the categories may be challenging to assess due to differences in biomarker selection as well as study design. Where meta-analysis has been decided as appropriate by the group, results will be extracted from the eligible studies and aggregated, with changes normalised and reported as percentage changes or standard mean differences in all studies. Where data are lacking, the authors of relevant studies will be contacted to provide further clarity on the data. If the data are not sufficiently homogenous, a critical/narrative synthesis will be focused from the data set alongside some binary elements of analysis. In this context, statements such as 'increase', 'decrease', or 'no change' in the accuracy of the proposed blood-based biomarkers will be described.

Confidence in cumulative evidence

The potential of publication bias will be minimised through a comprehensive search of unpublished studies, contacting respective authors in the field and including grey literature obtained via several further methods (e.g., snowballing of primary and review article reference lists). Conference presentations not carried through to publication will also be reviewed, with authors contacted. Further statistical tests, such as the Begg and Mazumbar's rank correlation test and Egger's linear

regression model, may be applied to each category and overall analyses. On publication bias being detected, Duval and Tweedie's trim and fill correction may be applied, and the resultant effect sizes and 95% confidence intervals examined in further detail.

The pooled data will be assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to evaluate the overall quality and 'certainty of recommendations' from the literature[33-35]. The GRADE approach will be used to determine the certainty and strength of evidence according to the categories (methodological and outcome based/results) in Table 2 and carried out in accordance with set recommendations. For example, observational studies will be assigned a 'low' certainty of recommendation prior to then either being upgraded or downgraded from this point, based on the quality of the evidence[36]. Studies will be upgraded for factors such as large effect sizes or mean test positivity is associated with more aggressive pre-cancerous lesions, blood-based biomarker characteristics and accuracy of the biomarker (sensitivity/specificity). Potential downgrading of studies for certainty of evidence may occur when there is substantial publication bias, indirect relationships with results (i.e., unexplained confounding) or inconsistencies between studies. From this process, qualitative ratings for the certainty of evidence and recommendations will be listed as 'high', 'moderate', 'low' or 'very low' and able to be interpreted according to the GRADE approach[34 36 37].

Contributorship statement

All authors have contributed to the focus of this systematic review topic and have revised and reviewed each draft of the protocol and approved the final manuscript.

Competing interests

Professor Young has a consultancy arrangement with a company that has developed a blood test for CRC detection (Clinical Genomics).

Funding

The authors have not declared a specific grant for this research from any funding agency in the public, commercial or non-for-profit sectors.

Data sharing statement

Data sharing not applicable as no datasets generated and/or analysed for this study

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Supplementary material: PubMed search 25th October 2021

Supplementary Table 1. Systematic review search results in PubMed

Search Number	Query	Results
1	Marker	1,520,779
2	Biomarker	1,009,617
3	"Biological marker"	3,104
4	"Molecular marker"	7,655
5	#1 OR #2 OR #3 OR #4	1,578,921
6	Blood	5,155,911
7	Serum	1,187,369
8	Plasma	987,178
9	Blood-based	3,792
10	Serum-based	1,221
11	Plasma-based	1,480
12	"Liquid biopsy"	5,495
13	#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12	5,937,796
14	Colorect*	199,866
15	Colon*	640,135
16	Bowel	536,671
17	Caec*	11,030
18	Rect*	244,366
19	"Large intestin*"	19,115
20	#14 OR #15 OR #16 OR #17 OR #18 OR #19	1,292,245
21	Adenoma*	120,722
22	Polyp	53,083
23	Serrated	4,934
24	#21 OR #22 OR #23	163,256
25	Diagnos*	5,574,405
26	Screen*	928,076
27	Detect*	2,567,713
28	"Early detection"	97,568
29	"Early diagnosis"	110,371
30	Test	2,972,332
31	#25 OR #26 OR #27 OR #28 OR #29 OR #30	9,851,696
32	#5 AND #13 AND #20 AND #24 AND #31	1172

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Where item can be found?
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Page 1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Page 3
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Page 1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 9
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Page 9
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	Pages 2 and 3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 3
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Page 4
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Page 5
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned	Page 5 and Supplementary Table 1

		limits, such that it could be repeated	
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 6
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Page 6
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Page 6
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Page 6 and 7
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 4 and 5
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Page 7
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	Page 7 and 8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	Page 8
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 8
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Page 8
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Page 8 and 9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 2 (abstract) and Page 9

