Blood-Based Biomarkers and Novel Technologies for the Diagnosis of Colorectal Cancer and Adenomas. A Narrative Review.

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Abstract

Introduction

Faecal tests are most commonly used in triage and screening for colorectal cancer (CRC), however there is a high false positive rate and poor sensitivity for colorectal adenomas (CRA). Blood-based biomarkers for CRC and CRA have recently shown great promise but none are in common use. This review aims to summarise the recent studies in this area and to describe their potential use in CRC and CRA diagnosis.

Methods

A systematic literature search regarding blood-based biomarkers in CRC and CRA was undertaken in line with the PRISMA 2020 guidelines. Medline and Embase were searched for eligible English language studies between 01/01/2017 - 01/03/2023. Conference abstracts and duplicates were removed. Key criteria included a range of terms describing CRC, CRA, liquid biopsy, blood-based tests, and diagnosis.

Results

12378 studies were found by the initial literature searches and reduced to 178 for data extraction after title, abstract and full text reviews. 60 focussed on proteomics, 53 on RNA species, 30 on cfDNA methylation, 7 on antigens and autoantibodies, and 28 on other novel techniques. There were 169 case-control studies and 9 cohort studies. Number of participants ranged from 100 to 54297, with a mean age of 58.26. CRC diagnostic sensitivity and specificity ranged from 9.10 to 100% and 20.40 to 100% respectively. CRA vs control diagnostic sensitivity and specificity ranged from 8.00 to 95.70% and 4.00 to 97.00% respectively.

Conclusion

There is a growing field of acceptably sensitive and specific blood-based tests for CRC and CRA. However, current studies demonstrate a broad range of heterogenous techniques and reporting quality which makes selecting the best candidates difficult. Further work should concentrate on larger validation studies and high-quality meta-analyses to determine which tests may realistically be worth progressing into clinical use.

Main Body

Introduction

Background and Aims

Colorectal cancer (CRC) is the third commonest cancer world-wide, accounting for 11% of global cancer diagnoses with approximately 1.8 million new cases each year.[1] CRC is the second most deadly cancer globally, leading to approximately 16,800 deaths per year in the UK, or 10% of all cancer deaths.[2] Most CRC is known to develop from benign neoplasms derived from over-proliferation of mucosal epithelial cells, known as “polyps”, which may grow slowly for 5 to 10 years or more before completing transformation into CRC.[3] The most common benign neoplasm of the colon and rectum at risk of causing CRC is a colorectal “adenoma” (CRA), a polyp originating from glandular cells whose function is to produce mucus which lines the colorectal mucosa.[4] Only a small number of all CRAs progress to become invasive cancers but this likelihood rises with time, increasing polyp size and differs by subtype of adenoma.[3, 5] CRC arising from CRAs is known as adenocarcinoma and represents 96% of CRC cases.[6] For this reason, diagnosis at the polyp stage or as an early CRC is obviously preferable and confers a survival benefit.[7]

CRC and CRAs are most commonly diagnosed because of symptoms which prompt further investigation or via CRC screening programmes.[8] Diagnosis is confirmed by direct tissue biopsy and histopathology, which is normally obtained by endoscopic examination of the colon and rectum. However, there have been efforts in recent years to reduce the burden placed on endoscopy resources by developing adequately sensitive and specific tests which help stratify a patient’s risk of CRC and polyps. Presently, faecal tests which detect the presence of trace blood in stool samples are most commonly used, with the faecal immunochemical test (FIT) superseding the faecal occult blood (FOB) test in recent years.[9] Particularly in symptomatic populations, FIT is a useful “rule-out” test, with a negative predictive value of up to 99.8%.[10] However, FIT still has a high false positive rate and is less useful in identifying high-risk colorectal polyps, with a sensitivity of approximately 40% even at low FIT positivity thresholds.[11]

Blood-based biomarkers for CRC have been available for many years, with protein antigen biomarkers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) utilised as adjuncts in diagnosis and follow-up.[12, 13] None currently in common use have been shown to have sufficient accuracy to replace faecal-based tests. However, research in recent years has identified several classes of blood-based biomarkers and related technologies for the diagnosis of CRC and CRAs which show great promise. This narrative review aims to provide an updated summary of the broad range of recent studies in this area and to describe their potential use in the future of CRC diagnosis and screening.

The groups of biomarkers involved are explained below and can broadly be classified under proteomics, antigens and auto-antibodies, circulating tumour cells (CTCs), circulating (cell-free) DNA (cfDNA), ribonucleic acid (RNA) tests, and other technologies such as Raman spectroscopy and fluorescence spectroscopy.

Proteomics

Proteomics simply describes the study of proteins, a field which has expanded rapidly with widespread access to enzyme-linked immunosorbent assay (ELISA) and other rapid protein-assay technologies.[14] Myriad protein biomarkers for CRC and colorectal polyps have been described which are often associated with pathways involved in inflammation, tissue growth, invasion, migration, metastasis, vascular development, cell adhesion and cell death.[15-18] This wide breadth of protein biomarker studies has yielded some promising results, particularly where multiple protein biomarkers are combined in panels, which have been shown to result in sensitivity and specificity as high as 90%.[16] However, no individual protein biomarkers have been shown to consistently outperform CEA or FIT sufficiently to enter common use.

Antigens and Auto-Antibodies

Antigens and auto-antibodies can be considered as an important subset of proteomics. Antigens are proteins presented on the surface of all human cells, which are able to bind with antibodies - proteins essential to the adaptive immune system by identifying “non-self” antigens which may represent foreign cells. Auto-antibodies are those antibodies which bind with “self” or “non-foreign” antigens. In CRC, the two most commonly used antigen biomarkers are CEA and CA19-9, as described above, which are aberrantly expressed by CRC tissue.[19] CEA comprises a set of related glycosyl-phosphatidyl-inositol cell-surface glycoprotein antigens, which are highly expressed during embryonic development but are not produced normally by the time of birth. CA19-9 is a sialylated tetrasaccharide antigen normally involved in cell-cell recognition processes. Both show relatively poor overall sensitivity (though this increases with advancing tumour stage), confer a poor survival rate if significantly raised, and are most commonly utilised in monitoring for recurrence.[12, 20] CEA is more specific for CRC, with CA19-9 more commonly used for pancreaticobiliary cancers. CEA sensitivity in the diagnosis of CRC is known to be 30-80% depending on cut-off and tumour stage, and specificity is >90%, though it has also been shown to be raised in benign colorectal conditions.[12] Many studies have examined both "tumour-associated" and "tumour-specific" antigens, as well as auto-antibodies against these antigens such as p53, c-myc, p62 and koc. However, the same pattern of low sensitivity and high specificity remains prevalent.[21]

Circulating tumour cells (CTCs)

CTCs are shed from the primary tumour and/or metastases and are detectable in peripheral blood samples.[22] This process appears to begin much earlier than previously thought, from oncogenesis onwards, and can be used to diagnose even early-stage cancers.[23] However, their presence in peripheral blood indicates an increased risk of distant spread and has been shown to confer poorer rates of long-term disease-free survival.[24] It has been suggested that this is because their presence in peripheral blood is indicative of readily-shed, circulating cancer cells which are therefore more likely to result in metastases. Detection of CTCs can be challenging due to their very low concentration and involves enrichment (isolation of CTCs) before detection, normally by staining and microscopy or polymerase chain reaction (PCR) techniques.[25] CTCs have previously been shown to have good diagnostic accuracy for CRC, with sensitivity and specificity of 82% and 97% respectively in one recent meta-analyses.[26] Specificity is high by the very nature of CTCs, however false positives do occur (in benign colorectal disease, for example) and have been attributed to circulating epithelial cells with borderline phenotype. The addition of further genomic analysis, such as fluorescence in-situ hybridisation (FISH), or single-cell analysis has been suggested to avoid this but at the cost of increased time and resources.[27]

Circulating (cell-free) DNA (cfDNA)

cfDNA has been detected in peripheral blood since the 1940s, even before the double-helix structure of DNA was described.[28] cfDNA is released frequently from apoptotic or necrotic cancer cells, and infrequently from living cells.[29] In recent years its use as a biomarker for CRC has been explored by investigating properties such as overall cfDNA level, methylation, integrity, microsatellite instability and somatic mutations of known oncogenes or tumour-suppressor genes (e.g. APC, KRAS, p53).[30] As for CTCs, cfDNA can be difficult to isolate and detect in blood owing to issues such as variable levels in plasma versus serum and a half-life ranging from minutes to hours, making detectable concentrations inconsistent.[31] However, reasonable sensitivity and specificity have previously been reported at 71-78% and 87-94% respectively, depending on the characteristic studied.[26] DNA methylation in particular has been highly studied because of its early and frequent occurrence in cancer, relatively easy detection via established techniques, stability in fixed samples, and cell-type specificity. Studies have tended to focus on hypermethylation of “promoter CpG islands”, which are DNA regions which regulate gene expression through transcriptional silencing. When hypermethylation of these areas occurs in association with tumour-suppressor genes their expression is downregulated and this has been shown to be common amongst the myriad genetic changes present in early cancer formation.[32] Two of the only FDA-approved tests commercially available for the diagnosis of CRC which do not involve the detection of blood utilise cfDNA methylation: Cologuard (genes NDRG4 and BMP3 in faecal DNA) and Epi-pro-Colon (gene SEPT9 in peripheral blood).

Ribonucleic Acid (RNA)

Many varieties have been investigated in recent years including RNA, messenger-RNA (mRNA), micro-RNA (miRNA), long non-coding RNA (lncRNA), circular RNA (circRNA), piwi-interacting RNA (piRNA) and other “small RNAs”. These may be isolated from serum, plasma, exosomes or other extracellular vesicles and are involved in regulating gene expression.[33]

RNA species comprise a single chain of nucleotides derived from a corresponding length of source DNA, however their size and function varies:

- mRNA (variable length) is created by direct transcription from DNA and codes for the formation of specific proteins by ribosomal translation. This is the first and main pathway by which genes are expressed – how genotype becomes phenotype.
- Small RNAs are short lengths of RNA comprising fewer than 200 nucleotides, of which most are thought to be non-coding.
- miRNAs (21-24 nucleotides in length) and piRNA (26-31 nucleotides) are short non-coding lengths of RNA which are known to act to silence RNA or regulate post-transcriptional gene expression.
- lncRNAs (>200 nucleotides in length) are thought to be mostly non-functional or biologically irrelevant but may be involved in transcriptional regulation.
- circRNAs are simply RNAs in a circular (rather than linear) structure and whose function may be as for any other RNA.

Most methods examining RNA species in clinical practice involve amplification by real-time reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) which remains comparatively costly, time-consuming and prone to issues with sample exclusion due to the relative lability of RNA species.[34] In RT-qPCR, the extracted target RNA of interest is first converted into a complimentary DNA (cDNA) strand by adding a specific RNA primer and the enzyme "reverse transcriptase". This cDNA template is then used to create exponential amplification of the original target RNA by use of further targeted primers and the enzyme "DNA polymerase" in repeated cycles. Diagnostic accuracy is improving with isolation of the most reliable markers and grouping into panels, however prior meta-analyses have continued to show overall sensitivity and specificity ranging between 70-80%.[35-38]

Other tests

Several other areas of research including metabolomics, lipidomics and specific analysis of standard clinical blood tests have also yielded promising results.[39-41] However, highly accurate peripheral blood-based tests are now emerging which involve novel technologies such as mass spectroscopy, Raman spectroscopy and fluorescence spectroscopy, often in conjunction with machine learning techniques due to the high dimensionality and scalability of data analysis required.

Metabolomics involves the study of metabolites: small molecules involved in – and produced by – cell physiology and metabolic processes, whilst lipidomics can be considered as a subset of metabolomics. Lipidomics involves the identification of pathological lipid profiles where metabolic processes such as fatty acid synthesis, desaturation, elongation and mitochondrial oxidation have been disrupted in cancer cells.[42]

Spectroscopic tests involve the interaction of electromagnetic radiation (EMR) with the sample being studied. EMR spectra are produced which can be used to measure how the received frequency or wavelength of the detected EMR has been altered compared with the emitted EMR due to interaction with a sample. Known molecules and other particles have been shown to alter EMR in specific patterns using these methods, revealing a constituent molecular fingerprint. Mass spectroscopy involves ionising a sample, accelerating the charged molecules by exposure to an appropriate electromagnetic field, then detecting the constituent molecular components by measuring their mass-to-charge ratio.[43] Fluorescence spectroscopy involves exposing a sample to a given wavelength of EMR as light, normally ultraviolet light, which excites electrons. The movement of electrons between energy levels causes them to emit light (i.e photons), and the comparison of detected vs emitted light EMR is used to infer the molecular constituents of a sample.[44] Raman spectroscopy employs similar basic principles, normally involving a laser light source.

Spectroscopic tests produce versions of what could be considered a sample's molecular fingerprint, which involves large amounts of data and may encompass multiple individual biomarkers of cancer and other pathologies. These tests have been shown to give sensitivity and specificity of greater than 90%, however, there remain issues with data analysis and interpretation, cost, stability, hardware inter-reliability and scalability of these technologies.[45-47]

Methods

A systematic literature search regarding blood-based biomarkers in CRC and CRA was undertaken in line with the PRISMA 2020 guidelines.[48] Medline and Embase were searched for eligible English language studies between 1st January 2017 and 1st March 2023. Conference abstracts and duplicates were removed. A detailed PRISMA flowchart can be seen in Figure 1 and the full search strategy with included terms can be seen in Appendix 1. Key criteria included a range of terms describing CRC, CRA, liquid biopsy, blood-based tests, and diagnosis.

Three reviewers then undertook a title and abstract review. The inclusion criteria were: adult patients aged 18 and over, both sexes, diagnosis of colorectal carcinoma and/or adenoma, blood collected prior to cancer or adenoma treatment, blood-based biomarker methodology explained in detail, non-cancer controls included, at least 100 subjects, all study types except review articles. Exclusion criteria were: no diagnosis of colorectal carcinoma or adenoma, non-colorectal carcinoma neoplasms, less than 100 subjects, non-blood-based biomarkers, multi-cancer detection studies where colorectal-specific subgroups were not reported separately, non-English language, published prior to 2017, review articles, in-vitro or animal models, test sensitivity and/or uptake not recorded or could not be calculated. A specific reference standard test was not specified because histopathology is obligatory for the diagnosis of CRC and CRA. Single reviewer sign-off was used at this stage, with eligible studies progressing to full text review.

Three reviewers undertook full text review using identical criteria, as above. Dual reviewer sign-off was required, with all conflicts discussed and resolved prior to a final decision. Two rounds of blinded data extraction from eligible studies were then undertaken between three reviewers including: study design, biomarker type, specific biomarker(s), blood component (plasma vs serum vs other), processing method, inclusion and exclusion criteria, sample size, population characteristics, follow-up period, and test diagnostic performance for CRC +/- CRA. Sensitivity, specificity and AUC were recorded with 95% confidence intervals (95% CI), p-value, positive predictive value (PPV), negative predictive value (NPV), true positives (TP), false positives (FP), true negatives (TN), false negatives (FN) and test cut-off value where these were available. Risk of bias was assessed for each paper using the relevant Newcastle-Ottawa scale (NOS) for case-control and cohort studies. All data conflicts were then discussed and resolved before being entered into the final results. Detailed statistical meta-analysis was not undertaken due to the markedly heterogenous nature of the biomarkers, technologies, study types and reporting quality involved. Instead, a narrative review has been favoured with the aim of describing current literature in the field of blood-based biomarkers and novel techniques for the diagnosis of CRC and CRA. Where diagnostic statistics have been provided, results from standard blood-based biomarker comparators (CEA and CA19-9) have been removed beforehand to reflect the true performance of test(s) described.

