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Innovative Tools and Methods

Blood-Based Circulating Tumor DNA Assays for Early Colorectal Cancer Detection: A Systematic Review of Performance in Asymptomatic Screening Populations

Caroline Ledertoug Kahn[1] | Maria Wissing Rasmussen[1] | Jakob Kleif[1,2,3] | Inge Søkilde Pedersen[4] | Christina Therkildsen[1]

[1]Gastro Unit, Hvidovre Hospital, Hvidovre, Denmark | [2]Institute for Clinical Medicine, University of Copenhagen, Copenhagen, Denmark | [3]Department of Surgery, Nordsjællands Hospital, Hillerød, Denmark | [4]Department of Molecular Diagnostics, Aalborg University Hospital, Aalborg, Denmark

Correspondence: Caroline Ledertoug Kahn (caroline.ledertoug.kahn@regionh.dk)

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ABSTRACT

Early detection of colorectal cancer (CRC) via population-based screening programs can reduce incidence and mortality. Current screening approaches are limited by cost, accessibility, compliance, and colonoscopy capacity. Blood-based screening of circulating tumor (ct) DNA may resolve some of these limitations. This systematic review evaluated the screening potential of blood-based ctDNA assays in asymptomatic screening populations. We systematically searched the PubMed database (final search June 20th, 2025) for studies detecting advanced adenomas (AAs) or CRC in asymptomatic cohorts, where a blood-based ctDNA assay had been evaluated with data on specificity, sensitivity, and/or AUC at early stages of disease. Newcastle-Ottowa scale was used for quality assessment. Only 20 studies of 454 unique hits met our inclusion criteria. Of these, 12 studies used assays targeting specific DNA alterations, while the remaining 8 studies used complex assays. Generally, comprehensive assays such as Shield, ColonSecure, and FMBT-CRC had the best performance for early-stage CRC detection with sensitivities of 71%–85% and specificities of 88%–90%. Despite being tested in a small cohort, the Coloscape assay was the only assay to reach sufficient AA detection with 59% sensitivity and 92% specificity. None of the reviewed ctDNA assays demonstrated sufficient sensitivities for both AA and early-stage CRC detection to be considered future screening tools. Screening tools based on ctDNA assays have not proved superior to fecal-based immunochemical test (FIT) yet. Future studies need to test ctDNA screening tools in large, prospective, asymptomatic cohorts emphasizing AA, and early-stage CRC.

1 | Introduction

Colorectal cancer (CRC) is the third most frequent and second-most lethal cancer type worldwide [1], emphasizing the need for targeted screening measures facilitating prevention through early detection. Early detection and prevention of CRC depend heavily on the efficacy of the implemented screening strategies [2, 3], which vary between countries and states with some having centrally administered national fecal immunochemical test (FIT) screening programs (detecting occult blood) of asymptomatic individuals and others having an unstructured opportunistic screening approach of both symptomatic, asymptomatic, and hereditarily predisposed individuals [4–8]. Both approaches have notable shortcomings and remain far from ideal [9]. The reasons

Abbreviations: AA, advanced adenoma; AUC, area under the curve; cfDNA, cell-free DNA; CRC, colorectal cancer; ctDNA, circulating tumor DNA; FIT, fecal immunochemical test; miRNA, microRNA; NOS, Newcastle-Ottawa scale; ROC, receiver operating curve; sDNA, stool DNA; UICC, Union for International Cancer Control.

What's New?

Early detection of colorectal cancer via population-based screening programs can reduce incidence and mortality, but current screening approaches are limited. This systematic review evaluated the screening potential of blood-based circulating tumor DNA assays in asymptomatic screening populations. The findings show that ctDNA assays have not proved superior to fecal-based immunochemical testing yet. None of the ctDNA assays reviewed showed sufficient sensitivities towards both advanced adenomas and early-stage colorectal cancer detection to be considered as future screening tools. ctDNA assays need to be developed and validated in a relevant target population and technologies improved if early-stage detection is to be achieved.

behind this are plentiful, but issues of cost, accessibility, compliance, and colonoscopy capacity are particularly highlighted in this debate [10–13]. Of note, FIT has shown high sensitivity (71%–91%) for CRC but still suffers with non-optimal specificity (90%–95%) and a low sensitivity for advanced adenoma (AA) (25%–40%) [14, 15]. In order to secure the colonoscopy capacity, some countries have increased test positivity threshold with the trade-off of missing CRCs [14, 16]. The abovementioned challenges call for improved screening approaches or new screening tools.

Blood-based screening for circulating tumor DNA (ctDNA) has emerged as a possible alternative with the potential to address these challenges. Particularly, since ctDNA methods depend on circulating fragments of tumor-derived DNA, they are generally more tumor-specific than FIT. ctDNA has been tested as a screening tool for various cancer types such as lung-, colorectal-, pancreatic-, breast-, and urothelial cancer using cancer-specific ctDNA targets [17–20]. For CRC, ctDNA has proven to be particularly efficient in distinguishing patients with metastatic disease from healthy individuals [21–23]. In the last decade, commercial ctDNA tests such as Epi proColon (now rebranded as ColoHealth), Colvera, Galleri, and Shield have gained attention as alternatives—especially in the USA—to FIT in an opportunistic screening setting [21, 24–27].