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (2015 when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

BMJ Open

Accuracy of blood-based biomarkers for screening advanced pre-cancerous colorectal lesions: a protocol for systematic review and meta-analysis.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-060712.R1
Article Type:	Protocol
Date Submitted by the Author:	22-Mar-2022
Complete List of Authors:	Lathlean, Timothy JH; Flinders Centre for Innovation in Cancer, Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University Wassie, Molla; Flinders Centre for Innovation in Cancer, Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University Winter, Jean; Flinders Centre for Innovation in Cancer, Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University Goyal, Rishabh; Flinders University College of Medicine and Public Health, Department of Medicine Young, Graeme; Flinders Centre for Innovation in Cancer, Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University Symonds, Erin; Flinders Centre for Innovation in Cancer, Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University; Flinders Medical Centre, Bowel Health Service, Gastroenterology Department
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Genetics and genomics, Research methods
Keywords:	GASTROENTEROLOGY, Gastrointestinal tumours < ONCOLOGY, Colorectal surgery < SURGERY

Accuracy of blood-based biomarkers for screening pre-cancerous colorectal lesions: a protocol for systematic review and meta-analysis.

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Word count: 3202 words

Keywords:

Colorectal neoplasms; Biomarkers, adenoma; Sensitivity and Specificity; Diagnostics; Bowel.

Ethics statement:

No ethical approval is required for this protocol as this will be a collation of previously published literature.

ABSTRACT

Introduction Colorectal cancer (CRC) is the third most diagnosed cancer and the second most common cause of cancer mortality worldwide. Most CRCs develop through either the adenoma-to-carcinoma or the serrated pathways, and therefore detection and removal of these pre-cursor lesions can prevent the development of cancer. Current screening programs can aid in the detection of CRC and adenomas; however, participation rates are suboptimal. Blood-based biomarkers may help to address these low participation rates in screening programs. Although blood-based biomarker tests show promise for cancer detection, limited attention has been placed on the sensitivity and specificity for detection of the pre-cursor lesions. The aim of this research is to conduct a systematic review and meta-analysis to evaluate the accuracy of blood-based biomarker tests in detecting advanced pre-cancerous lesions.

Methods and Analysis This protocol was informed by the PRISMA-P and results will be reported in line with the PRISMA guidelines. Literature searches will be conducted on PubMed, Embase and Web of Science. Two reviewers will conduct the searches, and independently screen them, according to title and abstract and then the full-text versions of those selected articles as well as the risk of bias via the QUADAS-2. The GRADE guidelines will be used to validate the certainty of evidence for recommendations based on the risk of bias findings. Meta-analysis will be conducted where appropriate on groups of studies with low heterogeneity.

Ethics and Dissemination No patient data will be included in our review and therefore ethics approval is not required. It is anticipated that the review will identify the most promising candidate biomarkers for clinical translation in the screening of advanced pre-cancerous lesions. The results will be published in a peer-reviewed journal.

PROSPERO Registration CRD42021285173

STRENGTH AND LIMITATIONS OF THIS STUDY

- Comprehensive review of blood-based biomarkers, involving a thorough search strategy to identify studies relating to detection of advanced adenomas;
- This review will take a thorough approach, carrying out screening, quality appraisal and data extraction according to the independent duplicate method;
- Meta-analysis of the accuracy of blood-based biomarkers for advanced adenomas will be conducted to identify potential biomarkers upholding high diagnostic accuracy; and,
- A potential limitation may be the limited numbers of studies focusing on advanced adenomas in place or in addition to CRC.