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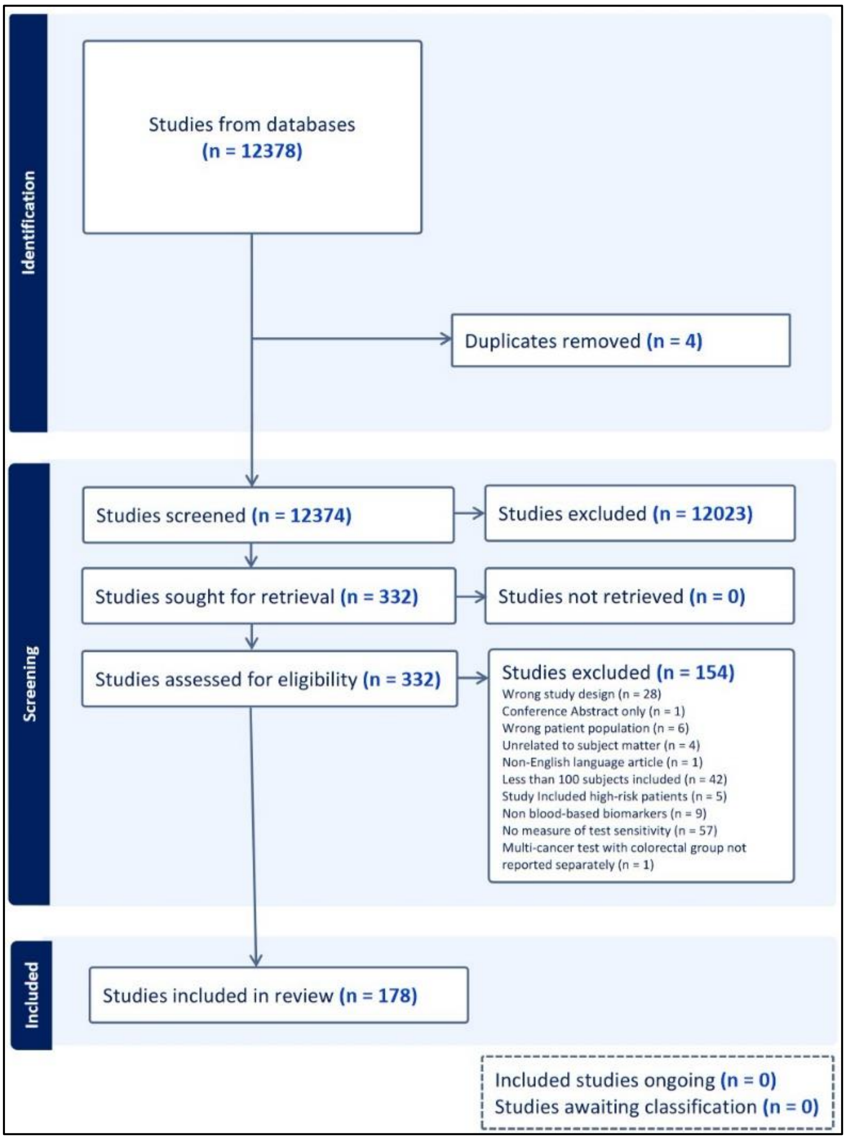
Results

A total of 12378 eligible studies were found by the initial literature searches and 4 duplicates were removed, leaving 12374 studies included in title and abstract review. 12042 studies were excluded at this stage, leaving 332 studies included in full text review. 154 papers were excluded at this stage, leaving 178 for data extraction. The review process is outlined in Figure 1.

Figure 1 – PRISMA Flowchart



Overall

Data were extracted from a total of 178 papers, comprising 60 focussed on proteomics, 53 on RNA species, 30 on cfDNA methylation, 7 on antigens and autoantibodies, and 28 on other novel techniques. 142 papers included data for CRC diagnosis alone, 2 for CRA alone, and 34 for both. There were 169 case-control studies and 9 cohort studies. 108 studies were obtained from China, 28 from Europe, 7 from Iran, 7 from Japan, 4 from the USA, 3 from multiple geographical areas, and 21 from other individual countries.

There were 23 studies involving symptomatic participants, 13 involving asymptomatic participants, 5 involving both populations, and 137 in which this was not stated. 112 studies used serum, 54 used plasma, 10 used whole blood and 2 used multiple blood sample types.

Number of participants ranged from 100 to 54297, with a mean age of 58.26 (95% CI 57.46 to 59.06) and male:female ratio of 1.34:1. CRC participants were distributed reasonably evenly between stages I+II (10732) and stages III+IV (11024) where this was recorded. CRC vs control diagnostic sensitivity, specificity and AUC ranged from 9.10 to 100%, 20.40 to 100% and 0.353 to 0.996 respectively. CRA vs control diagnostic sensitivity, specificity and AUC ranged from 8.00 to 95.70%, 4.00 to 97.00%, and 0.430 to 0.983 respectively.

Measures of uncertainty were poorly reported. 20 papers stated 95% CI for both sensitivity and specificity; 106 stated 95% CI for AUC; 58 stated p-values; 40 stated both PPV and NPV; and 4 stated TP, FP, TN and/or FN.

NOS score ranged from 4 to 8, with median 6 and interquartile range 6 to 7.

A breakdown of study characteristics, diagnostic results and risk of bias NOS for each blood-based biomarker subtype is provided below.

Proteomics

60 papers were obtained comprising 42856 participants (range 100 to 8415, mean 714.27). Mean age was 59.49, male:female ratio was 1.32:1 and CRC stage ratio was 0.86(I+II):1(III+IV). 48 papers involved CRC diagnosis, 12 papers involved both CRC and CRA diagnosis, and no papers involved CRA diagnosis alone. 7 papers

involved asymptomatic participants, 6 involved symptomatic participants, 1 involved both, and 46 did not record this information. 46 papers used serum, 13 used plasma and 1 used both. Study characteristics are summarised in Table 1.

CRC sensitivity, specificity and AUC ranged from 11.00 to 100%, 30.00 to 100%, and 0.530 to 0.990 respectively. CRC diagnostic data can be seen in Table 2.

CRA sensitivity, specificity and AUC ranged from 17.00 to 86.49%, 27.93 to 90.00%, and 0.532 to 0.790 respectively. CRA diagnostic data can be seen in Table 3.

NOS score ranged from 5 to 8, with median 6 and interquartile range 5 to 7. NOS data can be seen in Table 4.

An example of a paper reporting high AUC from this group is Liu et al (2020).[49] 313 participants were included, detailed population characteristics were given and results included sensitivity, specificity, AUC (with 95% CI), PPV, NPV, test cut-off value and p-value for CRC. They reported CRC diagnostic sensitivity, specificity and AUC of 86.76%, 97.76% and 0.968 (95% CI 0.949 to 0.986, $p<0.0001$) respectively for combined serum SYPL1 + CEA + CA19-9. NOS was 7. Sensitivity and specificity 95% CIs, TP/FP/TN/FN, and CRA diagnostic results were not directly reported.

An example of a paper reporting low AUC from this group is Jeun et al (2019).[50] 155 participants were included, detailed population characteristics were given and results included sensitivity, specificity and AUC (with 95% CI) for both CRC and CRA. They reported CRC diagnostic sensitivity, specificity and AUC of 44.4%, 86.7% and 0.670 (95% CI 0.570 to 0.770) respectively for plasma CCSP-2. They reported CRA diagnostic sensitivity, specificity and AUC of 43.3%, 86.7% and 0.670 (95% CI 0.530 to 0.800) respectively for plasma CCSP-2. NOS was 7. Sensitivity and specificity 95% CIs, test cut-off value, p-value, PPV/NPV and TP/FP/TN/FN were not directly reported.

<u>RNA Species</u>

53 papers were obtained (35 miRNA, 5 piRNA, 3 circRNA, 3 lncRNA, 3 mRNA, 2 other small RNA species, 1 RNA and 1 other long RNA species) comprising 15116 participants (range100 to 1899, mean 285.21). Mean age was 56.54, male:female ratio was 1.35:1 and CRC stage ratio was 1.14(I+II):1(III+IV). 42 papers involved CRC diagnosis, 9 papers involved both CRC and CRA diagnosis, and 2 papers

involved CRA diagnosis alone. 1 paper involved asymptomatic participants, 6 involved symptomatic participants, 2 involved both, and 44 did not record this information. 39 papers used serum, 12 used plasma and 2 used whole blood. Study characteristics are summarised in Table 5.

CRC sensitivity, specificity and AUC ranged from 45.20 to 100%, 34.00 to 100%, and 0.580 to 0.994 respectively. CRC diagnostic data can be seen in Table 6.

CRA sensitivity, specificity and AUC ranged from 63.20 to 95.00%, 27.30 to 97.00%, and 0.600 to 0.978 respectively. CRA diagnostic data can be seen in Table 7.

NOS score ranged from 4 to 8, with median 6 and interquartile range 6 to 7. NOS data can be seen in Table 8.

An example of a paper reporting high AUC from this group is Herreros-Villanueva et al (2019).[51] 297 participants were included, detailed population characteristics were given and results included sensitivity, specificity, AUC (with 95% CI), PPV and NPV for both CRC and CRA. They reported CRC diagnostic sensitivity, specificity and AUC of 91.00%, 90.00% and 0.950 (95% CI 0.903 to 0.991) respectively for combined plasma miRNA19a, miRNA19b, miRNA15b, miRNA29a, miRNA335, and miRNA1. They reported CRA diagnostic sensitivity, specificity and AUC of 95.00%, 90.00% and 0.920 (95% CI 0.868 to 0.959) respectively for combined plasma miRNA19a, miRNA19b, miRNA15b, miRNA29a, miRNA335, and miRNA1. NOS was 6. Sensitivity and specificity 95% CIs, TP/FP/TN/FN, test cut-off value and p-value were not directly reported.

An example of a paper reporting low AUC from this group is Zhou et al (2021).[52] 237 participants were included, detailed population characteristics were given and results included sensitivity, specificity, AUC (with 95% CI), test cut-off value and p-value for CRC. They reported CRC diagnostic sensitivity, specificity and AUC of 69.05%, 67.50% and 0.716 (95% CI 0.636 to 0.798) respectively for serum miRNA-135a. NOS was 6. Sensitivity and specificity 95% CIs, PPV/NPV and TP/FP/TN/FN were not directly reported.

Aberrant cfDNA Methylation

30 papers were obtained (13 involving mSEPT9, 17 other), comprising 16305 participants (range100 to 4077, mean 543.5). Mean age was 61.44, male:female

ratio was 1.22:1 and CRC stage ratio was 0.99(I+II):1(III+IV). 20 papers involved CRC diagnosis, 10 papers involved both CRC and CRA diagnosis, and no papers involved CRA diagnosis alone. 2 papers involved asymptomatic participants, 2 involved symptomatic participants, 3 involved both, and 23 did not record this information. 7 papers used serum, 22 used plasma and 1 used whole blood. Study characteristics are summarised in Table 9.

CRC sensitivity, specificity and AUC ranged from 39.90 to 96.80%, 50.00 to 99.50%, and 0.670 to 0.989 respectively. CRC diagnostic data can be seen in Table 10.

CRA sensitivity, specificity and AUC ranged from 12.20 to 64.30%, 45.50 to 95.60%, and 0.510 to 0.840 respectively. CRA diagnostic data can be seen in Table 11.

NOS score ranged from 5 to 8, with median 7 and interquartile range 6 to 7. NOS data can be seen in Table 12.

An example of a paper reporting high AUC from this group is Zhang et al (2021).[53] 268 participants were included, detailed population characteristics were given and results included sensitivity (with 95% CI), specificity (with 95% CI), and AUC (with 95% CI) for both CRC and CRA. They reported CRC diagnostic sensitivity, specificity and AUC of 80.00% (95% CI 66.70 to 93.30%), 97.10% (95% CI 91.40 to 100%) and 0.911 (95% CI 0.834 to 0.988) respectively for a 4-marker plasma DNA methylation panel. They reported CRA diagnostic sensitivity, specificity and AUC of 54.40% (95% CI 41.50 to 67.30%), 45.50% (95% CI 22.70 to 68.20%) and 0.614 (95% CI 0.457 to 0.770) respectively for a 4-marker plasma DNA methylation panel. NOS was 7. PPV and NPV, TP/FP/TN/FN, test cut-off value and p-value were not directly reported.

An example of a paper reporting low AUC from this group is Ma et al (2021).[54] 135 participants were included, detailed population characteristics were given and results included sensitivity, specificity, AUC (with 95% CI) and test cut-off value for CRC. They reported CRC diagnostic sensitivity, specificity and AUC of 74.00%, 50.00% and 0.710 (95% CI 0.620 to 0.800) respectively for plasma methylated SEPT9. NOS was 6. Sensitivity and specificity 95% CIs, PPV and NPV, TP/FP/TN/FN and p-value were not directly reported.

Antigens and Autoantibodies

7 papers were obtained, comprising 3873 participants (range 110 to 2283, mean 553.29). Mean age was 60.87, male:female ratio was 1.54:1 and CRC stage ratio was 0.69(I+II):1(III+IV). 7 papers involved CRC diagnosis, no papers involved both CRC and CRA diagnosis, and no papers involved CRA diagnosis alone. No papers involved asymptomatic participants, 2 involved symptomatic participants and 5 did not record this information. 7 papers used serum and none used plasma or whole blood. Study characteristics are summarised in Table 13.

CRC sensitivity, specificity and AUC ranged from 25.00 to 95.00%, 39.3 to 100%, and 0.542 to 0.940 respectively. CRC diagnostic data can be seen in Table 14.

NOS score ranged from 5 to 8, with median 7 and interquartile range 6.5 to 7. NOS data can be seen in Table 15.

An example of a paper reporting high AUC from this group is Cai et al (2022).[55] 288 participants were included, detailed population characteristics were given and results included sensitivity, specificity, AUC (with 95% CI), PPV, NPV and p-value for CRC. They reported CRC diagnostic sensitivity, specificity and AUC of 71.9%, 89.9% and 0.940 (95% CI 0.896 to 0.985) respectively for combined serum CST4 and DR-70. NOS was 7. Sensitivity and specificity 95% CIs, TP/FP/TN/FN and test cut-off value were not directly reported.

An example of a paper reporting low AUC from this group is Rao et al (2021).[56] 2283 participants were included, detailed population characteristics were given and results included sensitivity, specificity, AUC (with 95% CI), test cut-off value and p-value for CRC. They reported CRC diagnostic sensitivity, specificity and AUC of 74.1%, 39.3% and 0.580 (95% CI 0.556 to 0.604) respectively for serum CA24. NOS was 6. Sensitivity and specificity 95% CIs, PPV, NPV and TP/FP/TN/FN were not directly reported.

<u>Other – Including Novel Techniques</u>

28 papers were obtained, comprising 72105 participants (range 100 to 54297, mean 2575.18). Mean age was 57.94, male:female ratio was 1.43:1 and CRC stage ratio was 1.12(I+II):1(III+IV). 7 papers involved mixed methods utilising standard blood tests, 5 Raman spectroscopy, 5 metabolomics, 3 fluorescence spectroscopy, 3 novel cfDNA or nucleosome analysis, 2 CTCs, 1 lipidomics and 2 mixed standard serum

biomarkers. 26 papers involved CRC diagnosis, 2 papers involved both CRC and CRA diagnosis, and no papers focussed on CRA diagnosis alone. 1 paper involved asymptomatic participants, 7 involved symptomatic participants and 20 did not record this information. 12 papers used serum, 7 used plasma, 7 used whole blood and 2 used both serum and plasma. Study characteristics are summarised in Table 16.

Raman spectroscopy CRC sensitivity, specificity and AUC ranged from 51.00 to 95.70%, 30.50 to 100%, and 0.402 to 0.996 respectively.

Fluorescence spectroscopy CRC sensitivity, specificity and AUC ranged from 82.00 to 88.00%, 81.00 to 95.20% and 0.820 to 0.940 respectively.

Metabolomics CRC sensitivity, specificity and AUC ranged from 57.00 to 99.30%, 42.30 to 100%, and 0.742 to 0.996 respectively.

CTCs CRC sensitivity, specificity and AUC ranged from 39.10 to 95.20, 86.00 to 100%, and 0.695 to 0.940 respectively.

Novel cfDNA and nucleosome analysis CRC sensitivity, specificity and AUC ranged from 85.80 to 97.40%, 86.20 to 94.80%, and 0.940 to 0.988 respectively.

Remaining papers involved mixed methods of utilising standard blood tests and biomarkers. CRC sensitivity, specificity and AUC ranged from 41.00 to 100%, 20.40 to 95.60, and 0.571 to 0.992 respectively.

CRC diagnostic data is summarised in Table 17.

Two papers involved CRA diagnosis. A CTCs paper found CRA sensitivity 79.2%, specificity 84.70% and AUC 0.868. A cfDNA fragment analysis paper found CRA sensitivity 95.7%, specificity 94.8% and AUC 0.983. CRA diagnostic data is summarised in Table 18.

NOS score ranged from 5 to 8, with median 6 and interquartile range 6 to 7. NOS data can be seen in Table 19.

An example of a paper reporting high AUC from this group is Nishiumi et al (2017).[46] 573 participants were included, detailed population characteristics were given and results included sensitivity, specificity, AUC test cut-off value and p-value for CRC. They reported CRC diagnostic sensitivity, specificity and AUC of 99.3%, 93.8% and 0.996 respectively for a multiple logistic regression model based on 8 selected metabolites analysed by plasma gas chromatography/triple-quadrupole mass spectrometry. NOS was 7. Sensitivity, specificity and AUC 95% CIs, PPV, NPV, TP/FP/TN/FN and test cut-off value were not directly reported.