However, despite promising ctDNA test results in case–control studies, the tests generally underperform when validation is attempted in screening cohorts of primarily average-risk asymptomatic individuals from the general population aged 50–80 years. To date, only a few well-powered, prospective clinical trials have assessed a ctDNA assay in such populations, and no systematic review has yet compared these studies to evaluate the full screening potential of this tool [28]. With this review, we aimed to summarize and compare the performance of different blood-based ctDNA assays for early-stage CRC detection in a screening setting to evaluate their screening potential in an asymptomatic population.

2 | Materials and Methods

2.1 | Systematic Literature Search

The electronic bibliographic database, PubMed, was searched for published studies evaluating the performance of any ctDNA biomarker for early detection of CRC in a screening cohort. The search string included three categories including a disease domain [colorectal neoplasia], a detection/screening domain [early detection], and a biomarker domain [circulating tumor DNA] combined with a Boolean logical "AND." No constraints were made at this level regarding language, human samples, or publication type.

All search terms were used as MeSH terms and Title/Abstract level combined with "OR." The final search was performed on the 20th of June 2025. The entire search string is available in the Supporting Information. The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) checklist was used in order to adhere to transparency recommendations for reporting systematic reviews [29].

2.2 | Study Selection

All studies obtained from the search string were imported into Rayyan.AI (Rayyan Systems Inc). Title and abstract of all the identified studies were independently reviewed by two of the authors (CLK, MWR, or CT). Inclusion criteria comprised of published full-text articles that evaluated ctDNA targets or assays for CRC detection with results given as detection rates, sensitivity/specificity, and/or area under the curve (AUC) obtained through receiver operating curve (ROC) modeling in a CRC screening scenario. Exclusion criteria included are as follows: (1) retracted articles; (2) duplicated entries; (3) non-English entries; (4) murine/cell line studies (wrong study design or out of scope); (5) entries with no original data (e.g., reviews, editorials, etc.) (No original data); (6) studies utilizing other types of liquid biopsy biomarkers, such as cell-free (cf) DNA amount or fecal ctDNA (wrong type of biomarker); (7) studies only focusing on extracolonic cancers (wrong outcome); (8) articles regarding post-operative surveillance (wrong population), and lastly; (9) technical biomarker development studies (e.g., technical optimization, quality assessment of biomarkers, etc.) (Wrong study design). Articles aligning with the abovementioned criteria were included for full-text screening (Figure 1).

2.3 | Cohort Definitions

All studies eligible for full-text review were independently investigated by two authors. As the scope was to evaluate the potential of ctDNA assays as screening tools in a population-based screening setting, we strived to include studies reporting the ctDNA assays' ability to detect tumor development at *early stages* (adenoma or Stage I–II CRC)—preferentially in screening cohorts of primarily *asymptomatic individuals*. Hence, studies that had included participants from either organized or opportunistic screening settings were included. An organized CRC screening cohort was defined as a cohort of average-risk asymptomatic individuals between 50 and 80 years following a national or center-based CRC screening initiative where blood samples were drawn before a diagnostic colonoscopy. This type of cohort was termed cohort Type A ($n = 8$, Figure 2). Studies with a retrospective analytical approach, but utilizing a prospectively, consecutively collected cohort of asymptomatic individuals between the ages 50 and 80 years were categorized as cohort Type B ($n = 2$).