INTRODUCTION

Colorectal cancer (CRC) is the third most diagnosed cancer and the second most common cause of cancer mortality worldwide[1]. In Australia, the age-standardised CRC incidence rate is approximately 50 per 100,000 people[2]. CRC screening programs (colonoscopy and/or faecal based screening measures) have demonstrated efficacy in reducing both CRC incidence and mortality[3 4]. CRC develops through the adenoma (or sessile serrated) carcinoma pathway, where pre-cancerous lesions such as advanced adenomas and sessile serrated lesions may progress to CRC[5 6]. The purpose of screening is to detect CRC at an early stage, enabling earlier interventions, which can lead to more efficacious treatment options and better patient outcomes including reduced morbidity and mortality. Furthermore, screening can also assist in the detection of pre-cancerous lesions, such as adenomas and advanced sessile serrated lesions that can be removed at colonoscopy, preventing approximately 80% of cancers[7 8]. While colonoscopy is used as the main form of screening in several

countries[9], there are risks associated with the procedure such as bowel perforations (3.1/10,000 procedures) and major bleeding (14.6/10,000)[10]. Colonoscopy can also be expensive, and many countries have limited capacity and resources for this procedure. Therefore, implementation of less-invasive strategies for screening for pre-cancerous lesions is needed.

The faecal occult blood test, in particular the one using immunochemical test (FIT) technology, which detects the level of human haemoglobin (Hb) in the stool, has been shown to have benefit in the early detection and prevention of CRC[10-12]. Most organised CRC screening programs around the world use FIT, mainly focusing on people at average risk (i.e., no family history[13] and/or no previous pre-cancerous lesions[14-16]). As outlined in a recent review[16], FIT appears to maintain both high sensitivity (range 55-100%) and specificity (range 77-97%) across 12 previous studies in the detection of CRC[15 17]. While FIT has high accuracy for CRC detection, it can only detect up to 40% of advanced adenomas[14] and only 16% of advanced sessile serrated lesions[5 18] (depending on the positivity threshold applied and the number of samples collected). Even though FIT has good sensitivity for detecting CRC, the participation rate in FIT CRC screening programs is low, mainly due to faecal aversion and other issues leading to low acceptability in consumers[19]. An earlier study reported 78% of those surveyed preferring blood-based tests over faecal tests[19]. Furthermore, 83% of consumers would also prefer to have blood-based tests over colonoscopy, indicating the high acceptance rates of blood sampling over current screening options[20]. This highlights the need for blood-based biomarkers, which may improve participation in CRC screening, as well as potentially increasing sensitivity for detection of pre-cancerous colonic lesions.

Blood based biomarker tests can target the various changes occurring along the advanced adenoma to carcinoma pathway, contributing to aberrant protein, metabolic and immune functions[6 21]. Following these early initiating events, hyperproliferation of the colorectal epithelial cells can lead to the formation of polyps, which if left in place, can become adenomas and ultimately become invasive cancer. An alternate pathway (proposed more recently) is that of the alternative serrated pathway (15-30% of CRC)[22], where the precursor lesion is the sessile serrated lesion. A useful diagnostic blood biomarker should be sensitive and specific for detecting early neoplastic transformation as well as for CRC, and have clinical accuracy to allow for optimal detection of CRC and pre-cancerous lesions[13 17]. To date, there are no reviews investigating the accuracy of blood-based biomarkers for detection of advanced colonic adenomas and/or sessile serrated lesions. This project aims to investigate the sensitivity and specificity of blood-based biomarkers for the detection of advanced colonic adenomas and advanced sessile serrated lesions.

Objectives

1. To evaluate the accuracy (sensitivity and specificity) of blood-based biomarkers for detection of important pre-cancerous lesions, namely advanced colorectal adenomas and advanced sessile serrated lesions.
2. To determine if the accuracy of blood-based biomarkers is influenced by clinicopathological features of pre-cancerous lesions.

METHODS AND ANALYSIS

The protocol for this review was based on Cochrane guidelines[23 24], PRISMA guidelines[25 26] and other reviews already conducted in this area[27-29]. Registration was registered with PROSPERO (International Prospective Register of Systematic Reviews), an international database for systematic reviews prospectively registered by the Centre for Reviews and Dissemination of the University of

York (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021285173). This study commenced on October 2021 and is anticipated to be ready for publication in May 2022.

Patient and public involvement

The development of the research question and outcomes measures has been informed by patients' priorities, experience and preferences through regular contact with consumer groups, including Cancer Voices Australia. These consumers had previous experience either as a patient or support person for someone with colorectal cancer or had experience with having adenomas detected at colonoscopy. Provision for 'ad hoc' comments on the research process will also be facilitated due to regular contact with these groups. Patients will not be involved in the analysis and data collection of the systematic review and meta-analysis.