An example of a paper reporting low AUC from this group is Huang et al (2019).[57] 332 participants were included, detailed population characteristics were given and results included sensitivity, specificity, AUC (with 95% CI) and test cut-off value for CRC. They reported CRC diagnostic sensitivity, specificity and AUC of 41.00%, 72.00% and 0.571 (95% CI 0.730 to 0.828) respectively for whole blood red cell distribution width to lymphocyte ratio. NOS was 6. Sensitivity and specificity 95% CIs, PPV, NPV, TP/FP/TN/FN and p-value were not directly reported.

<u>CEA + CA19-9</u>

CEA was included as an isolated test for the diagnosis of CRC in 63 studies. Sensitivity, specificity and AUC ranged from 13.00 to 100%, 29.90 to 100%, and 0.469 to 0.869 respectively.

CA19-9 was included as an isolated test for the diagnosis of CRC in 34 studies. Sensitivity, specificity and AUC ranged from 9.10 to 81.20%, 30.00 to 100%, and 0.353 to 0.777 respectively.

Table 1 – Proteomics – Study Characteristics

Paper	Area	Study Design	Biomarker type	Specific Biomarker(s)	Specimen	Sample size	Population	Age CRC	Age CRA	Age Control	Male CRC	Male CRA	Male Control	Female CRC	Female CRA	Female Control	CRC stage I	CRC stage II	CRC stage III	CRC stage IV
Chen 2017	Germany	Case control	Proteomics	GDF-15, AREG, FasL, Flt3L, TP53	Plasma	598	Asymptomatic	67	64	62	29	56	44	12	50	62	14	3	21	3
Croner 2017	USA	Case control	Proteomics	A1AG, CEA, CO9, DPPIV, MIF, PKM2, SAA, TFRC	Plasma	4435	Symptomatic	70		63	92		539	55		650	25	50	45	27
Fei 2017	China	Case control	Proteomics	RBP4, THBS2	Serum	618	Symptomatic				248		108	154		108				
Li 2017	China	Case control	Proteomics	TFF3	Serum	204	Not stated	66	62	60	58	23	17	69	12	25	26	101 (II+III+IV)		
Song 2017	China	Case control	Proteomics	Cyr61	Serum	382	Not stated	59	57	57	82	46	102	55	27	70	29	43	45	20
Wang 2017	China	Case control	Proteomics	Five peptides - m/z peaks 1895.3, 2020.9, 2080.7, 2656.8, 3238.5	Serum	382	Not stated	63		62	107		107	84		84	8	21	87	75
Wang 2017	China	Case control	Proteomics	MIC-1/GDF15	Serum	987	Not stated				295	11	265	178	14	224	51	153	201	68
Wilhelmsen 2017	Denmark	Cohort	Proteomics	AFP, CA19-9, CEA, hs-CRP, CyFra21-1, Ferritin, Galectin-3, TIMP-1	Serum, Plasma	4698	Symptomatic													
Xie 2017	China	Case control	Proteomics	TFF3	Serum	870	Not stated	59	57	54	212	169	117	134	133	105	82 (I+II)		132 (III+IV)	
Yu 2017	China	Case control	Proteomics	MST1	Serum	324	Not stated	61		61	108		66	90		60	38	59	71	30
Chen 2018	China	Case control	Proteomics	TRIM72	Serum	100	Symptomatic				43			17			16 (I+II)		44 (III+IV)	
Ding 2018	China	Case control	Proteomics	MR, CD163	Serum	253	Not stated	65		62	84		45	82		42				
Duan 2018	China	Case control	Proteomics	SETD7	Serum	191	Symptomatic	69	60	58	65	21	19	50	17	19	26	45	18	26
Kasanga 2018	China	Case control	Proteomics	HSP90Î	Plasma	153	Not stated	-		-	45		55	32		21				
Peng 2018	China	Case control	Proteomics	CNPY2	Serum	631	Not stated	59		35	249		107	181		94	107	107	108	108
Rho 2018	USA + Japan	Case control	Proteomics	BAG4, IL6ST, VWF, EGFR, CD44	Plasma	900	Screening													
Shinozaki 2018	Japan	Case control	Proteomics	LRG-FTG	Serum	130	Not stated	63		39	43		25	37		25	2	11	18	46
Uchiyama 2018	Japan	Case control	Proteomics	PDA018, PDA052, PDA066, PDB001, PDB007	Serum	176	Not stated	70	70	68	28	30	30	28	30	30	14	14	14	14
Wang 2018	China	Case control	Proteomics	MACC1	Serum	347	Not stated	60		58	141			65			98 (I+II)		108 (III+IV)	
Aiyao 2019	China	Case control	Proteomics	IL-33	Serum	217	Not stated	46		48	46		60	50		54	71 (I+II)		50 (III+IV)	
Bhardwaj 2019	Germany	Case control	Proteomics	AREG. MASP1. OPN. PON3. TR	Plasma	259	Asymptomatic	66	66	65	36	65	66	20	36	36	17	6	26	7
Cao 2019	China	Case control	Proteomics	IQGAP3, B7-H4, COX	Serum	203	Asymptomatic				69			49			22	32	34	30
Hou 2019	China	Case control	Proteomics	IGFBP-3	Serum	120	Not stated	62		62	34		25	36		25	28 (I+II)		15 (III+IV	
Jeun 2019	Rep. of Korea	Case control	Proteomics	CCSP-2	Plasma	155	Not stated	60	61	60	47	17	20	41	13	17	26	22	18	22
Jiang 2019	China	Case control	Proteomics	ITIH3, ITIH4, TIMP-1	Plasma	257	Not stated	61		59	57		79	44		77	10	15	19	57
Li 2019	China	Case control	Proteomics	CXCL7	Serum	560	Not stated	62		61	166		178	114		102	50	95	106	29
Li 2019	China	Case control	Proteomics	Î²-catenin	Serum	327	Not stated				86	53	39	74	50	25	81(I+II)		79 (III+IV)	
Sun 2019	China	Case control	Proteomics	CPNE3	Plasma	124	Not stated				61			31			41 (I+II)		51 (III+IV)	
Sun 2019	China	Case control	Proteomics	fibrinogen: pre-albumin ratio	Serum	1365	Not stated	58	57	55	252	252	252	203	203	203	49	164	177	65
Ucuncu 2019	Turkey	Case control	Proteomics	CCR5, CCL5, PDGF, EphA7	Serum	110	Not stated	56		52	46		22	24		18				
Wang 2019	China	Case control	Proteomics	CCL20, IL-17A	Serum	347	Not stated													
Yamaguchi 2019	Japan	Case control	Proteomics	IL-9, Eotaxin, G-CSF, TNF-alpha, IL-4, IL-8, IP-10	Plasma	153	Not stated	61		61	36		51	30		36				
Bhardwaj 2020	Germany	Case control	Proteomics	275 protein biomarkers	Plasma	259	Asymptomatic	66	66	65	36	65	66	20	36	36	17	6	26	7
Bhardwaj 2020	Germany	Case control	Proteomics	A1AT, APOA1, HP, LRG1, PON3	Plasma	454	Asymptomatic	66	65	65	36	64	63	20	35	36	17	6	26	7
Hu 2020	China	Case control	Proteomics	ANXA2	Serum	103	Not stated				41			18			36 (I+II)		23 (III+IV)	
Li 2020	China	Case control	Proteomics	Netrin-1	Serum	430	Mixed	56	52	54	129	37	203	36	13	62	26	65	55	19
Liu 2020	China	Case control	Proteomics	SYPL1	Serum	313	Not stated	61	57	60	94	45	53	57	28	36	57 (I+II)		94 (III+IV)	
Paczek 2020	Poland	Case control	Proteomics	CXCL-8	Serum	105	Not stated				30		25	29		21	25 (I+II)		23	11
Qiu 2020	China	Case control	Proteomics	IGFBP-7	Serum	222	Not stated	63		56	68		75	47		32	12	54	39	6
Rasmussen 2020	Denmark	Case control	Proteomics	AFP, CA19-9, CEA, CyFra21-1, Ferritin, Galectin-3, hs-CRP, TIMP-1	Plasma	4698	Symptomatic				306	384		206	305					
Saridemir 2020	Turkey	Case control	Proteomics	AMDL DR-70	Serum	146	Not stated	59		55	53		21	42		30	40	19	36	
Wang 2020	China	Case control	Proteomics	ALDH1B1, UQCRC1, CTAG1, CENPF	Serum	315	Not stated	56	59	59	80	43	65	50	32	45	22	37	48	12
Wang 2020	China	Case control	Proteomics	B7-H1, IL-10	Serum	153	Not stated				61			28			29 (I+II)		60 (III+IV)	
Xu 2020	China	Case control	Proteomics	HE4, MASP-2, DKK-1	Serum	129	Not stated	54		54	48		42	21		18	36 (I+II)		33 (III+IV)	
Acevedo-Leon 2021	Spain	Case control	Proteomics	GSH, GSSG	Serum	140	Not stated	68		64	52		36	28		24	44	26	9	
Huang 2021	China	Case control	Proteomics	MMP-7, MMP-9, MMP-11, TIMP-1, TIMP-2, CEA, CA19-9	Serum	227	Not stated	61		60	79		68	33		47	5	34	37	36

Jiang 2021	China	Case control	Proteomics	ITGB4	Serum	2145	Not stated	67	59	55	85	338	618	62	194	848	82 (I+II)		32 (III+IV)	
Pan 2021	China	Case control	Proteomics	N-glycans	Serum	362	Not stated	62	60	58	105	66	64	58	32	37	24	46	47	46
Sebzda 2021	Poland	Case control	Proteomics	CB, ATA, TSA	Serum	220	Not stated	63		61	98		19	87		16	22	52	72	39
Wang 2021	China	Case control	Proteomics	GOLPH3	Serum	186	Not stated				66			70			29 (I+II)		73 (III+IV)	
Wang 2021	China	Case control	Proteomics	EphA2, VEGF	Serum	175	Not stated	61		45	62		39	44		30				
Wang 2021	China	Case control	Proteomics	BDNF	Serum	173	Not stated				49			32			3	11	25	42
Yu 2021	China	Case control	Proteomics	ANG	Serum	781	Not stated	60	56	54	228	98	165	141	35	114				
Kleif 2022	Denmark	Cohort	Proteomics	CEA, hsCRP, HE4, ferritin	Plasma	8415	Asymptomatic										112	48	65	17
Li 2022	China	Case control	Proteomics	CXCL5, STC2, CHI3L1	Serum	887	Not stated	64	52	45	217	184	80	175	158	73				
Ma 2022	China	Case control	Proteomics	CEA, IL-10, IL-17A, TNF-alpha, IFN-gamma, TGF-beta	Serum	182	Not stated				33	12	28	20	15	22	23 (I-II)		30 (III-IV)	
Shi 2022	China	Case control	Proteomics	ATPase, AMPase	Serum	135	Not stated				58			29			40 (I+II)		47 (III+IV)	
Wang 2022	China	Case control	Proteomics	proteins with mass:charge 2899.38 - 877.3	Serum	246	Not stated	60	58	51	92	48		9	14					
Chu 2020	China	Case control	Proteomics	L1CAM	Serum	374	Not stated	60		58	133		113	96		32	27	89	90	22
Voronova 2020	Russia	Case control	Proteomics	ApoA1, ApoA2, ApoB, AFP, B2M, CA 19-9, CA 15-3, CA 125, CEA, CYFRA 21-1, HE4, hsCRP, D-dimer, LRG 1, PSA, RANTES, sVCAM 1, TTR, VEGFR 1	Serum	305	Not stated	63		48	46		99	56		104	16 (I+II)		86 (III+IV)	

Table 2 – Proteomics – CRC Diagnostic Tests

Paper	Test 1	Sens (%)	Spec (%)	AUC	95% CI	Test 2	Sens (%)	Spec (%)	AUC	95% CI	Test 3	Sens (%)	Spec (%)	AUC	95% CI	Test 4	Sens (%)	Spec (%)	AUC	95% CI	Test 5	Sens (%)	Spec (%)	AUC	95% CI	Test 6	Sens (%)	Spec (%)	AUC	95% CI
Chen 2017	**GDF-15, AREG, FasL, Flt3L**	63.4	80	0.81	0.73-0.88	**GDF-15, AREG, FasL, Flt3L and TP53**	66.7	80	0.82	0.74-0.90																				
Croner 2017	**ITT CRC classifier panel**	80	83	0.86	0.82-0.90																									
Fei 2017	**RBP4**	74.9	81.7	0.853	0.822-0.883	**THBS2**	64.6	87.1	0.794	0.759-0.828	**CEA**	68.3	85.5	0.817	0.784-0.851	**CA19-9**	45.6	75.6	0.634	0.587-0.678	**RBP4+THBS2**	83.3	84.3	0.911	0.888-0.933	**RBP4 + THBS2 + CA19-9 + CEA**	87.1	92.7	0.961	0.947-0.975
Li 2017	**TFF3**	74.2	94.8	0.889	0.846-0.933	**CEA**	62.2	72.7	0.715	0.643-0.787																				
Song 2017	**Cyr61**	83	97	0.935	0.902-0.968	**CEA**	43	96	0.772	0.718-0.827	**CA19-9**	20	98	0.668	0.604-0.732															
Wang 2017	**Diagnostic panel**	95.6	87.9	0.932																										
Wang 2017	**MIC-1**	43.8	96.7	0.866	0.843-0.887	**CEA**	36.6	95.9	0.728	0.699-0.756																				
Wilhelmsen 2017	**CA19-9**			0.52		**CEA**			0.65		**hs-CRP**			0.65		**TIMP-1**			0.630		**AFP, CA19-9, CEA, hs-CRP, CyFra21-1, Ferritin, Galectin-3 and TIMP-1**			0.76		**CEA, CyFra21-1, Ferritin and hs-CRP + age + gender**	80	66	0.83	
Xie 2017	**TFF3 + CEA**	89.39	87.85	0.941	0.912-0.970																									
Yu 2017	**MST1**	82.4	93.8	0.934	0.871-0.997	**CEA**	37.3	93.8	0.773	0.647-0.899																				
Chen 2018	**TRIM72**	81.7	75	0.829	0.745-0.912	**CEA**	56.7	100	0.707	0.605-0.810	**CA19-9**	40	100	0.75	0.657-0.843	**TRIM72 + CEA + CA19-9**	88.3	82.5	0.928	0.858-0.970										
Ding 2018	**MR**	54.82	80.46	0.721		**CD163**	62.65	80.46	0.611		**MR + CD163**	69.28	77.01	0.797		**CEA**	60.24	79.31	0.716		**CA19-9**	36.75	88.51	0.6276						
Duan 2018	**SETD7**	92.17	81.08	0.9477	0.912-0.983																									
Kasanga 2018	**HSP90Î±**	64.9	92.1	0.872		**CEA**	38.9	97.4	0.764		**CA19-9**	9.1	94.7	0.585		**HSP90Î± + CEA**	85.6	-	0.968											
Peng 2018	**CNPY2 isoform 2**	72.6	58.5	0.687	0.625-0.749	**CEA**	40.8	93.6	0.714	0.666-0.762	**CA19-9**	19.8	98.9	0.638	0.584-0.693	**CNPY2 isoform 2 + CEA + CA19-9**	62.7	81.8	0.786	0.740-0.832										
Rho 2018	**BAG4, IL6ST, VWF, EGFR and CD44**	73	90	0.86																										
Shinozaki 2018	**LRG-FTG**	80	74	0.86		**CA19-9**			0.68		**CEA**			0.85		**CEA + LRG-FTG**	84	90	0.91											
Uchiyama 2018	**5 peptide panel (BLOTCHIP)**	82	93	0.888																										
Wang 2018	**MACC1**	66.9	88.7	0.859	0.817-0.902																									
Aiyao 2019	**IL-33**	80.45	80.93	0.844	0.793 - 0.895	**CEA**	57.39	98.48	0.839	0.788-0.890	**CA19-9**	43.29	99.36	0.739	0.673-0.804															
Bhardwaj 2019	**AREG + MASP1 + OPN + PON3 +TR**	71	80	0.82	0.74-0.89																									
Cao 2019	**IQGAP3**	89.8	58.8	0.799	0.736-0.861	**B7-H4**	88.1	62.4	0.795	0.731-0.858	**COX-2**	79.2	69.4	0.796	0.737-0.856	**IQGAP3 + B7-H4 + COX-2**	94.1	74.5	0.926	0.887-0.966	**CEA**	60.3	71.8	0.786	0.725-0.847	**CA19-9**	50.2	81.2	0.777	0.714-0.840
Hou 2019	**IGFBP-3**	70	85.5	0.826	0.721-0.931	**CEA**	60	80	0.757	0.633-0.881	**IGFBP-3 + CEA**	75	90	0.842																
Jeun 2019	**CCSP-2**	44.4	86.7	0.67	0.57-0.77																									
Jiang 2019	**ITIH3**	67.9	52.5	0.638	0.571-0.704	**ITIH4**	78.2	76.3	0.801	0.745-0.857	**CEA**	63.3	89.7	0.816	0.754-0.878	**TIMP-1**	72.3	87.8	0.832	0.776-0.888	**ITIH3 + ITIH4**	76.3	85.1	0.827	0.776-0.877	**ITIH3 + ITIH 4 + CEA + TIMP-1**	91.7	90.8	0.962	0.940-0.985
Li 2019	**CXCL7**	85	80.71	0.862	0.831-0.890	**CEA**	71.07	82.14	0.834	0.800-0.863	**CA125**	85.71	61.79	0.749	0.711-0.785	**CA19-9**	46.43	92.5	0.697	0.657-0.735	**Combination**	87.14	87.5	0.933	0.909-0.952					