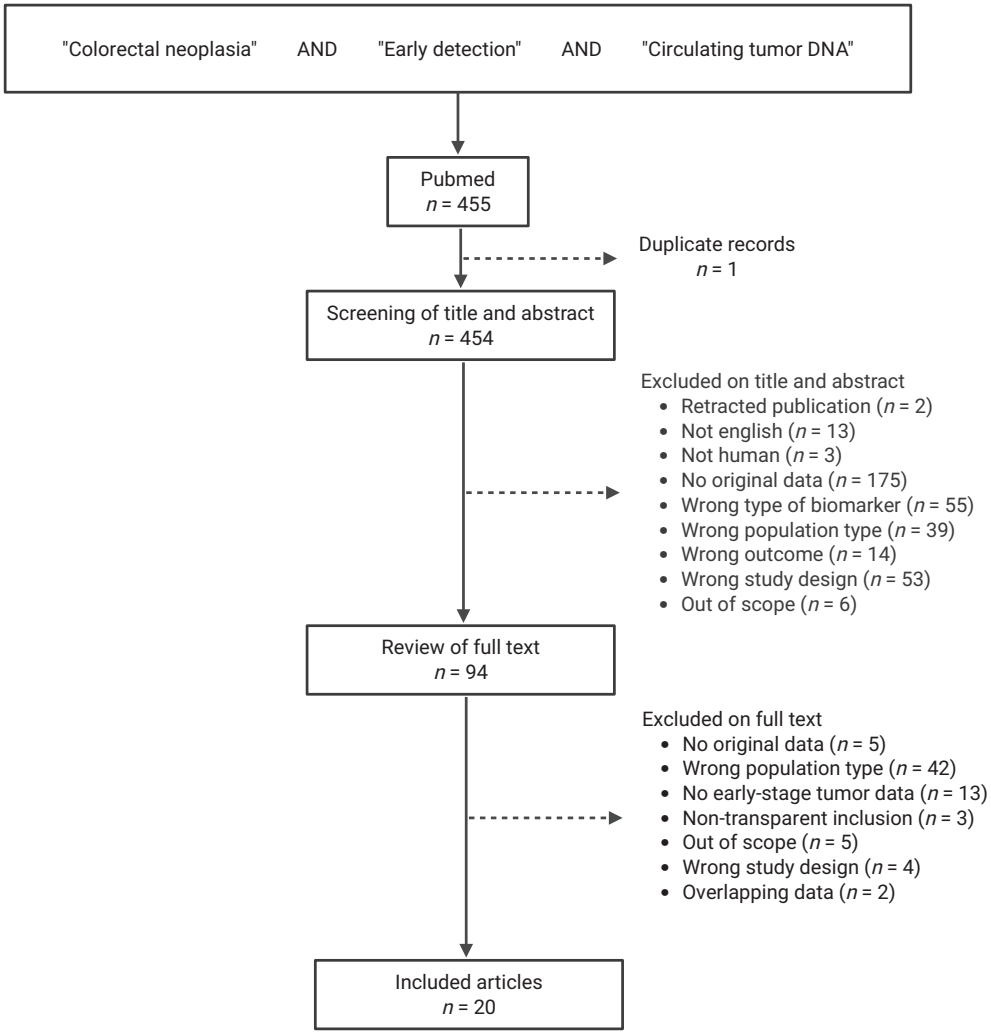

FIGURE 1 | Flowchart for selection of included studies. Flowchart depicting the process of including, screening, and excluding relevant studies. No original data included reviews, editorials, letters, and guidelines. Wrong type of biomarker included other types of blood-based biomarkers or liquid biopsies. Wrong population type included post-operative surveillance and studies with more than 50% symptomatic CRCs and no asymptomatic AA subset. Wrong outcome included extracolonic cancers or studies with no CRC specific data. Wrong study design included compliance or cost–benefit studies, studies on germline mutations, or technical biomarker development. Out of scope included articles in human papilloma virus or cell-free fetal DNA, case reports, or in vitro studies. Non-transparent inclusion included studies where data on inclusion was too sparse to assume whether this was a screening-relevant cohort. No early-stage tumor data included articles without data on Stage I–II and AA.

Opportunistic cohorts were defined as screening based on colonoscopy check-up recommendations or symptoms and could contain both asymptomatic (> 50%) and symptomatic participants (Figure 2). Opportunistic cohorts were divided into two subsets: one including individuals from a non-organized screening setting (primarily from countries without fecal-based screening) ($n = 9$) defined as cohort Type C; and one consisting of asymptomatic or opportunistic screening cohorts but spiked with post-diagnostically selected CRC cases (< 50%) ($n = 1$) [30], named cohort Type D.

Healthy cases were defined as individuals with non-advanced neoplasia, for example, non-advanced adenomas, inflammatory bowel syndrome, and diverticular disease, as well as people with no evidence of disease. Neoplasia diagnostics were required to be made via colonoscopy for prospectively included individuals or via cancer registries/medical records for retrospectively selected individuals to ensure available data on all study participants with a blood-based ctDNA test.

Any disagreements were resolved by consensus-based discussion with a minimum of two authors present.

2.4 | Quality Assessment

Quality assessment was performed for all 20 included studies by the three reviewing authors using a modified Newcastle-Ottawa scale (NOS) (https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) for cohort studies. The obtained number of stars for all included studies can be observed in Table S1 and further information regarding quality assessment can be found in the Supporting Information (section "Quality assessment"). The maximum number of stars obtainable in the modified NOS is