Eligibility criteria

Population

People over the age of 18 of either sex with a diagnosis of advanced adenomas and/or sessile serrated lesions based on colonoscopy findings, who have also had any blood-based biomarker test, will be included in the study. Advanced adenoma features are defined as advanced are polyp size ≥ 10mm, villous features, or high-grade dysplasia[30-32], whereas advanced sessile serrated lesions include those with dysplastic changes[33], and/or size ≥ 10mm[5]. These definitions for advanced pre-cancerous lesions match Australian[34] and US[35] guidelines. Studies investigating high-risk patients (e.g., familial risk and hereditary syndromes) will be excluded, as this does not represent the average population risk for development of colorectal adenomas or sessile serrated lesions. Furthermore, only studies published after 2006 will be included given the increase in the number of studies from this period and the changes in technology to accurately detect blood-based biomarkers.

Intervention

This review will consider studies that evaluate diagnostic accuracy (sensitivity and specificity) of blood-based biomarkers for detection of advanced adenomas and/or advanced sessile serrated lesions. The blood-biomarker study must apply wet-lab based methods (e.g., by testing a biological mechanism that is thought to be dysregulated in cancer) in blood samples collected from patients. Further, sufficient detailed methodology about the technique used for testing, sample preparation methods and analytical technique used is required in order to assess the reliability and the validity of each blood-based biomarker.

Comparison

The studies must compare the diagnostic accuracy of the blood-biomarkers from individuals with colonic pre-cancerous lesions alongside 1) individuals with no evidence of colonic neoplastic disease, and/or 2) individuals with CRC.

Outcomes

The main outcomes to be evaluated are:

i) Accuracy: the sensitivity and specificity of a blood-based biomarker test to detect advanced pre-cancerous lesions(s);
ii) How the accuracy of the test to detect advanced adenomas/ sessile serrated lesions compares to its ability to detect CRC.

Secondary outcomes of interest are:

a) Whether the blood test can detect adenomas/sessile serrated lesions in certain places of the colon (e.g., distal vs proximal);

b) The association between the blood test results and pathology of the pre-cancerous lesions; and,

c) Whether there have been investigations into the cause of false positive blood test results in participants without adenomas, sessile serrated lesions or CRC.

Measures of effect: Sensitivity will be presented on a 0 (least sensitive) to 1 (most sensitive) scale on Forest plots produced from the analysis. Concurrent analysis of sensitivity/specificity will be presented on a ROC/AUC curve for the combination of all the tests.

Studies

Given initial searches so far, it is anticipated that the level of evidence for this review is most likely to be based on observational (and some experimental studies), including cohort, case-control, and cross-sectional designs. The inclusion and exclusion criteria for this review are summarised in Table 1.

Table 1: Summary of inclusion and exclusion criteria

Inclusion criteria	
Population	Adult patients of both sexes with a diagnosis of colonic advanced adenomas and/or advanced sessile serrated lesions based on colonoscopy outcomes as a part of screening, diagnostic or surveillance, and who have also had blood collected prior to polypectomy for biomarker analysis.
Intervention/Exposure	Blood-based biomarker methodology is explained in detail, including the nature of the biomarker (e.g. DNA, miRNA), quantitation technique, preparation method, accuracy for patients with colonic adenomas/sessile serrated lesions.
Comparison	Blood-based biomarker studies in non-cancer controls and/or those with colorectal cancers.
Study type	Quantitative observational and experimental studies.
Exclusion criteria	
Population	Those without a diagnosis of advanced colonic adenomas/sessile serrated lesions based on colonoscopy (i.e. otherwise healthy controls or those with CRC). Those where colonoscopy has been completed for familial risk conditions.
Intervention/Exposure	Non blood-based biomarkers (e.g., faecal biomarkers).
Study type	Studies not in English, published prior to 2006, review articles, articles investigating blood-based biomarkers with *in-vitro* or animal models. Those not including a measure of test sensitivity.

Information sources

Information sources will be restricted to publications in English and articles published after 2006. Specific search strategies using medical subjective heading (MESH) will be utilized where appropriate. The following databases will be used for the literature search: PubMed, Embase (OVID interface) and Web of Science. Authors who have published conference abstracts of work not yet published in peer reviewed journals as a full text original research article will be contacted to identify relevant unpublished literature. Grey literature will be included in the review via a Google Scholar search. White papers and industry databases were deemed outside the scope of the review.