Li 2019	**Î²-catenin**			0.8		**CEA**			0.67		**Î²-catenin + CEA**	81.88	73.44	0.88																
Sun 2019	**exCPNE3**	67.5	84.4	0.791	0.698-0.885	**CEA**	54.3	93.7	0.728	0.640-0.816	**exCPNE3 + CEA**	84.8	81.2	0.833	0.758-0.907															
Sun 2019	**FPR**	72.7	74.3	0.801		**FAR**	59.3	72.5	0.707		**NLR**	51.2	64	0.565		**CEA**	53.6	87.5	0.746		**CA19-9**	48.8	75	0.652		**FPR+NLR+CEA+CA19-9**	83.5	68.8	0.843	
Ucuncu 2019	**PDGF-BB, EphA7, and CCL5**	87.9	87.5	0.894																										
Wang 2019	**CCL20**	93.1	89.64	0.936		**IL-17A**	24.8	98.3	0.879		**CCL20 + IL-17A**	93.3	93.3	0.976		**CEA**	46.7	86.7	0.596											
Yamaguchi 2019	**IL-9, Eotaxin, G-CSF and TNF-Î±**	65.2	83.9	0.819		**IL-4, IL-8, Eotaxin, IP-10 and TNF-Î±**	74.2	76.7	0.832																					
Bhardwaj 2020	**AREG + CEA + GZMB+ ITGAV + KRT19 + MCP1+ OPN+ PON3+ TR**	55	80	0.76	0.67 - 0.84																									
Bhardwaj 2020	**A1AT+APOA1+HP+LRG1+PON3**	68	80	0.79	0.70 - 0.86																									
Hu 2020	**Annexin A2**	88.1	68.2	0.852	0.779-0.926	**CEA**	62.7	84.1	0.836	0.761-0.910	**CA19-9**	27.1	97.7	0.693	0.592-0.795	**Annexin A2 + CEA**	86.4	84.7	0.931	0.887-0.976	**Annexin A2 + CA 19-9**	63.6	94.9	0.877	0.813-0.941					
Li 2020	**Netrin-1 (clinical)**	33.9	90	0.703	0.636-0.770	**Netrin-1 (screening)**	46	90	0.759	0.680-0.837																				
Liu 2020	**SYPL1**	86.09	91.01	0.948	0.923-0.974	**CEA**	52.32	92.14	0.654	0.585-0.722	**CA19-9**	24.5	100	0.567	0.495-0.639	**CEA + CA19-9**	54.31	90.11	0.658	0.590-0.726	**SYPL1 + CEA + CA19-9**	86.76	97.76	0.968	0.949-0.986					
Paczek 2020	**CXCL-8**	75	78	0.7775		**CEA**	49	96	0.7579		**CRP**	97	70	0.9101																
Qiu 2020	**IGFBP7**	93.9	64.5	0.815	0.754-0.877	**CEA**	78.3	29.9	0.541	0.465-0.617	**IGFBP7 + CEA**	99.1	57.9	0.815	0.755-0.875															
Rasmussen 2020	**AFP**	11	90	0.53		**CEA**	13	90	0.57		**CyFra21-1**	14	90	0.54		**hs-CRP**	11	90	0.57		**TIMP-1**	14	90	0.58						
Saridemir 2020	**DR-70**	47	88	0.68		**CEA**	30	85	0.469		**CA19-9**	20	92	0.549																
Wang 2020	**ALDH1**	62.31	73.87	0.7	0.63-0.77	**UQCRC1**	57.7	70.27	0.63	0.56-0.69	**CTAG1**	64.62	70.27	0.72	0.65-0.79	**CENPF**	64.34	67.27	0.67	0.61-0.74	**ALDH1 + UQCRC1 + CTAG1 + CENPF**	75.19	70	0.79	0.71-0.85					
Wang 2020	**B7-H1**	85.21	56.43	0.706		**IL-10**	72.24	41.87	0.571		**CEA**	57.09	81.65	0.743		**B7-H1+ IL-10**	90.63	75.18	0.879												
Xu 2020	**HE4**	69.6	84.1	0.81	0.736-0.883	**MASP-2**	98.6	52.2	0.738	0.650-0.827	**DKK-1**	78.3	91.3	0.876	0.815-0.936	**HE4 + MASP-2 + DKK-1**	87	89.9	0.939	0.899-0.978										
Acevedo-Leon 2021	**CEA**	26.3	100			**CA19.9**	17.5	100			**GSH**	78.8	100			**GSSG**	75	98.3			**GSSG/GSH**	98.8	98.3							
Huang 2021	**MMP-7**	87.4	51.3	0.708	0.640-0.775	**MMP-9**	82	46.1	0.669	0.599-0.739	**MMP-11**	51.4	73	0.639	0.567-0.712	**TIMP-1**	59.5	79.1	0.749	0.685-0.812	**TIMP-2**	53.2	72.2	0.648	0.577-0.720	**CEA + MMP-7 + TIMP-1**	70.3	91.3	0.89	0.849-0.930
Jiang 2021	**ITGB4**	52	89.4	0.761	0.685-0.837	**CEA**	32.7	95.7	0.632	0.546-0.718	**ITGB4+CEA**	71.4	85.4	0.762	0.686-0.837															
Pan 2021	**N-glycan machine learning model**	72																												
Sebzda 2021	**CB**	72	90	0.85		**TSA**	66	77	0.75		**ATA**	63	84	0.77		**CB + TSA + ATA**	88.2	100	0.95											
Wang 2021	**GOLPH3**	83.8	80	0.888	0.840-0.935	**CEA**	44.4	92	0.857	0.790-0.923	**CA19-9**	14.7	98	0.7	0.623-0.778	**GOLPH3 + CEA + CA19-9**	87.5	88	0.938	0.902-0.974										
Wang 2021	**EphA2**	45.28	79.71	0.622	0.545-0.694	**VEGF-A**	52.83	85.51	0.734	0.662-0.798	**CEA**	42.5	95.7	0.673	0.598-0.742	**EphA2 + VEGF-A + CEA**	60.4	92.8	0.781	0.712-0.839										
Wang 2021	**BDNF**	60.5	80.6	0.733	0.632-0.909	**CEA**	92.6	41.9	0.719	0.621-0.816	**BDNF + CEA**	85.2	67.7	0.823	0.737-0.909															
Yu 2021	**ANG**	67.8	71.8	0.74	0.705-0.744	**CEA**	36.9	96.8	0.77	0.735-0.802	**CA19-9**	12.2	100	0.636	0.598-0.674	**ANG + CEA + CA19-9**	78.9	68.1												
Kleif 2022	**Age, sex, CEA, hsCRP, HE4 and ferritin**	91	30	0.73	0.69-0.77																									
Li 2022	**CXCL5**	77.4	86.1	0.859	0.811-0.899	**STC2**	92.2	79.9	0.887	0.842-0.923	**CHI3L1**	59.1	84	0.783	0.728-0.831	**CXCL5, STC2 and CHI3L1 (Model 2)**	87.8	91.7	0.959	0.927-0.980	**CEA + CA19-9 (Model 1)**	59.1	83.3	0.804	0.750-0.851					
Ma 2022	**CEA**	59.5	78.8	0.713	0.65-0.77	**IL-17A**	97.1	62.3	0.88	0.88-0.92	**TNF-Î±**	96.2	54.7	0.88	0.83-0.91	**TGF-Î²**	98.1	40.8	0.61	0.54-0.67	**IL-10**	83	55.3	0.8	0.74-0.84	**CEA + IL-17A + TNF-Î±**	96.2	80.4	0.935	0.89-0.96
Shi 2022	**CEA**	67.8	93.5	0.869	0.811-0.926	**ATPase**	95.4	69.9	0.839	0.762-0.917	**AMPase**	75.9	73.9	0.769	0.680-0.859	**CEA + ATPase**	94.3	80.4	0.94	0.901-0.978	**CEA + AMPase**	97.7	73.9	0.933	0.893-0.974	**CEA + ATPase + AMPase**	92	87	0.956	0.918-0.986
Wang 2022	**Protein panel (classification tree model)**	81.8	66.75			**CEA**	55.6	91.3			**CA19-9**	65.4	65.2																	
Chu 2020	**L1CAM**	43.2	90.3	0.781	0.734-0.828																									
Voronova 2020	**15 marker SVM model**	95	97	0.99	0.96-1	**15 marker LDA model**	100	97	0.99	0.97-1																				

Table 3 – Proteomics - CRA Diagnostic Tests

Paper	Test 1	Sens (%)	Spec (%)	AUC	95% CI	Test 2	Sens (%)	Spec (%)	AUC	95% CI	Test 3	Sens (%)	Spec (%)	AUC	95% CI	Test 4	Sens (%)	Spec (%)	AUC	95% CI	Test 5	Sens (%)	Spec (%)	AUC	95% CI
Chen 2017	**GDF-15, AREG, FasL, Flt3L**	18.9	80	0.58	0.51-0.65	**GDF-15, AREG, FasL, Flt3L,TP53**	22	80	0.6	0.52-0.69															
Uchiyama 2018	**5 peptide panel (BLOTCHIP)**			0.532																					
Bhardwaj 2019	**AREG + MASP1 + OPN + PON3 +TR**	36	80	0.6	0.51 - 0.69																				
Jeun 2019	**CCSP-2**	43.3	86.7	0.67	0.53-0.80																				
Li 2019	**Beta-catenin**	86.41	51.56	0.74		**CEA**			0.59		**Beta-catenin + CEA**			0.73											
Bhardwaj 2020	**AREG + CEA + GZMB+ ITGAV + KRT19 + MCP1+ OPN+ PON3+ TR**	28	80	0.58	0.47 - 0.68																				
Bhardwaj 2020	**HP + LRG1 + PON3**	41	80	0.65	0.56 - 0.73																				
Rasmussen 2020	**CEA + Hs-CRP (HRA)**	19	90	0.63		**AFP + CEA + CyFra21-1 + Hs-CRP (HRA)**	17	90	0.63																
Wang 2020	**ALDH1**	75.68	63.06	0.74	0.66-0.82	**UQCRC1**	86.49	27.93	0.65	0.57-0.72	**CTAG1**	59.46	56.36	0.62	0.55-0.68	**CENPF**	62.67	67.27	0.7	0.63-0.77	**ALDH1 + UQCRC1 + CTAG1 + CENPF**	75.68	63.64	0.79	0.69-0.87
Jiang 2021	**ITGB4**	58.52	60.88	0.623	0.593-0.653																				
Pan 2021	**Machine learning model**	58																							
Yu 2021	**ANG (AA)**	66.2	64.9			**ANG (AA+CRC)**	65.3	71.7																	

Table 4 – Proteomics – Risk of Bias NOS

Paper	NOS - Selection	NOS - Comparability	NOS - Exposure	NOS - Overall score
Chen 2017	3	1	3	7
Croner 2017	3	1	3	7
Fei 2017	3	1	2	6
Li 2017	3	0	3	6
Song 2017	3	1	2	6
Wang 2017	3	2	2	7
Wang 2017	3	1	2	6
Wilhelmsen 2017	3	2	3	8
Xie 2017	3	1	2	6
Yu 2017	3	2	2	7
Chen 2018	3	1	2	6
Ding 2018	3	1	2	6
Duan 2018	3	1	2	6
Kasanga 2018	3	1	2	6
Peng 2018	3	0	2	5
Rho 2018	3	0	3	6
Shinozaki 2018	3	0	2	5
Uchiyama 2018	3	2	3	8
Wang 2018	2	1	2	5
Aiyao 2019	2	1	2	5
Bhardwaj 2019	3	1	3	7
Cao 2019	3	1	3	7
Hou 2019	3	2	2	7
Jeun 2019	3	1	3	7
Jiang 2019	3	1	2	6
Li 2019	3	1	2	6
Li 2019	3	0	2	5
Sun 2019	3	0	2	5
Sun 2019	3	1	2	6
Ucuncu 2019	3	0	2	5
Wang 2019	3	0	2	5
Yamaguchi 2019	3	2	2	7
Bhardwaj 2020	3	1	3	7
Bhardwaj 2020	3	1	3	7
Hu 2020	3	1	2	6
Li 2020	3	1	2	6
Liu 2020	3	1	3	7
Paczek 2020	3	1	2	6
Qiu 2020	3	0	2	5
Rasmussen 2020	3	1	3	7
Saridemir 2020	3	1	3	7
Wang 2020	3	1	3	7
Wang 2020	3	2	2	7
Xu 2020	3	2	2	7
Acevedo-Leon 2021	3	1	2	6
Huang 2021	3	1	3	7
Jiang 2021	3	1	3	7
Pan 2021	3	1	3	7
Sebzda 2021	3	1	2	6
Wang 2021	3	0	2	5
Wang 2021	3	1	3	7
Wang 2021	2	1	2	5
Yu 2021	3	1	2	6
Kleif 2022	4	1	3	8
Li 2022	3	1	3	7
Ma 2022	2	1	2	5
Shi 2022	3	0	2	5
Wang 2022	3	0	2	5
Chu 2020	2	1	2	5
Voronova 2020	3	0	2	5