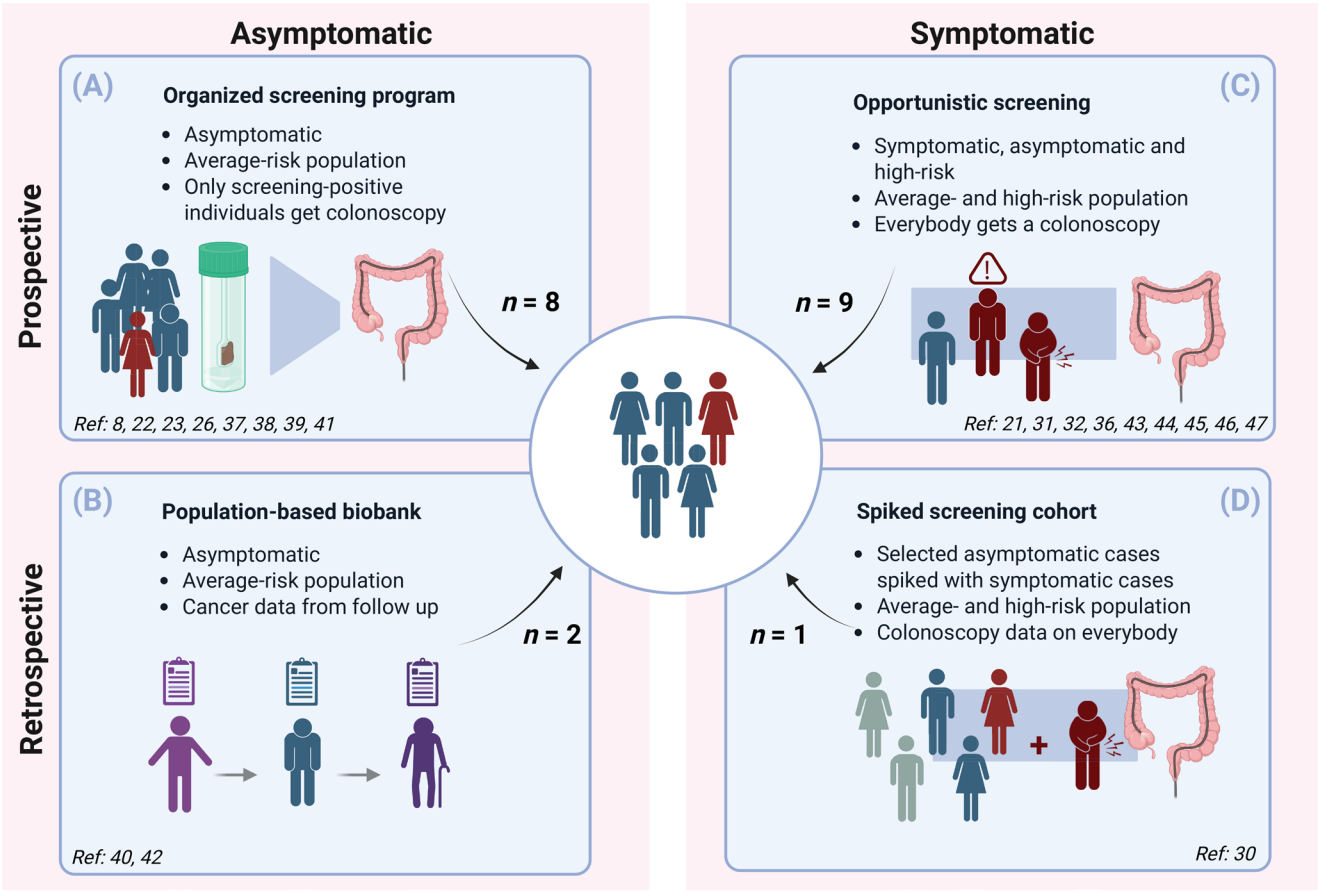

FIGURE 2 | Types of CRC screening cohorts in the included studies. Schematic depicting the four types of cohorts included in this review. Type (A) are prospective cohorts deriving from organized screening programs of all asymptomatic individuals at average risk of CRC. Of note, only fecal-test-positive individuals have received a colonoscopy if the cohort is derived from a fecal-test based screening approach. Type (B) are retrospective cohorts where blood samples were taken from asymptomatic individuals from the general population in relation to other study protocols. CRC data have then been assessed from medical records or registries. Hence, adenomas are completely missed in this sense. Type (C) consists of prospective opportunistically screened cohorts where all individuals receive a colonoscopy based on symptoms, a hereditary predisposition, a positive fecal-based test or a request from patients or general practitioners. This type of cohort is thus spiked with early-stage CRC that may be more severe as they have resulted in symptoms. Type (D) cohorts include cohorts of Type (A), (B), and (C) where the cohorts have been spiked with CRC cases selected post diagnosis. These studies may therefore—like the studies of cohort Type (C)—reach higher detection rates. Altogether, these cohort types result in the screening populations used to evaluate ctDNA detection of CRC and AA in this systematic review. Number and references to studies are shown for each cohort type.

11, whereas the maximum number of stars in the original NOS is 10. Only studies of good (9 stars) or fairly good quality (5–9 stars) were included.

2.5 | Data Extraction

Data were extracted by the three reviewing authors with two independent data extractions per study entry. The extracted data included are as follows: (1) study data; (2) cohort data; (3) methodological data; and (4) result/outcome data (sensitivity, specificity, AUC).

2.6 | Definitions

AAs did not have a specific definition but were considered AA if the studies defined adenomas as advanced. If a study had the correct cohort type but did not distinguish between non-advanced adenoma and AA and instead had pooled adenoma data, this data was included [31, 32]. Since non-advanced adenomas may have a lower overall detection rate, caution is advised when interpreting adenoma data from these studies.

Early-stage CRC was defined as CRC of UICC Stage I and II (though some studies also included Stage 0/carcinoma in situ with these stages). In case TNM scoring was used, early-stage CRC was defined as T0, T1, and T2 with M0 and regardless of N stage.