Search strategy

The search will be conducted by two authors (RG, TL) and informed by subject-specific expertise (ES, JW, MW, GY). The keyword search strategy was developed for PubMed and identified appropriate MESH keywords to ensure completeness of the search. If MESH search terms do not add any further hits, these will be removed and only keywords used to improve the precision of the search approach. Different search terms may be used to reflect differences across the three databases. The initial PubMed search strategy is included in the supplementary material (Supplementary Table 1).

Two authors (RG, TL) under the guidance of the other authors (ES, JW, MW, GY) will search all information sources to identify suitable studies and then independently screen the full-text articles of all eligible studies. Information extracted (using a specified extraction template) will be completed by RG, with accuracy checked by TL. There will be no blinding by author, research group and/or institution in the included studies.

Data management

The complete record for each eligible study (including citation, abstract and other identifiable information) will be imported into Endnote 20 (Clarivate Analytics). The full text version for all included studies will be obtained and store in Endnote 20. Screening will be carried out utilizing a predetermined template to reflect the above stated inclusion and exclusion criteria.

Selection process

The study selection process aims to reflect the best practice guidelines outlined in the Cochrane handbook[23]. Initial screening aims to only include studies aligning with the inclusion criteria readily identifiable from the title and abstract. The reason for exclusion for each study will be recorded. Where eligibility is unclear, the reviewers will obtain and review the full text of the article, using the predetermined screening template to ascertain eligibility for inclusion. Studies identified as unclear will be checked to determine the eligibility of the study.

Data collection process

Data will be extracted from studies by two independent reviewers (RG, MW) using a standardised extraction form, based on the Cochrane data extraction template[23] as a guide and entered into a Microsoft Excel spreadsheet. The data extracted will include study population (including age, gender, country, reason for colonoscopy), type of blood-based biomarker (e.g. methylated DNA), test method (e.g. serum vs plasma; analysis technique such as digital droplet PCR), type of study, number of participants, pathology details (e.g., type of pre-cancerous lesion, stage of CRC, how patients were classified as 'healthy' or without cancer), and outcomes of significance to the review objectives (i.e., the sensitivity/specificity of the blood-based biomarker for colonic adenomas/sessile lesions as well as CRC and non-neoplastic controls). Data extraction domains will involve: 1) article details (author, title, country of origin), 2) population, 3) methods, and 4) results. A standardised extraction sheet will be tested for completeness on a subset of studies, after which any relevant updates will be made prior to full extraction of all eligible studies. The extracted data will be reviewed for accuracy by TL and any discrepancies discussed and resolved as a group.

Data items

A summary of data items to be extracted from included studies is outlined in Table 2. Where data is not identifiable or unclear, attempts will be made to contact the corresponding author for clarification. On attempts to contact the author being unsuccessful within a set timeframe and the clarification potentially having an impact on the eligibility, the study will be deemed ineligible based on ambiguity. If there is evidence of overlapping samples, where the same cohort appears to have

been used for multiple studies, the authors will be contacted to confirm eligibility. Where other types of biomarkers (e.g. tissue-based biomarkers) or blood-based biomarkers used for other advanced cancers are used, only the blood-based biomarkers specific to colonic advanced pre-cancerous lesions (and CRC) will be used for extraction.

Table 2: Summary of items to be extracted from eligible studies using the standardised extraction form

Information area	Data extracted
Background	Authors
	Year of publication
	Name of the blood-based biomarker
	Blood-based biomarker type (e.g., cfDNA, miRNA etc.)
Methodology	Specimen type (e.g., serum vs plasma)
	Study type
	Number of participants included
	Cohort included (e.g. no neoplasia detected, advanced adenoma, CRC)
	Technique used for blood-biomarker assay (e.g. qPCR)
Results	Accuracy of blood-based biomarker (sensitivity, specificity) for advanced adenoma/sessile serrated lesion
	Accuracy of blood-based biomarker (sensitivity, specificity) for CRC