Table 5 – RNA Species – Study Characteristics

Paper	Area	Study Design	Biomarker type	Specific Biomarker(s)	Specimen	Sample size	Population	Age CRC	Age CRA	Age Control	Male CRC	Male CRA	Male Control	Female CRC	Female CRA	Female Control	CRC stage I	CRC stage II	CRC stage III	CRC stage IV
Krawczyk 2017	Poland	Case control	miRNA	miR-506, miR-4316	Plasma	126	Not stated	68		59	38		39	18		31				
Liu 2017	China	Case control	miRNA	miR-206	Serum	118	Not stated				44			29				47	26	
Ng 2017	China	Case control	miRNA	miR-139-3p	Serum	207	Not stated													
Wang 2017	China	Case control	miRNA	miRNA-135a-5p	Serum	150	Not stated	63	61	60	32	21	27	28	19	23	37 (I+II)		23	
Wang 2017	China	Case control	miRNA	miR-210	Serum	370	Not stated	58		56	151			117			38	93	126	11
Bilegsaikhan 2018	China	Case control	miRNA	miR-338-5p	Serum	210	Symptomatic	59	61	35	47	36	49	33	14	31	12	30	34	4
He 2018	China	Case control	miRNA	miR-101	Serum	389	Not stated				171			92			153 (I+II)		110	
Liu 2018	China	Case control	miRNA	miR-27a, miR-130a	Plasma	350	Not stated	53	53	53	111	31	82	59	19	48	130	20	16	4
Tan 2018	China	Case control	miRNA	miR-199a	Serum	167	Not stated				65			42			43 (I+II)		41	23
Herreros-Villanueva 2019	Spain	Case control	miRNA	miRNA19a, miRNA19b, miRNA15b, miRNA29a, miRNA335, miRNA1.	Plasma	297	Mixed	72	63	60	50	73	51	46	28	49	20	23	34	14
Huang 2019	China	Case control	miRNA	miR-200c, miR-125b	Serum	125	Symptomatic	51		51	38		31	29		27	31 (I+II)		36 (III+IV)	
Marcuello 2019	Spain	Case control	miRNA	miR-15b-5p, miR-18a-5p, miR-29a-3p, miR-335-5p, miR-19a-3p, miR-19b-3	Serum	264	Asymptomatic	62	62	62	44	51	55	15	23	25	30	13	14	2
Sabry 2019	Egypt	Case control	miRNA	miRNA-210, miRNA-21, miRNA-126, VEGF, HIF-alpha	Serum	187	Not stated	52	50	48	19	34	52	16	17	49	4	14	12	5
Sahami-Fard 2019	Iran	Case control	miRNA	miR-143-3p, -424-5p	Serum	124	Not stated	61		59	32		35	30		27	34 (I-II)		28 (III-IV)	
Sun 2019	China	Case control	miRNA	miR-30a-5p	Serum	248	Not stated	56	54	56	87			51			28	55	34	21
Tan 2019	China	Case control	miRNA	miR-144-3p, miR-425-5p, miR-1260b	Plasma	255	Not stated	61		59	43		75	58		59	63 (I+II)		38 (III+IV)	
Wang 2019	China	Case control	miRNA	miR-663	Serum	378	Not stated	59			45			81			26	36	35	29
Zhang 2022	USA	Case control	miRNA	hsa-miR-5100, hsa-miR-1343-3p, hsa-miR-1290, hsa-miR-4787	Serum	1899	Not stated													
Zhao 2022	China	Case control	miRNA	miR-199a-5p, miR-627-5p	Serum	150	Not stated	67	63	64	41	42	20	19	18	10	37 (I-II)		23 (III-IV)	
Wang 2022	China	Case control	miRNA	miR-377-3p, miR-381-3p	Serum	347	Not stated				113			62			69 (I+II)		106 (III+IV)	
Shaker 2022	Egypt	Case control	miRNA	miR-944 & EphA7	Serum	300	Symptomatic	52	40	45	89	29	54	61	21	46	19	76	33	22
Nakamura 2022	Japan, USA, Spain	Case control	miRNA	miR-193a-5p, miR-210, miR-513a-5p, miR-628-3p	Plasma	259	Not stated	44		42	69		60	80		50	29	38	54	28
Du 2022	China	Case control	miRNA	miR-654-5p, miR-126, miR-10b, miR-144	Serum	319	Not stated				49		51	51		69	24	17	30	29
Shi 2021	China	Case control	miRNA	miRNAs (miR-126, miR-1290, miR-23a, miR-940)	Serum	135	Not stated				63		22	37		13	30	35	25	10
Elaguizy 2020	Egypt	Case control	miRNA	miRNA-18a, miRNA-21, miRNA-92a	Serum	100	Not stated	50		45	27		28	23		22	18 (I-II)		32 (III-IV)	
BaderElDin 2020	Egypt	Case control	miRNA	miRNA-21, miRNA-26a, miRNA-146a, Let-7c	Serum	129	Not stated	46		42	44		24	40		21	34	50		
Han 2021	China	Case control	miRNA	miR-15b, miR-16, miR-21, miR-31	Serum	628	Not stated	52	52	52	59	59	76	64	58	74				
Zhou 2021	China	Case control	miRNA	miR-135a, MMP-13	Serum	237	Not stated	54		55	78		72	39		48	42 (I+II)	-	49 (III)	26 (IV)
Abdul-Maksoud 2021	Egypt	Case control	miRNA	miR-29c, miR-149	Serum	240	Symptomatic	60	59	59	58	56	61	22	24	19	55 (I/II)	-	14	11
Wang 2020	China	Case control	miRNA	miR-1207-5p	Plasma	142	Not stated				40	24	16	24	18	20	29 (I+II)		35 (III+IV)	
Wu 2020	China	Case control	miRNA	miR-34c, miR-141	Serum	128	Not stated	47		45	44		38	20		26	22 (I+II)		42	0
Shi 2020	China	Case control	miRNA	miR-92a -1	Serum	216	Not stated				67			81			74 (I+II)		74 (III+IV)	
Jin 2020	China	Case control	miRNA	miR-4516, miR-21-5p	Serum	130	Not stated	51		42	38		28	42		22	44 (I+II)		36 (III+IV)	
Huang 2020	China	Case control	miRNA	miR-203a-3p, miR-145-5p, miR-375-3p, miR-200c-3p	Serum	270	Not stated	60		59	72		68	63		67	37 (I+II)		98 (III+IV)	
Cui 2020	China	Case control	miRNA	miR-1539	Serum	100	Not stated	59		56	29		29	22		20	19 (I+II)		31 (III+IV)	
Li 2020	China	Case control	circRNA	hsa_circ_0001900, hsa_circ_0001178, hsa_circ_0005927	Plasma	182	Not stated	66		63	62		45	40		35	54 (I+II)		48 (III+IV)	
Pan 2019	China	Case control	circRNA	hsa-circ-000477	Serum	200	Not stated	60		60	81		24	39		21	31	45	30	14
Lin 2019	China	Case control	circRNA	circ-CCDC66, circ-ABCC1, circ-STIL	Plasma	129	Not stated	63		59	24		31	21		30	19 (I+II)		26 (III+IV)	
Ding 2020	China	Case control	lncRNA	B3GALT5-AS1	Serum	251	Not stated				69			49			72 (I+II)		46 (III+IV)	
Ismail 2019	Egypt	Case control	lncRNA	H19, HOTAIR	Serum	116	Symptomatic	53			44			12						
Xu 2021	China	Case control	lncRNA	lncRNA 01410	Serum	139	Not stated	55	55	48	30	32		21	6		16 (I+II)		35 (III+IV)	
Vychytilova-Faltejskova 2018	Czech Republic	Case control	piRNA	piR-5937, piR-28876	Serum	679	Not stated	65		60	220		143	183		133	84	119	105	95
Qu 2019	China	Case control	piRNA	piR-001311, piR-004153, piR-017723, piR-017724, piR-020365	Serum	440	Not stated													
Mai 2020	China	Case control	piRNA	piRNA-54265	Serum	1045	Not stated													
Wang 2020	China	Case control	piRNA	piR-020619, piR-020450	Serum	680	Not stated													

Sabbah 2021	Egypt	Case control	piRNA	piRNA-823	Serum	159	Not stated	59		61	47		40	37		35	19	25	21	19
AbdelGhafar 2020	Egypt	Case control	mRNA	AEG-1	Serum	164	Symptomatic	57		55	57		43	29		35	5	39	42	0
Rodriguez-Cobos 2021	Spain	Case control	mRNA	mRNA Np73, TAp73, 133p53	Plasma	120	Not stated	71	62	59	22	24	10	20	25	19	5	9	23	5
Rodia 2018	Italy	Case control	mRNA	LGALS4, CEACAM6, TSPAN8, COL1A2	Whole blood	231	Mixed	67	62	63										
Xie 2021	China	Case control	RNA	m6A RNA	Whole blood	169	Not stated				69			36			6	20	31	26
Roberts 2018	USA	Cohort	small RNAs	miR-335-5p + un-annotated small RNA	Plasma	329	Not stated		58	58		87	79		71	92				
Wu 2021	China	Case control	tRNA-derived small RNAs	5-tRF-GlyGCC	Plasma	195	Not stated	59		52	63		54	42		36	11	34	32	25
Guo 2022	China	Case control	Extracellular vesicle long RNAs	exLRs - 17 gene diagnostic signature	Plasma	194	Not stated	61	56	60	48	24	54	24	18	26	22	31	3	16

Table 6 – RNA Species – CRC Diagnostic Tests

Paper	Test 1	Sens (%)	Spec (%)	AUC	95% CI	Test 2	Sens (%)	Spec (%)	AUC	95% CI	Test 3	Sens (%)	Spec (%)	AUC	95% CI	Test 4	Sens (%)	Spec (%)	AUC	95% CI	Test 5	Sens (%)	Spec (%)	AUC	95% CI	Test 6	Sens (%)	Spec (%)	AUC	95% CI
Krawczyk 2017	**miR-506**	60.7	76.8	0.747	0.662-0.820	**miR-4316**	83.9	60.9	0.744	0.658-0.817	**miR-506 + miR-4316**	76.8	75	0.751	0.666-0.824															
Liu 2017	**miR-206**	82.19	80	0.846																										
Ng 2017	**miR-139-3p**	96.6	97.8	0.9935	0.9868-1.000																									
Wang 2017	**miR-135a-5p**	76.67	88	0.818		**CEA**	58.33	86	0.709		**CA19-9**	58.33	68	0.627		**miR-135a-5p + CEA**	90	76	0.836											
Wang 2017	**miR-210**	74.6	73.5	0.821	0.778-0.859	**CEA**	49.2		0.701	0.651-0.747																				
Bilegsaikhan 2018	**miR-338-5**	76.3	92.5	0.923	0.882 - 0.964	**CEA**	66.3	78.8	0.787	0.716 - 0.857	**MiR-338-5 + CEA**	85	88.8	0.932	0.882 - 0.964															
He 2018	**miR-101**	68	71.7	0.732	0.658-0.806																									
Liu 2018	**miR-27a**	81.82	90.91	0.866	0.774-0.957	**miR-130a**	69.32	100	0.816	0.730-0.901	**miR-27a + miR-130a**	85.23	90.91	0.899	0.854-1.019															
Tan 2018	**miR-199a**	77.6	83.3	0.864																										
Herreros-Villanueva 2019	**miRNA19a, miRNA19b, miRNA15b, miRNA29a, miRNA335, miRNA1**	91	90	0.95	0.903-0.991	**miRNA19a, miRNA19b, miRNA15b, miRNA29a, miRNA335, miRNA1 (CRC+AA)**	85	90	0.92	0.871-0.962																				
Huang 2019	**miR-200c**	72.13	80.1	0.856	0.830-0.922	**miR-125b**	74.26	72.58	0.815	0.806-0.915	**miR-200c + miR-125b**	84.72	78.12	0.879	0.818-0.940															
Marcuello 2019	**6-miRNA**	81	56	0.74	0.65-0.82	**FIT +ve**	81	73	0.85	0.78-0.91	**6-miRNA + FIT**	81	78	0.88	0.83-0.94															
Sabry 2019	**miR-210**	88.6	90.1	0.934	0.873-0.995	**miR-21**	91.4	95	0.973	0.946-1	**miR-126**	88.6	50.5	0.665	0.571-0.759	**VEGF**	65.7	78.2	0.758	0.658-0.859	**HIF-alpha**	91.4	94.1	0.97	0.942-0.998					
Sahami-Fard 2019	**miR-424-5p**	72.6	79	0.703	0.605-0.801	**miR-143-3p**	74.2	61.3	0.724	0.632-0.813																				
Sun 2019	**miR-30a-5p**	77.5	78.3	0.858																										
Tan 2019	**miR-144-3p, miR-425-5p, miR-1260b**	93.8	91.3	0.954	0.914-0.994	**CEA**	35.4	87.2			**CA19-9**	22.9	87.2																	
Wang 2019	**miR-663**	83.1	73.58	0.806																										
Zhang 2022	**hsa-miR-5100, hsa-miR-1343-3p, hsa-miR-1290, hsa-miR-4787**	91.6	95	0.955																										
Zhao 2022	**miR-627-5p**	87	100	0.97		**miR-199a-5p**	93	70	0.9		**CEA**	32	100	0.7		**CA19-9**	12	100	0.54		**Combined model**	87	100	0.98						
Wang 2022	**miR-377-3p**	84	64	0.798	0.751-0.845	**miR-381-3p**	70.3	74.4	0.792	0.745-0.838	**miR-377-3p + miR-381-3p**	90.9	58.7	0.807	0.761-0.852															
Shaker 2022	**miRNA-944**	100	78.9	0.9	0.814-1.03	**EPHA7**	100	72	0.86	0.750-0.986																				
Nakamura 2022	**miR-193a-5p, miR-210, miR-513a-5p, miR-628-3p**	82	86	0.88	0.82-0.93																									
Du 2022	**MiR-654-5p, miR-126, miR-10b, miR-144**	91	34	0.913																										
Shi 2021	**miR-126**	84	88.57	0.94	0.90-0.98	**miR-1290**	85	88.57	0.92	0.87-0.97	**miR-23a**	91	74.29	0.89	0.83-0.95	**miR-940**	90	77.14	0.88	0.82-0.94	**Combined**	90	88.57	0.95	0.91â€' 0.99					
Elaguizy 2020	**miRNA-18a**	84	84	0.906		**miRNA-21**	90	90	0.918		**miRNA-92a**	66	68	0.672		**miRNA-18a + miRNA-21**	88	92	0.966		**miRNA-18a + miRNA-21 + miRNA-92a**	86	90							
BaderElDin 2020	**Let-7c**	77.6	100	0.855	0.77 - 0.941	**mi-RNA-21**	80.7	74.1	0.936	0.884 - 0.989	**mi-RNA-26a**	77.6	96.2	0.918	0.857 - 0.989	**mi-RNA-146a**	78	100	0.805	0.708 - 0.903	**Combined**	82.1	96.2	0.95	0.898 - 1.002					
Han 2021	**miR-15b**	81.33	91.8	0.86	0.82-0.91	**miR-16**			0.58	0.51-0.65	**miR-21**	91.95		0.75	0.69-0.81	**miR-31**	97.62		0.75	0.68-0.82	**miR-15b, miR-16, miR-21**	95.06	94.44							
Zhou 2021	**miR-135a**	69.1	67.5	0.716	0.636-0.798	**MMP-13**	45.2	99.2	0.723	0.616-0.830																				
Wang 2020	**miR-1207-5p**	95.31	94.44	0.985	0.987 -1.000																									
Wu 2020	**miR-34c**	84.38	68.75	0.857	0.795-0.919	**miR-141**	70.31	96.88	0.876	0.810-0.941	**miR-34c + miR-141**	84.38	93.75	0.929	0.884-0.974															
Shi 2020	**miR-92a-1**	81.8	95.6	0.914																										
Jin 2020	**miR-4516**	94.4	89.8	0.9584		**miR-21-5p**	90.63	86.2	0.9278		**CEA**	85.7	84.9	0.774		**miR-4516 + miR-21-5p**	92.11	87.6	0.9425											

Huang 2020	R-203a-3p, miR-145-5p, miR-375-3p and miR-200c-3p) for	81.3	73.3	0.893	0.846-0.940																									
Cui 2020	miR-1539	92.2	40.8	0.673	0.568-0.779																									
Li 2020	circPanel	67.65	90	0.874	0.816-0.918	CEA	63.73	80	0.724	0.653-0.788	circPanel + CEA	82.35	83.75	0.903	0.851-0.942															
Pan 2019	circulating exosomal hsa-circ-00047	80.91	82.86	0.88	0.815-0.940																									
Lin 2019	circ-CCDC66, circ-ABCC1 and circ-STIL	64.4	85.2	0.78	0.689-0.854																									
Ding 2020	CEA	61	72.7	0.718		CA19-9	17.8	83	0.47		B3GALT5-AS1	89	53.4	0.762		B3GALT5-AS1 + CEA	94.9	50			B3GALT5-AS1 + CA19-9	58.5	71.6			B3GALT5-AS1 + CEA + CA19-9	94.9	43.2		
Ismail 2019	HOTAIR	92.9	100	0.93	0.83-1	H19	92.9	100	0.93	0.83-1																				
Xu 2021	lncRNA01410	86.3	84	0.851		CEA	25.5	92	0.584		CA19-9	25.5	92	0.584		lncRNA01410 + CEA	89.8	77.3	0.832		CEA + CA19-9 + lncRNA01410	92.6	70	0.812						
Vychytilova-Faltejskova 2018	piR-hsa-28876	66	65.3	0.707		piR-hsa-5937	73.6	65.3	0.767		piR-hsa-28876 + piR-hsa-5937	70.4	71.4	0.765																
Qu 2019	piRNA panel	78	76	0.854	0.797-0.900	CEA + CA19-9	53	89	0.768	.0.704-0.825	piRNA panel + CEA + CA19-9	84	85	0.897	0.846-0.935															
Mai 2020	piRNA-54265	85.7	65.1	0.896	0.874-0.914																									
Wang 2020	piR-020619 and piR-020450	86	92	0.883		CEA	40	93	0.658		CA19-9	27	95	0.596																
Sabbah 2021	piR-823	83.3	89.3	0.933																										
AbdelGhafar 2020	Metadherin mRNA	91.9	92.3	0.976	0.958-0.993	CEA	60.5	79.4	0.808	0.743-0.872	CA 19-9	58.1	79.5	0.731	0.651-0.810	FOB	50	42.3	0.538	0.450-0.627										
Rodriguez-Cobos 2021	â^†Np73 EV mRNA	61.9	79.3	0.679		133p53 EV mRNA	47.5	85.2	0.65		CEA	100	85	0.857																
Rodia 2018	LGALS4, CEACAM6, TSPAN8 and COL1A2 (all adenomas + CRC)	73	89	0.88		LGALS4, CEACAM6, TSPAN8, COL1A2 (HRAs + CRC)	75	87	0.88																					
Xie 2021	m6-A-RNA	80	95.3	0.946	0.914-0.977	CEA	72.4	81.2	0.817	0.754-0.881	CA125	47.6	95.3	0.732	0.659-0.806	CA19-9	65.7	85.9	0.771	0.700-0.842	m6-A-RNA + CEA + CA125 + CA19-9	91.4	93.8	0.977	0.961-0.994					
Wu 2021	5-tRF-GlyGCC	85.7	72.2	0.882	0.83-0.92	CEA			0.762		CA19-9			0.557		5-tRF-GlyGCC + CEA + CA19-9	86.1	84	0.926	0.87-0.96										
Guo 2022	ex-LRs d-signature	92.5	94.4	0.983	0.969-0.997																									