2.7 | Overlapping Studies

At least three of the published ctDNA biomarker assays (Colvera, Epi proColon/ColoHealth, and Shield) were commercially available at the time of this review, and some of these biomarkers had been heavily studied (and reviewed [33, 34]) in overlapping and increasing populations. When information on overlapping data sets was missing, the study

with the highest number of study participants was included. Regarding studies published by LaPointe and colleagues, the largest study cohort was chosen between Pedersen et al. and Symonds et al. [30, 35] (e.g., Petersen et al.), as well as a study focusing solely on sessile serrated lesions from the same research team [36].

3 | Results

3.1 | Literature Search Results

In total, 454 unique records published between February 1998 and June 2025 were identified in PubMed, of which 94 were suitable for full-text review following the title and abstract screening phase (Figure 1). Twenty studies met our inclusion criteria and were found eligible for this systematic review.

3.2 | Study Characteristics

The 20 studies tested ctDNA assays targeting DNA hotspot mutations in *KRAS* and/or *BRAF* ($n = 3$), DNA methylations in *BCAT1* and *IKZF1* (the Colvera assay, $n = 3$), DNA methylations in *SEPT9* (including the Epi proColon assay, $n = 6$), and more complex assays of multiple targets ($n = 8$). Study cohorts derived from North-American (USA), South-American (Brazil), Australian (Australia), European (Italy, Spain, Norway, Greece, The Netherlands, France, Germany, and Denmark), and Asian (China, Taiwan, and United Arab Emirates) countries (Table 1). The number of participants ranged from 52 to 27,010 across the studies with the number of CRC cases ranging from 12 to 123 and AA cases from 13 to 2567. Most studies used plasma samples ($n = 18$), while a couple of studies used serum samples ($n = 2$).

3.3 | Quality Assessment

The overall quality of the included studies ranged from 5 to 11 stars with only a single study [38] getting the maximum score of 11 stars (Table S1). The main reasons for scores falling lower than 11 were the cohort not being an organized screening cohort ($n = 4$ [8, 30, 41, 45]), ascertainment bias through conscious selection of the cohort (by spiking with CRC cases, retrospective selection, or selection of healthy controls from a different source than CRC cases) ($n = 10$ [8, 23, 30, 37, 40–45, 47]), non-transparency or no description in the methods section regarding blood sampling, ctDNA technology and/or ctDNA classification ($n = 11$ [21, 22, 31, 32, 36, 39, 40, 43–47]), and commercial conflicts of interest ($n = 7$ [21–23, 26, 30, 36, 37, 39, 44]). A total of 3 studies did not find and classify/interpret the results of the ctDNA analyzes in a blinded manner despite financial support from the commercial company owning the ctDNA assay [21, 39, 44].

Half of the studies featured opportunistic cohorts of both asymptomatic and symptomatic participants ($n = 10$ [21, 30–32, 36, 43–47]). The other half all had cohorts consisting of asymptomatic participants included before diagnostic examinations, of which 8 studies prospectively included organized screening cohorts [8, 22, 23, 26, 37–39, 41] while the 2 other studies had retrospective follow-up/registry-based cohort designs in a general population [40, 42].

3.4 | All CRC Detection

For organized screening cohorts (Type A and B), sensitivities for all CRC cases ranged between 47% and 79.2% and specificities ranged between 73% and 91.6% (Table 2). The FMBT-CRC test by Freenome had the highest sensitivity (79.2%) and the second highest specificity (91.5%). The Epi proColon test had the highest specificity (91.6%) in the study by Church et al. although this specificity was not seen in the other studies validating Epi proColon [22, 26].

The performance was improved for some biomarkers when considering the opportunistic screening cohorts (Type C and D), with sensitivities for all CRC cases ranging between 45% and 92.4% and specificities ranging between 17.9% and 100% (Table 2). A 20-marker methylation assay showed the highest sensitivity (92.4%) [47], although the same assay presented the lowest specificity of all assays (17.9%). In contrast, a *KRAS/BRAF* assay by Junca et al. showed the highest specificity (100%), but a low sensitivity of 45% [46]. This inverse association between sensitivity and specificity held mostly true throughout all studies, with the exceptions of the ColonSecure and Shield tests that both had sensitivities and specificities above 80% [21, 31].

3.5 | Early-Stage CRC Detection

Generally, the sensitivity of the blood-based ctDNA assays decreased when limiting the results to early-stage CRC. For organized screening cohorts, the sensitivities ranged between 37.2% and 71.1% (Table 2). An exception was, however, observed in the study by West-Szymanski et al. where the AUC increased from 0.728 (for all CRC) to 0.769 (for early-stage CRC) [40]. Though the sensitivity also dropped for the Freenome assay, it continued to have the highest sensitivity for early-stage CRC detection, being the only assay with a sensitivity above 70% [22].

For the opportunistically screened cohorts, sensitivities also dropped, ranging from 34.8% to 93.75% (Table 2) where the study by Gallardo-Gómez et al. stands out as the only one reaching an increased sensitivity when focusing only on early-stage CRC (from 92.4% to 93.8%) [47]. Though the sensitivities also dropped for the ColonSecure and Shield assays, they continued to have high sensitivities over 70% for early-CRC detection [21, 31].