CfDNA: circulating cell free DNA, CRC: colorectal cancer

Risk of bias

A formal risk of bias assessment will be conducted via the QUADAS-2 tool, as recommended in the Cochrane Handbook[23], which is used for evaluating potential bias (quality appraisal) of studies assessing diagnostic test accuracy. The following domains will be assessed: i) patient selection, ii) type of blood biomarker, iii) reference standard and iv) flow of patients through the study and timing of the index test(s) and reference standard ("flow and timing"). The tool will be completed according to four phases: 1) state the review question; 2) develop review specific guidance; 3) review the published flow diagram for the primary study or construct a flow diagram if none is reported; and, 4) judgement of bias and applicability. Each domain will then be assessed in terms of the risk of bias and the first three domains are also assessed for applicability. To help reach a judgement on the risk of bias, signalling questions will be included, such as "were the participants representative of the general population of those with advanced colorectal lesion?" and "was there an acceptable reference standard referred to?". These identify aspects of study design related to the potential for bias and aim to help reviewers make risk of bias judgements.

Data synthesis

Studies will be synthesised using a best evidence synthesis. Meta-analysis of diagnostic test accuracy can be carried out; however more sophisticated methods may be required to simultaneously analyse outcome measures (i.e., sensitivity and specificity). Methods such as bivariate model and hierarchical summary receiver operating characteristic (HSROC) model may be carried out.

In order to be considered for meta-analysis, the outcomes and the methodology of eligible studies must maintain homogeneity. For example, they need to have used the same type of test with the same type of comparators (i.e., a DNA methylation test for adenomas vs non-cancer controls). The process for meta-analysis will be more clear pending data extraction, based on assumptions of homogeneity remaining true. The remainder of this section is based on such an assumption of homogeneity in outcomes and methodology of the studies; however, some changes may be needed following data extraction.

The heterogeneity of the eligible studies will be assessed according to outcome categories, such as:

1. the sensitivity of the test to detect pre-cancerous lesion (advanced adenoma and /or sessile serrated lesion);
2. the blood test methodology has been clearly described;
3. the blood test considers the anatomical status (i.e. distal vs proximal) of the pre-cancerous lesion (advanced adenoma/sessile serrated lesion);
4. the association between the blood test results and pathological or histological features of the pre-cancerous lesion (advanced adenoma/sessile serrated lesion);
5. the association between the blood test results and CRC status;
6. the assessment of factors influencing the accuracy of the blood test;
7. the quality of the studies included according to the QUADAS-2 tool.

Where homogeneity is sufficient between groups within these categories, then inclusion within meta-analysis either as a large group or several subgroups will be determined by all authors. If the eligible studies are clearly homogenous, then each reviewer will place studies into appropriate sub-groups for analysis. This process will be done by each reviewer independently to determine which factors will allow the best and most accurate comparisons to be made. Relevant sub-groupings are likely to be made based on the methodological factors listed in Table 2 above. For example, sub-groupings according to whether the patient group(s) have different classifications of colonic adenoma(s)/sessile serrated lesion(s) would be considered, as appropriate for the eligible studies. If suitable, reviewers can include studies in more than one sub-group (i.e., different genomic analysis, different technique), however, in this instance the subgroups will not be used in the same meta-analysis. Where reviewers agree on what is to be grouped for each meta-analysis, this process will be carried out. If there is not broad consensus on the groups, further discussion will take place. If the reviewers disagree, the authors as a group will determine suitability of meta-analysis or meta-synthesis.

On a meta-analysis being deemed appropriate by the reviewers, a statistical test of heterogeneity will be carried out, providing an I^2 value in the heterogeneity of the sample[36].The I^2 value will be reported as a percentage and interpreted as suggested in the Cochrane Handbook for Systematic Reviews[23]. Significance in the measure of heterogeneity as calculated by the chi squared test, will be set at $p \leq 0.10$. In the event significance was reported, the I^2 statistic will be then explored to define the magnitude of heterogeneity about the finding, where 0-40, 30-60, 50-90 and 75+ are suggestive of low, moderate, substantial, and considerable heterogeneity, respectively[23]. In the instance of statistical heterogeneity, leave-one-out sensitivity analyses may be performed; however, groups considered to exceed the minimal value for heterogeneity will be ineligible for meta-analysis and hence considered for meta-synthesis instead.