Table 7 – RNA Species – CRA Diagnostic Tests

Paper	Test 1	Sens (%)	Spec (%)	AUC	95% CI	Test 2	Sens (%)	Spec (%)	AUC	95% CI	Test 3	Sens (%)	Spec (%)	AUC	95% CI	Test 4	Sens (%)	Spec (%)	AUC	95% CI	Test 5	Sens (%)	Spec (%)	AUC	95% CI	Test 6	Sens (%)	Spec (%)	AUC	95% CI
Herreros-Villanueva 2019	miRNA19a, miRNA19b, miRNA15b, miRNA29a, miRNA335, and miRNA1	95	90	0.91	0.868-0.959																									
Marcuello 2019	6-miRNA	81	63	0.8	0.72-0.87	FIT +ve	81	35	0.65	0.56-0.73	6-miRNA + FIT	81	69	0.81	0.75-0.88															
Zhao 2022	miR-627-5p	84	93	0.84		miR-199a-5p	76	53	0.76		CEA	50	33	0.5		CA19-9	43	4	0.43		Integrated model	86	77	0.86		Integrated model (AA+CRC)	92	87	0.92	
Shaker 2022	miRNA-944	84.2	27.3	0.6	0.424-0.776	EPHA7	63.2	78.8	0.68	0.497-0.862																				
Abdul-Maksoud 2021	Combined miR-92c/miR-149	95	90	0.96	0.86-0.95	miR-29c	91.3	83.7	0.9	0.86-0.95	miR-149	93.8	81.2	0.93	0.89-0.97	CEA	58.8	53.7	0.57	0.48-0.66										
Wang 2020	miR-1207-5p	90.48	80.56	0.953	0.912 -0.994																									
Wang 2020	piR-020619 and piR-020450	72.5	76.6	0.779																										
Rodriguez-Cobos 2021	â^†Np73 EV mRNA	75.5	69	0.723		CEA			0.529																					
Rodia 2018	LGALS4, CEACAM6, TSPAN8 and COL1A2 (LRAs)	79	97	0.91																										
Roberts 2018	miR-335-5p isoform + un-annotated small RNA	71.1	70.3	0.755																										
Guo 2022	ex-LRs d-signature	71.43	96.15	0.978	0.940-1.000																									

Table 8 – RNA Species – Risk of Bias NOS

Paper	NOS - Selection	NOS - Comparability	NOS - Exposure	NOS - Overall score
Krawczyk 2017	3	0	2	5
Liu 2017	2	0	2	4
Ng 2017	3	0	3	6
Wang 2017	3	2	2	7
Wang 2017	3	1	2	6
Bilegsaikhan 2018	3	1	3	7
He 2018	4	1	2	7
Liu 2018	3	1	2	6
Tan 2018	3	0	2	5
Herreros-Villanueva 2019	3	1	2	6
Huang 2019	2	1	2	5
Marcuello 2019	3	1	3	7
Sabry 2019	3	1	3	7
Sahami-Fard 2019	3	1	2	6
Sun 2019	3	2	2	7
Tan 2019	3	2	2	7
Wang 2019	2	2	2	6
Zhang 2022	3	0	2	5
Zhao 2022	3	2	3	8
Wang 2022	3	1	2	6
Shaker 2022	3	1	2	6
Nakamura 2022	3	1	2	6
Du 2022	3	1	2	6
Shi 2021	3	1	2	6
Elaguizy 2020	3	1	2	6
BaderElDin 2020	3	1	2	6
Han 2021	3	1	3	7
Zhou 2021	3	2	2	6
Abdul-Maksoud 2021	2	1	3	6
Wang 2020	3	1	3	7
Wu 2020	3	1	2	6
Shi 2020	3	2	2	7
Jin 2020	3	1	3	7
Huang 2020	4	1	2	7
Cui 2020	3	1	2	6
Li 2020	3	1	2	6
Pan 2019	3	1	2	6
Lin 2019	3	1	2	6
Ding 2020	2	1	2	5
Ismail 2019	3	1	3	7
Xu 2021	3	0	2	5
Vychytilova-Faltejskova 2018	3	0	2	5
Qu 2019	3	0	2	5
Mai 2020	3	0	3	6
Wang 2020	3	2	2	7
Sabbah 2021	3	1	3	7
AbdelGhafar 2020	2	1	3	6
Rodriguez-Cobos 2021	3	0	3	6
Rodia 2018	2	1	2	5
Xie 2021	3	0	2	5
Roberts 2018	3	1	3	7
Wu 2021	3	1	2	6
Guo 2022	3	2	2	7

Table 9 – DNA Methylation – Study Characteristics

Paper	Area	Study Design	Biomarker type	Specific Biomarker(s)	Specimen	Sample size	Population	Age CRC	Age CRA	Age Control	Male CRC	Male CRA	Male Control	Female CRC	Female CRA	Female Control	CRC stage I	CRC stage II	CRC stage III	CRC stage IV
Siri 2022	Iran	Case control	DNA methylation	SDC2	Whole blood	130	Not stated	56		54	38		31	27		34	19	24	13	9
Nguyen 2022	Vietnam	Case control	DNA methylation	Multiple	Plasma	317	Not stated	60		48	99		64	60		94	12	42	53	5
Klein 2021	USA	Case control	DNA methylation	Multiple	Plasma	4077	Not stated													
Nagai 2017	Japan	Case control	DNA methylation	LINE-1	Plasma	167	Not stated	63		51	65		34	49		19	57 (I+II)		57 (III+IV)	
Rasmussen 2017	Denmark	Case control	DNA methylation	ALX4, BMP3, NPTX2, RARB, SDC2, SEPT9, VIM	Plasma	295	Symptomatic	68		65	119		55	74		47	27	54	72	34
Fu 2018	China	Case control	DNA methylation	mSEPT9	Plasma	558	Not stated				61	71	139	37	30	114	26	31	31	8
Rokni 2018	Iran	Case control	DNA methylation	BMP3	Plasma	100	Not stated	59	59	50			25			25				
Suehiro 2018	Japan	Case control	DNA methylation	TWIST1, SEPT9	Serum	138	Asymptomatic	71	68	55	13	48	10	5	22	15	14	1	3	
Xie 2018	China	Case control	DNA methylation	mSEPT9	Plasma	248	Not stated	66		66	74		65	49		60	5	36	58	4
Chen 2019	China	Case control	DNA methylation	SEPT9, SDC2	Serum	225	Asymptomatic	61		33	75			36			13	49	39	7
Jensen 2019	Denmark	Case control	DNA methylation	C9orf50, KCNQ5, CLIP4	Plasma	434	Symptomatic	73		67	81		46	62		45	25	75	33	10
Leung 2019	Hong Kong	Cohort	DNA methylation	mSEPT9	Plasma	282	Symptomatic													
Li 2019	China	Case control	DNA methylation	SFRP2	Serum	117	Not stated				44			18			13	27	17	5
Pasha 2019	Egypt	Case control	DNA methylation	RUNX3, SFRP1	Serum	165	Not stated				52	28	26	33	12	14	9	39	34	3
Sun 2019	China	Case control	DNA methylation	mSEPT9	Plasma	650	Asymptomatic				30		285	20		315	3	24	22	8
Bagheri 2020	Iran	Case control	DNA methylation	TFPI2, NDRG4	Serum	100	Not stated	56		54	26		22	24		28	16	19	9	6
Cho 2020	Korea	Case control	DNA methylation	FAM123A, GLI3, PPP1R16B, SLIT3, TMEM90B	Plasma	157	Not stated										17	24	33	23
Song 2020	China	Case control	DNA methylation	mSEPT9, CA724, SNCG, AFP	Plasma	750	Not stated				183	104	177	108	60	118	45	90	109	47
Zhao 2020	China	Case control	DNA methylation	SFRP2, SDC2	Plasma	318	Not stated	62	58	40	64	71	56	58	34	36	19	38	44	8
Cai 2021	China	Case control	DNA methylation	ColonAiQ	Plasma	507	Not stated										23	50	72	16
Ma 2021	China	Case control	DNA methylation	mSEPT9	Plasma	135	Mixed	67		63	63		12	40		20	27	37	33	3
Young 2021	Australia	Case control	DNA methylation	BCAT1, IKZF1, IRF4	Plasma	1620	Mixed	67	64	60	97	387	418	87	229	402	41	57	51	33
Zhang 2021	China	Case control	DNA methylation	SEPT9, SDC2	Serum	187	Not stated	57	56	37	79	41	32	46	29	60	22	38	28	37
Zhang 2021	China	Case control	DNA methylation	twist1, fbn1, c9orf50, sfmbt2, kcnq5, fam72c, itga4, kcnj12, znf1	Plasma	268	Not stated	60	57	46	81	15	29	94	16	33	26	44	21	84
Alizadeh-Sedigh 2022	Iran	Case control	DNA methylation	FBN1, SPG20, ITF2, RUNX3, SNCA, MLH1, mSEPT9	Plasma	120	Not stated	56		54	37		28	33		22				
Jafarpour 2022	Iran	Case control	DNA methylation	ITGA4	Serum	396	Not stated	57		47	119		77	79		121				
Lu 2022	China	Case control	DNA methylation	mSEPT9	Plasma	326	Not stated	60		58	113		98	67		48	20	33	57	27
Lu 2022	China	Case control	DNA methylation	mSept9	Plasma	738	Not stated	61		61	397		69	219		53	91	170	267	88
Walker 2022	UK, USA	Case control	DNA methylation	5hmC	Plasma	2106	Not stated	66		62	391		255	406		318	161	319	222	95
Lin 2021	China	Case control	DNA methylation	MYO1-G	Plasma	674	Not stated	56		45	149		189	123		213	18	53	140	60

Table 10 – DNA Methylation – CRC Diagnostic Tests

Paper	Test 1	Sens (%)	Spec (%)	AUC	95% CI	Test 2	Sens (%)	Spec (%)	AUC	95% CI	Test 3	Sens (%)	Spec (%)	AUC	95% CI	Test 4	Sens (%)	Spec (%)	AUC	95% CI	Test 5	Sens (%)	Spec (%)	AUC	95% CI
Siri 2022	**SDC2**	81.5	69.2	0.847																					
Nguyen 2022	**SPOT-MAS**	96.8	97	0.989																					
Klein 2021	**CCGA MCED panel**	82				**Overall cancer vs no cancer**	51.5	99.5																	
Nagai 2017	**LINE-1**	65.8	90	0.81		**CEA**	54.4																		
Rasmussen 2017	**ALX4, BMP3, NPTX2, RARB, SDC2, SEPT9, VIM**	90.7	72.5	0.86																					
Fu 2018	**mSEPT 9 (1/3 algorithm)**	80.61	86.17			**mSEPT 9 (2/3 algorithm)**	61.22	98.42																	
Rokni 2018	**BMP3**	40	94																						
Suehiro 2018	**FIT +ve**	44.4				**mTWIST1**	44.4				**FIT + mTWIST1**	72.2													
Xie 2018	**FOBT**	61.4	70.3	0.658	0.578-0.723	**CEA**	35	62.6	0.485	0.411-0.559	**CA19-9**	17.9	55.7	0.353	0.283-0.423	**mSEPT9**	61.8	89.6	0.757	0.701-0.807	**FOBT + mSEPT9**	84.1	62.2	0.807	0.740-0.875
Chen 2019	**mSEPT9**	73	95.6	0.854	0.800-0.907	**SDC2**	71.2	95.6	0.881	0.835-0.928	**SEPT9 +SDC2 (ColoDefense)**	86.5	92.1	0.922	0.883-0.961										
Jensen 2019	**C9orf50, KCNQ5 and CLIP4**	91	99																						
Leung 2019	**mSEPT9**	73.9	72.5			**CEA**	48.2	79.3																	
Li 2019	**SFRP2**	69.4	87.3	0.821	0.744-0.898																				
Pasha 2019	**RUNX3**	60	82.5	0.672		**SFRP1**	77.65	70	0.752		**CEA**	43.53	85	0.568		**CA19**	21.18	90	0.432		**RUNX3/SFRP1 /CEA/CA19-9**	84.71	67.5	0.792	
Sun 2019	**mSEPT9**	73	94.5	0.835	0.758-0.913	**FOBT**	58.7	91.9																	
Bagheri 2020	**TFPI2**	88	92			**NDRG4**	86	92																	
Cho 2020	**ANKRD13, FAM123A, GLI3, PCDHG, PPP1R16B, TMEM90B**	74.4	85.7	0.866	0.814-0.917	**FAM123A, GLI3, PPP1R16B, SLIT3, TMEM90**	57.3	95	0.839	0.782-0.897															
Song 2020	**mSEPT9**	76.6	94.6	0.86		**CEA**	40.3	95	0.74		**mSEPT9 + CEA**	86.4	92.8												
Zhao 2020	**SFRP2**	63.1	90.1	0.787	0.729-0.846	**SDC2**	56.6	95.6	0.765	0.704-0.826	**SpecColon (combined)**	76.2	87.9	0.856	0.806-0.905										
Cai 2021	**ColonAiQ**	86.1	92	0.93																					
Ma 2021	**mSEPT9**	74	50%	0.71	0.62-0.80																				
Young 2021	**BCAT1**	47.3	94.6	0.71	0.662-0.757	**IRF4**	50	97.8	0.739	0.691-0.787	**IKZF1**	59.2	95.7	0.775	0.730-0.820	**Any 3**	73.9	90.1	0.82	0.781-0.859					
Zhang 2021	**mSEPT9 + mSDC2**	61.3	90.2	0.758		**mSEPT9 + mSDC2 + CEA + CA19-9 + AFP**	77.4	89.1	0.84																
Zhang 2021	**4 marker model**	80	97.1	0.911	0.834-0.988																				
Alizadeh-Sedigh 2022	**FBN1**	81.5	66.2	0.808	0.701-0.915	**SPG20(a)**	73.3	94	0.825	0.719-0.896	**SPG20(c)**	67.9	90	0.674	0.508-0.839	**SNCA(c)**	39.9	89.7	0.67	0.567-0.763	**SEPT9(a)**	58.7	100	0.763	0.663-0.845
Jafarpour 2022	**ITGA4**	54	89	0.7	0.64-0.75																				
Lu 2022	**mSEPT9**	77	88	0.82	0.78-0.86	**FIT**	88	80	0.83	0.79-0.88															
Lu 2022	**mSEPT9**	72.94	81.97	0.826	0.792-0.860	**CEA**	43.96	96.72	0.789	0.751-0.826	**CA19-9**	14.99	96.61	0.59	0.539-0.641	**mSept9 + CEA + CA19-9**	78.43	86.07	0.878	0.849-0.906					
Walker 2022	**5hmC classifiers**	81	84	0.9	0.87-0.93																				
Lin 2021	**MYO1-G**	84.3	94.5	0.94	0.93-0.96	**CEA**	54.8	97.1	0.87	0.84-0.90															

Table 11 – DNA Methylation – CRA Diagnostic Tests

Paper	Test 1	Sens (%)	Spec (%)	AUC	95% CI	Test 2	Sens (%)	Spec (%)	AUC	95% CI	Test 3	Sens (%)	Spec (%)	AUC	95% CI	Test 4	Sens (%)	Spec (%)	AUC	95% CI	Test 5	Sens (%)	Spec (%)	AUC	95% CI	Test 6	Sens (%)	Spec (%)	AUC	95% CI
Fu 2018	**mSEPT 9**			0.532																										
Suehiro 2018	**FIT (HRA)**	24.3				**FIT (LRA)**	8				**mTWIST1 (HRA)**	50				**mTWIST1 (LRA)**	30				**FIT + mTWIST1 (HRA)**	64.3				**FIT + mTWIST1 (LRA)**	45.7			
Pasha 2019	**RUNX3**	17.5				**SFRP1**	30																							
Sun 2019	**mSEPT9**	17.1	94.5			**FOBT**	12.2	91.9																						
Song 2020	**mSEPT9**			0.51																										
Zhao 2020	**SFRP2**	50	90.1			**SDC2**	33.3	95.6			**SpecColon**	58.3	87.9																	
Cai 2021	**ColonAiQ**	42.1		0.84																										
Young 2021	**Any 3 (AA)**	15.7																												
Zhang 2021	**mSEPT9 + mSDC2**	39.3	90.2			**mSEPT9 + mSDC2 + CEA + CA19-9 + AFP**	39.3	89.1																						
Zhang 2021	**4 marker model**	54.4	45.5	0.614	0.457-0.770																									