3.6 | AA Detection

AAs were in general more difficult to detect across the assays investigated. Sensitivities for AAs ranged between 11.2% and 58.8% among organized screening populations (Table 2). The detection of AAs was below 25% for almost all studies ($n = 7$) and there was no data on AAs for the two retrospective studies [40, 42]. Only one assay performed fairly well: the ColoScape

TABLE 1 | Study cohort characteristics.

Author, year (ref)	Assay name/genetic targets	Cohort Type (A–D)	Cohort size (*n*)	Event size (*n*)	Age median (range)	% females	Country
Cohorts A and B							
m*SEPT9*							
Church 2014 [26]	Epi proColon	A	6874	CRC: 53 AA: 314	61–67 (50–80)[a]	34%–55%[b]	USA and Germany
Potter 2014 [37]	Epi proColon	A	1544	CRC: 44 AA: 621	NA	47%	Germany
Lima 2023 [8]	Methylated *SEPT9* and *BMP3*	A	262	AA: 44	58 (NA)	63%	Brazil
KRAS/BRAF							
Perrone 2014 [38]	*KRAS*	A	170	AA: 19	62 (49–74)	45%	Italy
BCAT1/IKZF1							
Kahn 2025 [23]	Colvera	A	774	CRC: 91 AA: 149	64 (56–70)	44%	Denmark
Assays							
Scimia 2018 [39]	ColoScape	A	52	AA: 13	NA	NA	Italy
West-Szymanski 2024 [40]	5hmC (32 marker panel)	B	200	CRC: 67	64 (55–75)	46%	USA
Shaukat 2025 [22]	FMBT-CRC (Freenome test)	A	27,010	CRC: 72 AA: 2567	57 (45–86)	56%	USA and United Arab Emirates
Petit 2023 [41]	Methylated *SDC2*, *NPY*, *IKZF1*, and *SEPT9*	A	537	AA: 137	64 (37–92)	45%	Australia
Brenne 2023 [42]	HUNT-CRCd	B	69	CRC: 34	Total: 70[c] (≥ 20)	49%	Norway
Cohorts C and D							
m*SEPT9*							
Warren 2011 [32]	m*SEPT9*	C	300	AA: 104	56 (22–84)	NA	USA
Chen 2017 [43]	Abbott MS-9	C	60	CRC: 51	NA	42%	Taiwan
Johnson 2014 [44]	Epi proColon	C	301	CRC: 101 AA: 193	NA (50–84)	52%	USA

(Continues)

TABLE 1 | (Continued)

Author, year (ref)	Assay name/genetic targets	Cohort Type (A–D)	Cohort size (*n*)	Event size (*n*)	Age median (range)	% females	Country
KRAS/BRAF							
Galanopoulos 2017 [45]	*KRAS* and *BRAF*	C	92	AA: 30[d]	NA (38–85)	45%	Greece
Junca 2020 [46]	*KRAS/BRAF*	C	130	CRC: 20 AA: 39	NA (29–92)	36%	France
BCAT1/IKZF1							
Cock 2019 [36]	Colvera	C[e]	1882	AA: 34	NA (28–86)	49%	Australia
Pedersen 2015 [30]	Colvera	D	2105	CRC: 129 AA: 357	62 (33–90)	46%	Australia and the Netherlands
Assays							
Gallardo-Gómez 2023 [47]	20-gene marker assay	C[f]	105	CRC: 24 AA: 42	63 (50–75)	47%	Spain
Zhao 2023 [31]	ColonSecure	C[g]	3493	CRC: 103 AA: 1735	54–62 (20–90)[h]	49%	China
Chung 2024 [21]	Shield	C	7861	CRC: 65 AA: 1116	60 (45–84)	54%	USA

Note: Cohort Types: (A) Prospective organized screening of asymptomatic individuals; (B) Retrospective (follow-up/registry based) screening of asymptomatic individuals; (C) Prospective opportunistic screening of asymptomatic and symptomatic (and high-risk) individuals; (D) Prospective screening cohorts spiked with CRCs selected post diagnosis. The targets of Shield, ColonSecure, and Freenome remain unknown for commercial purposes but are stated to include DNA alterations (mutations/methylations/CNV/SNP, or a mix including several of these) that are analyzed by machine learning algorithms and classifiers to give positive/negative scores for each sample.
Abbreviations: AA, advanced adenomas; CRC, colorectal cancer.
[a]Median age was 67 for CRC cases and 61 for non-CRC cases.
[b]The percentage of females were given separately for CRC cases (34%) and non-CRC cases (55%).
[c]Cohort characteristics given for total cohort, while result data were given separately for training and validation cohorts.
[d]Adenomas assumed as advanced adenomas.
[e]Same cohort as Pedersen S et al. but only sessile serrated lesions included here.
[f]Individuals are assumed to be prospectively selected from an opportunistic screening approach.
[g]Only "high-risk" individuals were included in the study. "High-risk" was based on family/personal history of colorectal neoplasia, IBD, or symptoms.
[h]Median age was 62 for CRC cases and 54 for non-CRC cases.