Comparisons in some of the categories may be challenging to assess due to differences in biomarker selection as well as study design. Where meta-analysis has been decided as appropriate by the group, results will be extracted from the eligible studies and aggregated, with changes normalised and reported as percentage changes or standard mean differences in all studies. Where data are lacking, the authors of relevant studies will be contacted to provide further clarity on the data. If the data are not sufficiently homogenous, a critical/narrative synthesis will be focused from the data set alongside some binary elements of analysis. In this context, statements such as 'increase', 'decrease', or 'no change' in the accuracy of the proposed blood-based biomarkers will be described.

Confidence in cumulative evidence

The potential of publication bias will be minimised through a comprehensive search of unpublished studies, contacting respective authors in the field and including grey literature obtained via several further methods (e.g., snowballing of primary and review article reference lists). Conference presentations not carried through to publication will also be reviewed, with authors contacted. Further statistical tests, such as the Begg and Mazumbar's rank correlation test and Egger's linear regression model, may be applied to each category and overall analyses. On publication bias being detected, Duval and Tweedie's trim and fill correction may be applied, and the resultant effect sizes and 95% confidence intervals examined in further detail.

The pooled data will be assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to evaluate the overall quality and 'certainty of recommendations' from the literature[36-38]. The GRADE approach will be used to determine the certainty and strength of evidence according to the categories (methodological and outcome based/results) in Table 2 and carried out in accordance with set recommendations. For example, observational studies will be assigned a 'low' certainty of recommendation prior to then either being upgraded or downgraded from this point, based on the quality of the evidence[39]. Studies will be upgraded for factors such as large effect sizes or mean test positivity is associated with more aggressive pre-cancerous lesions, blood-based biomarker characteristics and accuracy of the biomarker (sensitivity/specificity). Potential downgrading of studies for certainty of evidence may occur when there is substantial publication bias, indirect relationships with results (i.e., unexplained confounding) or inconsistencies between studies. From this process, qualitative ratings for the certainty of evidence and recommendations will be listed as 'high', 'moderate', 'low' or 'very low' and able to be interpreted according to the GRADE approach[38-40].

Ethics and dissemination of results

No patient data will be included in our review and therefore ethics approval is not required. It is anticipated that the review will identify the most promising candidate biomarkers for clinical translation in the screening for advanced pre-cancerous lesions. The results will be published in a peer-reviewed journal and presented at appropriate domestic/international conferences.

Contributorship statement

All authors have contributed to the focus of this systematic review topic and have revised and reviewed each draft of the protocol and approved the final manuscript. Specifically, ES and GY were responsible for the initial conceptualisation of this work. TL, ES, MW, JW, ES and RG led the planning, design as well as the conduct and reporting of this manuscript. All authors made substantial contributions to the drafting and critical revision of the work and all authors approved the final manuscript.

Competing interests

Professor Young has a consultancy arrangement with a company that has developed a blood test for CRC detection (Clinical Genomics).

Funding

The authors have not declared a specific grant for this research from any funding agency in the public, commercial or non-for-profit sectors.

Data sharing statement

353 Data sharing not applicable as no datasets generated and/or analysed for this study

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Supplementary Table 1. Systematic review search results in PubMed

Search Number	Query	Results
1	Marker	1,520,779
2	Biomarker	1,009,617
3	"Biological marker"	3,104
4	"Molecular marker"	7,655
5	#1 OR #2 OR #3 OR #4	1,578,921
6	Blood	5,155,911
7	Serum	1,187,369
8	Plasma	987,178
9	Blood-based	3,792
10	Serum-based	1,221
11	Plasma-based	1,480
12	"Liquid biopsy"	5,495
13	#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12	5,937,796
14	Colorect*	199,866
15	Colon*	640,135
16	Bowel	536,671
17	Caec*	11,030
18	Rect*	244,366
19	"Large intestin*"	19,115
20	#14 OR #15 OR #16 OR #17 OR #18 OR #19	1,292,245
21	Adenoma*	120,722
22	Polyp	53,083
23	Serrated	4,934
24	#21 OR #22 OR #23	163,256
25	Diagnos*	5,574,405
26	Screen*	928,076
27	Detect*	2,567,713
28	"Early detection"	97,568
29	"Early diagnosis"	110,371
30	Test	2,972,332
31	#25 OR #26 OR #27 OR #28 OR #29 OR #30	9,851,696
32	#5 AND #13 AND #20 AND #24 AND #31	1172