Table 12 – DNA Methylation – Risk of Bias NOS

Paper	NOS - Selection	NOS - Comparability	NOS - Exposure	NOS - Overall score
Siri 2022	3	2	3	8
Nguyen 2022	3	0	2	5
Klein 2021	4	1	3	8
Nagai 2017	3	0	3	6
Rasmussen 2017	4	1	3	8
Fu 2018	3	1	3	7
Rokni 2018	3	0	3	6
Suehiro 2018	3	1	3	6
Xie 2018	3	2	2	7
Chen 2019	3	1	3	7
Jensen 2019	3	1	3	7
Leung 2019	3	0	3	6
Li 2019	3	1	2	5
Pasha 2019	3	1	3	7
Sun 2019	3	1	3	7
Bagheri 2020	3	1	3	7
Cho 2020	3	1	2	6
Song 2020	3	1	2	6
Zhao 2020	3	1	3	7
Cai 2021	3	1	3	7
Ma 2021	3	0	3	6
Young 2021	3	1	3	7
Zhang 2021	3	1	3	7
Zhang 2021	3	1	3	7
Alizadeh-Sedigh 2022	3	1	2	6
Jafarpour 2022	3	1	3	7
Lu 2022	3	1	3	7
Lu 2022	3	1	3	7
Walker 2022	3	2	3	8
Lin 2021	3	0	3	6

Table 13 – Antigens and Autoantibodies – Study Characteristics

Paper	Area	Study Design	Biomarker type	Specific Biomarker(s)	Specimen	Sample size	Population	Age CRC	Age Control	Male CRC	Male Control	Female CRC	Female Control	CRC stage I	CRC stage II	CRC stage III	CRC stage IV
Huajun 2018	China	Case control	Antigen	APE1-Aabs, CEACAM-1	Serum	110	Not stated	63	60	41	26	19	24				
Fitzgerald 2019	Republic of Ireland	Case control	Antigen	CADM1, HMGB1, ICLN, p53, SEC 16, ZNF 700, ZNF768	Serum	114	Symptomatic	67	67	12	20	12	17	2	5	12	5
Rao 2021	China	Case control	Antigen	CEA, CA24-2, and CA19-9	Serum	2283	Not stated	61	57	1004	416	574	287	604 (I+II)		541	405
Luo 2020	China	Case control	Antigen	NSE, CEA, CA19-9, CA125, CA242	Serum	656	Not stated	61	56	218	158	140	140				
Zhao 2020	China	Case control	Autoantibodies	anti-TOPO48	Serum	230	Not stated	56		61		34		30	20	30	15
Fan 2017	Taiwan	Case control	Autoantibodies	anti-SLP2, anti-p53, anti-SEC61B, anti-PLSCR1	Serum	192	Not stated	66	66	51	60	41	40	3	39	35	15
Cai 2022	China	Case control	Autoantibodies	CST4, goat anti-DR-70	Serum	288	Symptomatic	57	55	15	13	17	19	11	21		

Table 14 – Antigens and Autoantibodies – CRC Diagnostic Tests

Paper	Test 1	Sens (%)	Spec (%)	AUC	95% CI	Test 2	Sens (%)	Spec (%)	AUC	95% CI	Test 3	Sens (%)	Spec (%)	AUC	95% CI
Huajun 2018	**APE1-AAbs**	62.7	85.4			**CEACAM-1**	51	98.1			**CEA**	47	97.48		
Fitzgerald 2019	**SEC 16 IgM**	25	97.3			**ZNF 768 IgM**	33.3	94.6			**ZNF 700 IgG**	25	91.9		
Rao 2021	**CEA**	71.7	50	0.637	0.614-0.660	**CA24-2**	74.1	39.3	0.58	0.556-0.604	**CA19-9**	81.2	30	0.565	0.540-0.590
Luo 2020	**NSE**	63.41	79.53	0.766	0.732-0.798	**CEA**	37.71	90.6	0.682	0.644-0.717	**CA19-9**	34.92	78.19	0.56	0.521-0.599
Zhao 2020	**anti-TOPO48**	72.3	100	0.835	0.747-0.924	**anti-P53**	41.2	76.2	0.766	0.614-0.919	**CEA (I+II)**	86.2	75.8	0.663	0.504-0.822
Fan 2017	**anti-SLP2**	51.1	80	0.675	0.597-0.753	**anti-p53**	41.3	80	0.638	0.558-0.718	**anti-SEC61B**	30.4	80	0.696	0.619-0.774
Cai 2022	**CST4**	53.1	96.9	0.933	0.886-0.980	**CR-70**	28.1	92.2	0.76	0.660-0.860	**CST4 + DR-70**	71.9	89.9	0.94	0.896-0.985

Paper	Test 4	Sens (%)	Spec (%)	AUC	95% CI	Test 5	Sens (%)	Spec (%)	AUC	95% CI	Test 6	Sens (%)	Spec (%)	AUC	95% CI
Huajun 2018	**APE1-AAbs + CEACAM-1**	82.2	82.38			**APE1-AAbs + CEA**	70.9	83.01							
Fitzgerald 2019	**CADM1, HMGB1), ICLN, p53, SEC 16, ZNF 700, and ZNF768**	70.8	86.5												
Rao 2021	**CEA + CA24-2 + CA19-9**	88.3	35.6	0.641	0.618-0.664										
Luo 2020	**CA125**	25.14	88.93	0.59	0.552-0.688	**CA242**	66.2	59.73	0.651	0.613-0.688	**NSE + CEA + CA19-9 + CA125 + CA242**	69.3	84.6	0.827	0.796-0.855
Zhao 2020	**anti-TOPO48 + anti-P53 (I+II)**	95		0.925	0.863-0.987	**anti-TOPO48 + CEA (I+II)**	82		0.862	0.762-0.965					
Fan 2017	**anti-PLSCR1**	35.9	80	0.542	0.457-0.627	**anti-SLP2, anti-p53, anti-SEC61B, anti-PLSCR1,CEA**	64.1	80							
Cai 2022															

Table 15 – Antigens and Autoantibodies – Risk of Bias NOS

Paper	NOS - Selection	NOS - Comparability	NOS - Exposure	NOS - Overall score
Huajun 2018	4	1	2	7
Fitzgerald 2019	3	1	3	7
Rao 2021	3	1	2	6
Luo 2020	3	0	2	5
Zhao 2020	3	2	3	8
Fan 2017	3	1	3	7
Cai 2022	3	1	3	7

Table 16 – Other – Study Characteristics

Paper	Area	Study Design	Biomarker type	Specific Biomarker(s)	Specimen	Sample size	Population	Age CRC	Age CRA	Age Control	Male CRC	Male CRA	Male Control	Female CRC	Female CRA	Female Control	CRC stage I	CRC stage II	CRC stage III	CRC stage IV
Peng 2023	China	Case control	Raman Spectroscopy	SERS	Serum	100	Not stated													
Hong 2020	China	Case control	Raman Spectroscopy	SERS	Serum	150	Not stated				68		28	41		12	25	34	47	3
Jenkins 2022	UK	Cohort	Raman Spectroscopy	SERS	Serum	705	Symptomatic	67		64	84		69	66		81	79 (I+II)		72 (III-IV)	
Moisoiu 2019	Romania	Case control	Raman Spectroscopy	SERS	Serum	148	Not stated				55		25	43		14	5	13	55	22
Woods 2022	UK	Cohort	Raman Spectroscopy	SERS	Serum	541	Symptomatic													
Gayer 2023	Russia	Case control	Fluorescence spectroscopy	UV/Vis protein fluorescence	Plasma	289	Symptomatic													
Soares 2017	Brazil	Case control	Fluorescence spectroscopy	Blood fluorescence spectroscopy + machine learning	Plasma	299	Symptomatic													
Yin 2021	China	Case control	Fluorescence spectroscopy	3D fluorescence + TM-PLS-DA classification model	Plasma	225	Not stated													
Nishiumi 2017	Japan	Case control	Metabolomics - gas chromatography / mass spectrometry	29 metabolites by gas chromatography/triple-quadrupole mass spectrometry	Plasma	573	Not stated	68		68	170		178	112		113	159	123		
Hata 2017	Japan	Case control	Metabolomics	GTA-446	Serum	1141	Not stated				136		567	89		349	91	49	71	13
Jaberie 2020	Iran	Case control	Metabolomics	A1AT + A1AT activity	Plasma	163	Not stated	58		60	59		28	54		22	31	36	26	8
Pan 2022	China	Case control	Metabolomics	Sphingolipids: TGs,TC, LDL and HD	Serum	126	Not stated	58	53	55	42	6	31	23	5	20	8	23	18	11
Zhang 2020	China	Case control	Metabolomics	PON1	Plasma	374	Not stated	61		57	180		48	104		42	103 (I+II)		181 (III+IV)	
Liu 2020	China	Case control	Lipidomics	11 lipid species	Serum	103	Not stated	58		56	31		32	20		20	51 (I+II)			
Tsai 2019	Taiwan	Cohort	CTCs	CTC count	Whole blood	667	Asymptomatic	64	62	48							65	93	115	39
Luo 2021	China	Case control	CTCs	CTC, CTEC	Whole blood	135	Not stated	58		52	69		15	46		5	25 (I-II)		90 (III-IV)	
Rasmussen 2018	Denmark	Cohort	Circulating cf nucleosomes	CCFNs + multiple epigenetic signals	Serum	4105	Not stated													
Salem 2020	Egypt	Case control	cfDNA integrity index	DNA integrity index	Serum	150	Not stated	52	50	51	48	12	21	42	18	9	6	21	30	33
Ma 2021	China	Case control	cfDNA fragment analysis	cfDNA	Plasma	621	not stated													
Choi 2018	Korea	Case control	Immune antibodies and cells	Multiple	Whole blood	305	Symptomatic	63		43	73		61	58		112	30	20	47	11
Savage 2022	UK	Cohort	Standard blood tests	FBC, biochem, tumour markers, age, sex + machine learning	Multiple	54297	Symptomatic	69		69										
Li 2021	China	Case control	Standard blood tests	FBC, CEA and AFP	Whole blood + Serum	1164	Not stated	52		52	355		355	227		227				
StojkovicLalosevic 2019	Serbia	Case control	FBC cell ratios	NLR, PLR, MPV	Whole blood	600	Not stated	62		60	160		150	140		150	82	74	92	52
Li 2019	China	Case control	FBC cell ratios	Inflammatory Cell Ratios + CEA	Whole blood	1502	Not stated	64			423		423	328		328	84	301	287	79
Huang 2019	China	Case control	FBC cell ratios	RLR	Whole blood	332	Not stated	54	53	53	97	58	51	65	34	27	75 (I+II)		87 (III+IV)	
Zhu 2018	China	Case control	Platelet indicies	PC, MPV, PDW, PCT	Whole blood	1935	Not stated	61	59	60	467	312	383	316	151	316	136	247	322	78
Song 2020	China	Case control	Mixed serum biomarkers	36 serum biomarkers + machine learning	Serum	1010	Not stated				217	200	212	133	100	148	56	108	106	30
Battista 2021	Italy	Case control	Mixed serum biomarkers	17 serum biomarkers	Serum	345	Not stated	70		69	159		58	89		39	74	61	76	37

Table 17 – Other – CRC Diagnostic Tests

Paper	Test 1	Sens (%)	Spec (%)	AUC	95% CI	Test 2	Sens (%)	Spec (%)	AUC	95% CI
Peng 2023	**Random Forest Algorithm**	86.7	100	0.996						
Hong 2020	**SERS/SVM**	87.5	100			**SERS/SVM + CEA**	90	100		
Jenkins 2022	**Raman-CRC**	95.7	69.3	0.842		**FIT**	90.9	83.5	0.93	
Moisoiu 2019	**PCA-LDA model**	83.3	64.1							
Woods 2022	**EMSC-3**	89.6	55.7	0.754		**EMSC-4**	54.2	70.2	0.667	
Gayer 2023	**Classifier - test**	82	81	0.82	0.68-0.96					
Soares 2017	**Complete hierarchical classifier**	86.4	95.2	0.901						
Yin 2021	**TM-PLS-DA model**	88	95	0.94						
Nishiumi 2017	**Model 1**	99.3	93.8	0.996		**CEA**	18.1	96		
Hata 2017	**GTA-446**	83.3	84.8	0.91						
Jaberie 2020	**A1AT**	75.2	90	0.86	0.80-0.91	**CEA**	70.8	70	0.74	0.67-0.81
Pan 2022	**ST(d18:1/18:0)**	81.5	81.5	0.817	0.724-0.910	**CerP(d18:1/17:0)**			0.811	0.725-0.896
Zhang 2020	**CEA**	78.2	60	0.818		**CA12-5**	58.5	50	0.581	
Liu 2020	**11 lipid panel**	100	88.5	0.981	0.952-1.000					
Tsai 2019	**CTC assay**	95.2	86	0.94						
Luo 2021	**CTC**	87.8	90	0.889		**CTEC**	39.1	100	0.695	
Rasmussen 2018	**ccfn, 5-methylcytosine DNA, CEA, age and gender**	57	90	0.84						
Salem 2020	**DII**	93.3	90	0.95	0.89-1					
Ma 2021	**cfDNA stacked model**	97.4	94.8	0.988						
Choi 2018	**Binary logistic regression analysis**	85.8	86.2	0.94						
Savage 2022	**Machine learning model (LGI)**	97.8	20.4							
Li 2021	**Logistic regression model**	89.5	83.5	0.865	0.857-0.877	**SVM model**	86.5	83	0.865	0.857-0.874
StojkovicLalosevic 2019	**NLR**	74.1	73	0.79	0.736-0.884	**PLR**	74	80	0.846	0.801-0.891
Li 2019	**NLR**	69.13	65.21	0.723	0.698-0.747	**PLR**	57.23	85.39	0.779	0.756-0.802
Huang 2019	**RLR**	41	72	0.571	0.730-0.828	**CEA**	37	97	0.779	0.493-0.619
Zhu 2018	**PC**	62	72	0.706	0.677-0.735	**MPV**	69	59	0.663	0.632-0.964
Song 2020	**Artificial neural network**	98.9	95.6	0.992	0.987-0.997					
Battista 2021	**Combined SVM, XGB, MLP models**	100	92.3							

Paper	Test 3	Sens (%)	Spec (%)	AUC	95% CI	Test 4	Sens (%)	Spec (%)	AUC	95% CI
Peng 2023										
Hong 2020	**CEA**	49	77.1							
Jenkins 2022										
Moisoiu 2019										
Woods 2022	**POL-8-norm_vec**	75	48.8	0.671		**NO_EMSC**	51	30.5	0.402	
Gayer 2023										
Soares 2017										
Yin 2021										
Nishiumi 2017	**CA19-9**	9.3	95.6							
Hata 2017										
Jaberie 2020	**A1AT activity**	84.1	100	0.94	0.89-0.97					
Pan 2022	**ST(d18:1/16:0)**			0.776	0.685-0.867					
Zhang 2020	**CA19-9**	53.5	78	0.593		**PON1**	91.1	42.3	0.742	
Liu 2020										
Tsai 2019										
Luo 2021	**CEA**	28.7	100	0.696		**CA19-9**	26.1	95	0.695	
Rasmussen 2018										
Salem 2020										
Ma 2021										
Choi 2018										
Savage 2022										
Li 2021										
StojkovicLalosevic 2019	**MPV**	74	88	0.816	0.764-0.869	**NLR + PLR + MPV**	96	70	0.904	0.869-0.938
Li 2019	**LMR**	72.89	73.8	0.8	0.778-0.821	**CEA**	58.28	85.54	0.792	0.769-0.813
Huang 2019	**CEA + RLR**	56	90	0.782	0.734-0.831					
Zhu 2018	**PCT**	64	80	0.765	0.738-0.791	**CEA**	41	90	0.74	0.713-0.767
Song 2020										
Battista 2021										