TABLE 2 | —Study outcome data for each of their reported biomarkers.

Author, year (ref)	Case size (*n*)	Cohort Type (A–D)	Assay name/ genetic targets	CRC—all			CRC—early (Stage 0–II)			Advanced adenoma		
				Sens (%)	Spec (%)	AUC	Sens (%)	Spec (%)	AUC	Sens (%)	Spec (%)	AUC
Cohorts A and B												
m*SEPT9*												
Church 2014 [26]	CRC: 53 AA: 314	A	Epi proColon	50.9	91.6	—	44.4	91.6	—	11.2	91.6	—
Potter 2014 [37]	CRC: 44 AA: 621	A	Epi proColon	68.2	78.2	—	58.6	78.2	—	21.6	78.2	—
Lima 2023 [8]	CRC: 36 AA: 44	A[a]	Methylated *SEPT9*	—	—	—	—	—	—	15.9	88.4	—
KRAS/BRAF												
Perrone 2014 [38]	AA: 19	A	*KRAS*	—	—	—	—	—	—	15.8	100	—
BCAT1 + *IKZF1*												
Kahn 2025 [23]	CRC: 91 AA: 149	A	Colvera	71.4	89.7	—	60.0	89.7	—	12.1	89.7	—
Assays												
Scimia 2018 [39]	AA: 13	A	ColoScape	—	—	—	—	—	—	58.8	92.3	—
Lima 2023 [8]	CRC: 36 AA: 44	D[a]	Methylated *BMP3*	—	—	—	—	—	—	13.3	84.1	—
West-Szymanski 2024 [40]	CRC: 67	B	5hmC (32 marker panel)	47.0	73.0	0.728	—	73.0	0.769[b]	—	—	—
Shaukat 2025 [22]	CRC: 72 AA: 2567	A	FMBT-CRC (Freenome test)	79.2	91.5	—	72.1	91.5	—	12.5	91.5	—
Petit 2023 [41]	AA: 137	A[a]	Methylated *IKZF1*, and *SEPT9*	—	—	—	—	—	—	2.2	94.6	—
Petit 2023 [41]	AA: 137	A[a]	Methylated *SDC2*, and *NPY*	—	—	—	—	—	—	13.9	89.5	—
Petit 2023 [41]	AA: 137	A[a]	Methylated *SDC2*, *NPY*, *IKZF1*, and *SEPT9*	—	—	—	—	—	—	14.6	85.2	—
Brenne 2023 [42]	CRC: 34	B	HUNT-CRCd	47.1	77.1	0.669	37.2	77.1	—	—	—	—

(Continues)

TABLE 2 | (Continued)

Author, year (ref)	Case size (*n*)	Cohort Type (A–D)	Assay name/ genetic targets	CRC—all			CRC—early (Stage 0–II)			Advanced adenoma		
				Sens (%)	Spec (%)	AUC	Sens (%)	Spec (%)	AUC	Sens (%)	Spec (%)	AUC
Cohorts C and D												
m*SEPT9*												
Warren 2011 [32]	AA: 104	C	m*SEPT9*	—	—	—	—	—	—	11.5	94.3	—
Chen 2017 [43]	CRC: 51	C	Abbott MS-9	47.0	89.0	0.766	34.8	89.0	—	—	—	—
Johnson 2014 [44]	CRC: 101 AA: 193	D	Epi proColon	73.3	81.5	—	69.6	81.5	—	21.2	81.5	—
KRAS/BRAF												
Galanopoulos 2017 [45]	AA: 30	C	*KRAS*	—	—	—	—	—	—	10	97.2	—
Galanopoulos 2017 [45]	AA: 30	C	*BRAF*	—	—	—	—	—	—	20	88.6	—
Junca 2020 [46]	CRC: 20 AA: 39	C	*KRAS/BRAF*	45	100	—	38.5	100	—	2.6	100	—
BCAT1 + IKZF1												
Cock 2019 [36]	AA: 34	C	Colvera	—	—	—	—	—	—	8.8	93.0	—
Pedersen 2015 [30]	CRC: 129 AA: 357	C	Colvera	65.9	93.9	—	56.3	93.9	—	6.2	93.9	—
Assays												
Gallardo-Gómez 2023 [47]	CRC: 24 AA: 42	C	20-gene marker assay	92.4	17.9	0.553	93.8	17.9	—	92.9	17.9	—
Zhao 2023 [31]	CRC: 103 AA: 1735	C	ColonSecure	86.4	88.1	—	85.0	88.1	—	10.3	88.1	—
Chung 2024 [21]	CRC: 65 AA: 1116	C	Shield	83.1	89.9	—	71.1[c]	89.9	—	13.2	89.9	—

Note: Cohort Types: (A) Prospective organized screening of asymptomatic individuals; (B) Retrospective (follow-up/registry based) screening of asymptomatic individuals; (C) Prospective opportunistic screening of asymptomatic and symptomatic (and high-risk) individuals; (D) Prospective screening cohorts spiked with CRCs selected post-diagnosis.