Supplementary Table 2. Systematic review search results in EMBASE

Search Number	Query	Results
1	Marker	1,494,869
2	Biomarker	476,589
3	"Biological marker"	361,140
4	"Molecular marker"	21,712
5	#1 OR #2 OR #3 OR #4	2,040,222
6	Blood	2,577,094
7	Serum	208,082
8	Plasma	187,378
9	Blood-based	780,242
10	Serum-based	195,019
11	Plasma-based	162,152
12	"Liquid biopsy"	9,905
13	#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12	7,562,205
14	Colorect*	337,010
15	Colon*	986,230
16	Bowel	284,284
17	Caec*	14,509
18	Rect*	378,999
19	"Large intestin*"	110,687
20	#14 OR #15 OR #16 OR #17 OR #18 OR #19	1,650,217
21	Adenoma*	159,256
22	Polyp	78,303
23	Serrated	5,746
24	#21 OR #22 OR #23	214,866
25	Diagnos*	7,360,031
26	Screen*	1,504,388
27	Detect*	3,358,994
28	"Early detection"	104,936
29	"Early diagnosis"	192,795
30	Test	3,442,978
31	#25 OR #26 OR #27 OR #28 OR #29 OR #30	12,342,669
32	#5 AND #13 AND #20 AND #24 AND #31	1,895

Supplementary Table 3. Systematic review search results in Web of Science

Search Number	Query	Results
1	Marker	1,021,771
2	Biomarker	214,492
3	"Biological marker"	3,633
4	"Molecular marker"	12,620
5	#1 OR #2 OR #3 OR #4	1,188,941
6	Blood	2,771,325
7	Serum	1,195,351
8	Plasma	1,647,445
9	Blood-based	4,414
10	Serum-based	1,379
11	Plasma-based	4,984
12	"Liquid biopsy"	6,077
13	#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12	4,935,835
14	Colorect*	281,808
15	Colon*	885,669
16	Bowel	222,847
17	Caec*	12,419
18	Rect*	436,618
19	"Large intestin*"	12,692
20	#14 OR #15 OR #16 OR #17 OR #18 OR #19	1,601,963
21	Adenoma*	94,489
22	Polyp	44,085
23	Serrated	9,215
24	#21 OR #22 OR #23	132,732
25	Diagnos*	3,184,460
26	Screen*	1,089,598
27	Detect*	4,159,272
28	"Early detection"	78,759
29	"Early diagnosis"	78,824
30	Test	5,969,003
31	#25 OR #26 OR #27 OR #28 OR #29 OR #30	12,151,435
32	#5 AND #13 AND #20 AND #24 AND #31	821

Supplementary Table 4. Systematic review search results in PubMed

Search Number	Query	Results
1	Marker	1,520,779
2	Biomarker	1,009,617
3	"Biological marker"	3,104
4	"Molecular marker"	7,655
5	#1 OR #2 OR #3 OR #4	1,578,921
6	Blood	5,155,911
7	Serum	1,187,369
8	Plasma	987,178
9	Blood-based	3,792
10	Serum-based	1,221
11	Plasma-based	1,480
12	"Liquid biopsy"	5,495
13	#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12	5,937,796
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20	#14 OR #15 OR #16 OR #17 OR #18 OR #19	1,292,245
21	Adenoma*	120,722
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30	Test	2,972,332
31	#25 OR #26 OR #27 OR #28 OR #29 OR #30	9,851,696
32	#5 AND #13 AND #20 AND #24 AND #31	1172

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Where item can be found?
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Page 1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Page 3
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Page 1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 9
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Page 9
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	Pages 2 and 3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 3
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Page 4
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Page 5
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned	Page 5 and Supplementary Tables

		limits, such that it could be repeated	
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 6
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Page 6
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Page 6
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Page 6 and 7
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 4 and 5
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Page 7
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	Page 7 and 8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	Page 8
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 8
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Page 8
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Page 8 and 9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 2 (abstract) and Page 9

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (2015 when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.