Paper	Test 5	Sens (%)	Spec (%)	AUC	95% CI	Test 6	Sens (%)	Spec (%)	AUC	95% CI
Peng 2023										
Hong 2020										
Jenkins 2022										
Moisoiu 2019										
Woods 2022	**Raw spectra**	65.6	42.1	0.543						
Gayer 2023										
Soares 2017										
Yin 2021										
Nishiumi 2017										
Hata 2017										
Jaberie 2020										
Pan 2022										
Zhang 2020	**PON1 + CEA + CA12-5**	57	100	0.861		**PON1 + CEA + CA12-5 + CA19-9**	76.1	82.4	0.867	
Liu 2020										
Tsai 2019										
Luo 2021	**CTC, CTEC, CEA, CA19-9**			0.935						
Rasmussen 2018										
Salem 2020										
Ma 2021										
Choi 2018										
Savage 2022										
Li 2021										
StojkovicLalosevic 2019										
Li 2019	**PLR + LMR + CEA**	76.81	85.69	0.892	0.874-0.908					
Huang 2019										
Zhu 2018	**CA19-9**	16	94	0.612	0.580-0.643	**CEA + PCT**	70	83	0.835	0.812-0.857
Song 2020										
Battista 2021										

Table 18 – Other – CRA Diagnostic Tests

Paper	Test 1	Sensitivity (%)	Specificity (%)	AUC	95% CI
Tsai 2019	**CTC assay**	79.2	84.7	0.868	
Ma 2021	**cfDNA stacked model**	95.7	94.8	0.983	0.968-0.999

Table 19 – Other – Risk of Bias NOS

Paper	NOS - Selection	NOS - Comparability	NOS - Exposure	NOS - Overall score
Peng 2023	3	0	2	5
Hong 2020	3	1	2	6
Jenkins 2022	4	1	3	8
Moisoiu 2019	3	0	2	5
Woods 2022	3	0	3	6
Gayer 2023	3	1	3	7
Soares 2017	3	0	3	6
Yin 2021	3	0	2	5
Nishiumi 2017	3	1	3	7
Hata 2017	3	1	2	6
Jaberie 2020	3	1	2	6
Pan 2022	3	0	2	5
Zhang 2020	3	2	2	7
Liu 2020	3	1	2	6
Tsai 2019	3	1	3	7
Luo 2021	3	1	2	6
Rasmussen 2018	4	0	3	7
Salem 2020	3	1	2	6
Ma 2021	3	0	2	5
Choi 2018	3	1	2	6
Savage 2022	4	2	2	8
Li 2021	3	1	2	6
StojkovicLalosevic 2019	3	2	2	7
Li 2019	3	1	2	6
Huang 2019	3	1	2	6
Zhu 2018	3	2	2	7
Song 2020	3	1	2	6
Battista 2021	3	1	3	7

Discussion

This review describes recent progress in the field of blood-based testing for CRC and CRA over more than 6 years, including both isolated biomarkers and novel approaches such as spectroscopic techniques. The aim was to provide an update regarding the potential accuracy of these tests and consider how they may be utilised in the diagnosis of CRC and CRA at a time when faecal-based testing remains prevalent and a heavy burden is being placed on services providing radiological imaging and direct visualisation by colonoscopy.

This review found the largest area of research remains in the traditional biomarker field of proteomics. However, this was closely followed by papers involving RNA species (particularly small/microRNAs) and aberrant DNA methylation. Some studies are also now concentrating on the detection of multiple biomarkers and/or multiple cancers by spectroscopic techniques, including Raman and fluorescence spectroscopy, or through highly dimensional and scalable data analysis by machine learning. Though most papers concentrated on CRC detection alone (142 papers), many also included data for both CRC and CRA detection (34 papers), and a small number for CRA detection alone (2 papers). The large number of studies obtained (178 papers) suggests an expanding area of research when compared with similar reviews such as Nikolaou et al in 2018, who described 51 papers over 5 years.[58]

Reported diagnostic accuracy was shown to vary widely and should be considered in context, derived from a broad range of population sizes, biomarker types and reporting quality. Reported test sensitivity, specificity and AUC ranged between 9.10 to 100%, 20.40 to 100%, and 0.353 to 0.996 respectively for CRC vs controls. For comparison, several recent meta-analyses focussing solely on specific blood-based protein biomarkers, small RNA species and aberrant DNA methylation have found pooled AUC values of 0.760 to 0.890, 0.730 to 0.780, and 0.880 to 0.960 respectively.[26, 37, 59-70] Likewise, across 63 papers which included isolated CEA and CA19-9 tests for the diagnosis of CRC vs controls, reported AUC ranged from 0.469 to 0.869, and 0.353 to 0.777 respectively. This compares with previous studies which have reported AUC values for CEA and CA19-9 of 0.700 to 0.856, and 0.580 to 0.650 respectively for the diagnosis of CRC.[12, 20, 71, 72]

This review found that reported test sensitivity, specificity and AUC for CRA vs controls ranged between 8.00 to 95.70%, 4.00 to 97.00%, 0.430 to 0.983 and respectively. Data regarding CRA detection is more difficult to contextualise, with few studies regarding blood-based biomarkers having previously examined this specific population in detail. However, both CRC and CRA detection rates are reasonably well described for FIT, the most common faecal test currently in use for both screening and as an adjunct to triage symptomatic patients. In large meta-analyses FIT sensitivity for CRC of 79% (95% CI 69 to 86%) and specificity 94% (95% CI 92 to 95%) has been described in asymptomatic adults and sensitivity of 91% (95% CI 88 to 92%), specificity 75% (95% CI 69 to 80%) in symptomatic adults.[73, 74] However, FIT is significantly less useful in identifying high-risk CRA. Even at low positive detection thresholds, sensitivity of 40% (95% CI 33 to 47%) and specificity 90% (95% CI 87 to 93%) has been described in asymptomatic populations.[11] The optimal threshold for a positive FIT result is unclear and low thresholds (around 20ug/g) produce high false positive rates resulting in the increased use of colonoscopy. Furthermore, very little has been published regarding true FIT sensitivity at higher screening thresholds (around 80 to 120ug/g), which may be as low as 47% and 25% for CRC and high risk CRA respectively.[75-77]

Several limitations must be taken into consideration regarding the range of papers obtained in this review. The REMARK criteria for structuring studies describing clinical biomarkers were generally followed with correct layout, description of biomarker subtype, reporting of testing methods and statistical analysis.[78] However, marked test and population heterogeneity resulted in a broad range of diagnostic results for both CRC and CRA. This broad heterogeneity, along with overall poor reporting of complete data such as detailed population characteristics, specific inclusion/exclusion criteria and measures of uncertainty (such as 95% CIs and p-values) in particular meant a reliable and meaningful meta-analysis would be impractical. Test heterogeneity was demonstrated not only in the broad classification of biomarker types but also in the wide range of specific biomarker subtypes and myriad individual biomarkers within each subtype. Incomplete population characteristics, inclusion/exclusion criteria and different populations are also troublesome as they may all influence the diagnostic accuracy of a test.[79, 80] Furthermore, several studies demonstrated reporting bias by including CRA populations but failing to report their outcomes, potentially due to poor or statistically insignificant results, which was also reflected in the poor reporting of measures of uncertainty. It is also interesting to note that several of the best diagnostic results relied on combination of their primary biomarker with CEA and/or CA19-9.

However, this narrative review represents a useful large-scale overview of recent studies regarding blood-based testing for CRC and CRA. It suggests a growing area of research with diagnostic accuracies being reported which are commonly equivalent or superior to current faecal-based tests at a time when blood-based testing is not widespread.[81, 82] Though a wide range of diagnostic sensitivities are reported - both in the literature regarding FIT and in our data - this review does tentatively suggest that several blood-based biomarkers and novel technologies report comparable or superior results for both CRC and CRA detection when compared with FIT. These results are particularly encouraging for the detection of CRA, which is important in screening as a precursor lesion to CRC and for which FIT has been shown to have poor diagnostic accuracy.[11]

Individual studies with promising results are not uncommon and clearly (given the lack of common blood-based testing for CRC) it has been the case for many years in biomarker research that exceedingly few tests progress to clinical use. One reason for this is a lack of reliable systematic reviews and meta-analyses of promising tests. For example, this review found and excluded only 18 high quality meta-analyses from the original 12374 papers returned. Low participant numbers, a lack of large validation studies, uncertain inter-reliability and reproducibility between labs, bias in reporting of subgroup results and inconsistent or unclear diagnostic thresholds are also issues, some of which were encountered in this review.[83, 84] It is also known that independent external validation, increased study population size and focussed meta-analysis are all shown to decrease reported detection rates.[85-87]

Further work should concentrate on larger collaborative studies with rigorous methodology, independent external validation and clear test positivity thresholds. Inclusion/exclusion criteria should be well defined, with adequate description of both CRC/CRA groups (symptomatic vs asymptomatic) and controls (confirmed clean colon at endoscopy vs healthy community controls). High-quality systematic reviews and meta-analyses should be prioritised, aiming to ameliorate the influence of bias demonstrated in smaller studies and provide a more accurate picture of a biomarker's potential.

Compared with running initial case-control studies, there is a significant increase in resources required to then progress potential biomarkers through to clinical use.[81] This review demonstrates the breadth of current research in blood-based biomarkers and novel technologies for the detection of CRC / CRA, and it may be inefficient to progress any test to clinical use without properly considering its competitors. Therefore, once there are sufficient numbers, it would also be beneficial to consider umbrella reviews of high-quality systematic reviews and meta-analyses, comparing the best available evidence for each test to reveal the most promising candidates.[88] Cost-effectiveness analysis would then need to be done before considering the use of any new test within the NHS.

Real-world feasibility is important not only where there is potential for increased diagnostic accuracy in symptomatic patients but also because there may be significant benefits for CRC screening uptake. Despite the benefits of early diagnosis, faecal-based tests remain unpopular and only 63% of adults in England and Wales who receive bowel screening kits complete them, with 12% of all CRC diagnoses via bowel screening overall.[89] It has been suggested that 97% of screening participants who refuse colonoscopy would be receptive to a non-invasive test and of these 83% would prefer a blood test.[90] If an acceptably accurate blood-based test were clinically available it may improve bowel screening uptake and rates of early diagnosis.

Conclusion

In summary, blood-based testing continues to show great promise and may eventually be feasible to replace or complement FIT both for screening and in the diagnosis of CRC/CRA in symptomatic patients. This review suggests a growing field of acceptably sensitive and specific tests which may be comparable or superior to current faecal-based testing. However, current studies demonstrate a broad range of heterogenous tests, techniques and reporting quality which makes selecting the best candidates difficult. Further work should concentrate on larger validation studies and high-quality meta-analyses to determine which tests may realistically be worth progressing into clinical use.

Appendix 1

Initial searches run 26/10/2022 – Repeated at monthly intervals until 01/03/2023

The searches below were run in OVID Medline Ovid MEDLINE(R) Epub Ahead of Print and In-Process, In-Data-Review & Other Non-Indexed Citations <October 25, 2022> AND Embase <1996 to 2022 October 24> Limits on both databases were English language only and date range of 2017 -2023 as requested. Conference abstracts were removed from EMBASE. Both sets of references were exported into ENDNOTE and reviewed for duplicates which were removed.

A total of 15888 references were found in the searches and 3561 duplicates were removed leaving 123 exported to Covidence. Covidence also checks for duplicates when references are imported but did not identify any duplicates.

Ovid MEDLINE 2017-2023, English language only

Ovid MEDLINE(R) Epub Ahead of Print and In-Process, In-Data-Review & Other Non-Indexed Citations <October 25, 2022>

Ovid MEDLINE(R) <1996 to October 25, 2022>

https://ovidsp.ovid.com/athens/ovidweb.cgi?T=JS&NEWS=N&PAGE=main&SHAREDSEARCHID=3HCldg0ZhiM4wD6KHOVlQ6XQk2M7wdTyNkjoO7ApBxOU4HGIZmp8az7WflIpHrVQ8

1	exp Colorectal Neoplasms/	179579
2	colon.ti,ab.	142365
3	colorect*.ti,ab.	164141
4	bowel*.ti,ab.	143177
5	caec*.ti,ab.	7618
6	(rectal or rectum).ti,ab.	90875
7	2 or 3 or 4 or 5 or 6	460310
8	cancer*.ti,ab.	1844565

9	carcino*.ti,ab.	667234
10	adenocarcinoma*.ti,ab.	139649
11	adenoma*.ti,ab.	64669
12	((Sessile or serrated) adj polyp*).ti,ab.	1282
13	tumo?r*.ti,ab.	1561351
14	pre-malignan*.ti,ab.	2118
15	8 or 9 or 10 or 11 or 12 or 13 or 14	2894233
16	7 and 15	268324
17	1 or 16	298947
18	exp Liquid Biopsy/	2834
19	"liquid biops*".ti,ab.	5891
20	"blood test*".ti,ab.	21751
21	exp Biomarkers, Tumor/bl [Blood]	48957
22	"blood serum".ti,ab.	11137
23	"peripheral blood*".ti,ab.	149363
24	"blood sample*".ti,ab.	138085
25	"blood plasma".ti,ab.	13071
26	(Blood-based adj4 screen*).ti,ab.	128
27	(Blood-based adj4 test*).ti,ab.	483
28	(Blood-based adj2 biomarker*).ti,ab.	1583
29	(Blood-based adj4 detect*).ti,ab.	257
30	Epi Procolon.ti,ab.	34
31	Cellmax.ti,ab.	15
32	Galleri.ti,ab.	6

33	Guardant.ti,ab.	34
34	exp Spectrum Analysis, Raman/	23945
35	"raman spectroscop*".ti,ab.	30124
36	"Vibrational spectroscop*".ti,ab.	4567
37	"raman scat*".ti,ab.	13909
38	18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37	421914
39	17 and 38	13996
40	limit 39 to yr="2017 - 2023"	4337
41	remove duplicates from 40	4324
42	limit 41 to english language	4171

Exported to Endnote for deduplication with EMBASE

Embase <1996 to 2022 October 24>

https://ovidsp.ovid.com/athens/ovidweb.cgi?T=JS&NEWS=N&PAGE=main&SHAREDSEARCHID=3awRrJds8HJhQtFsSJeyUl31Vlb86XWGJFeygXHc57LE6CebB6wDCkggDxtVJRma8

1	exp colorectal tumor/	32359
2	colon.ti,ab.	221078
3	colorect*.ti,ab.	257074
4	bowel*.ti,ab.	246945
5	caec*.ti,ab.	10055
6	(rectal or rectum).ti,ab.	153975

7	2 or 3 or 4 or 5 or 6	727684
8	cancer*.ti,ab.	2715295
9	carcino*.ti,ab.	942809
10	adenocarcinoma*.ti,ab.	224290
11	adenoma*.ti,ab.	97962
12	((Sessile or serrated) adj polyp*).ti,ab.	3130
13	tumo?r*.ti,ab.	2244016
14	pre-malignan*.ti,ab.	3823
15	8 or 9 or 10 or 11 or 12 or 13 or 14	4138413
16	7 and 15	422822
17	1 or 16	427552
18	exp liquid biopsy/	9029
19	"liquid biops*".ti,ab.	10100
20	"blood test*".ti,ab.	41978
21	exp tumor marker/	330994
22	"blood serum".ti,ab.	16117
23	"peripheral blood*".ti,ab.	241685
24	"blood sample*".ti,ab.	221180
25	"blood plasma".ti,ab.	17786
26	(Blood-based adj4 screen*).ti,ab.	257
27	(Blood-based adj4 test*).ti,ab.	939
28	(Blood-based adj2 biomarker*).ti,ab.	2552
29	(Blood-based adj4 detect*).ti,ab.	467
30	Epi Procolon.ti,ab.	59

31	Cellmax.ti,ab.	39
32	Galleri.ti,ab.	9
33	Guardant.ti,ab.	956
34	exp Spectrum Analysis, Raman/	48547
35	"raman spectroscop*".ti,ab.	25375
36	"Vibrational spectroscop*".ti,ab.	3875
37	"raman scat*".ti,ab.	9790
38	18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37	892624
39	17 and 38	43724
40	limit 39 to yr="2017 - 2023"	17573
41	limit 40 to english language	17106
42	limit 41 to conference abstracts	5390
43	41 not 42	11716

Total combined:	15,888
De-Duplicated (MEDLINE record preferred)	
Duplicates removed:	3,561
Total initially export to COVIDENCE:	12,327
Additional papers exported by March 2023:	51
Total exported to COVIDENCE:	**12,378**

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