Abbreviations: AA, advanced adenomas; AUC, area under the curve; CRC, colorectal cancer; Sens, sensitivity; Spec, specificity.

[a]Study design included an AA subset of prospectively included asymptomatic individuals that data was extracted from (cohort Type A), along with a selected CRC subset. The CRC subset that did not have data extracted would therefore be cohort Type D, but the AA extracted data belong to cohort Type A.

[b]Stage I: 0.767, Stage II: 0.771.

[c]Had CRC data with unknown staging but 5/7 were "malignant polyps" and were therefore assumed to be Stage 0 (carcinoma in situ). The early-stage data therefore includes this group. The sensitivity is 80.6% if the unknown group is not included in early-stage CRC.

assay by Scimia et al. showing a sensitivity of 58.8% and a specificity of 92.3% [39].

The same was true for opportunistic screening cohorts, with half of the studies falling below even 10% ($n = 4$). Sensitivities for AAs ranged between 2.2% and 92.9%, with detection again being below 25% for almost all studies ($n = 8$) (Table 2). The only assay in cohorts C and D to have a sensitivity above 25% was the 20-marker test by Gallardo-Gómez et al. with a sensitivity of 92.9% and a specificity of 17.9% [47].

4 | Discussion

Despite decades of research on the use of blood-based ctDNA as a CRC screening tool, only 10 studies have tested ctDNA assays in an organized screening population of asymptomatic individuals (cohort Type A/B). Whether this is due to publication bias, financial challenges, lack of clinically relevant infrastructure, or poor study design, the issue still stands today that the existing technology for ctDNA detection in a screening setting is lacking. We found 10 studies with opportunistically screened cohorts of symptomatic and asymptomatic individuals that are clinically relevant with careful interpretation (cohort Type C/D). Though not all ctDNA assays were tested in all cohort types, the assays in cohorts C and D were able to reach higher performances. As symptomatic CRCs would be expected to be of higher UICC stages and therefore shed more ctDNA [48], we are not surprised to find that screening tools developed in opportunistic screening cohorts perform worse when detecting asymptomatic early-stage CRC and AAs.

4.1 | Early-Stage Versus Late-Stage CRC Detection

In general, all assays included in this review had previously been developed and tested in case–control studies—many including symptomatic and often late-stage CRC cases, thus reaching much higher sensitivities for all CRC than observed in here, when looking at early-stage CRC. For instance, the LUNAR-2 assay (assumed to later be developed and described as the Shield test) showed a sensitivity of 93% for all CRC (84% for Stage I and 94% for Stage II) in a cohort of retrospectively selected CRC patients [49], while the sensitivity dropped to 83% (65% for Stage I and 100% for Stage II) in an asymptomatic cohort of opportunistically screened individuals [21]. Likewise, a meta-analysis including 34 studies using methylated *SEPT9* as target for CRC detection (with no restrictions on cohort type or stage distribution) showed a sensitivity for all CRC of 67.9% (ranging between 24% and 90%) [34], which is somewhat higher than reported in the studies included in here with sensitivities of 47%–73% for all CRC and 35%–70% for early-stage CRC. The variability between the study results remains high in both their and our review highlighting performance dependencies on cohort ascertainment, study designs, laboratory methods, positivity thresholds, and laboratory staff. In all case groups (all CRC, early-stage CRC, AA), the range from lowest to highest reported sensitivity and specificity grew. The studies on mSEPT9 are an example of this, suggesting that cohort ascertainment influences assay performance more in opportunistically included cohorts compared to organized screening cohorts—perhaps due to heterogeneity between symptomatic and asymptomatic CRCs.

The two exceptions having better detection of early-stage CRC were West-Szymanski et al. and Gallardo-Gómez et al. that investigated epigenetic markers of 32 and 20 genes, respectively [40, 47] with only West-Szymanski et al. having an asymptomatic study cohort. In both studies, the majority of their cohorts consisted of stage I–II CRCs (61% in Gallardo-Gómez et al. and 55% in West-Szymanski et al.) and were used for assay/model development and validation (with a training/discovery part and a validation part). Their assays being developed for mostly early-stage CRC therefore might explain why they are the exceptions. However, due to the low specificities, this does not compete with already implemented screening tools.

Interestingly, only one of the reviewed ctDNA assays (ColonSecure) proved equally efficient in the detection of early-stage CRC (sensitivity of 85% for Stage I–II) as the FIT, which has a sensitivity of 73% for Stage I CRC and 80% for Stage II CRC [31, 50]. The ColonSecure assay was, however, evaluated in a high-risk cohort and these results thus need further validation in an average-risk cohort. Additionally, it would be useful to see FIT benchmarked against ColonSecure (along with the other highest-performing ctDNA assays in this review) in a set-up where all participants were required to take both tests to allow direct comparison. Thus far, only stool (s) DNA-based assays have done this comparison [51], while others have added the ctDNA test to an already existing FIT screening program to evaluate optimization potential [23, 52, 53]. If blood-based ctDNA assays are not able to compete with FIT when it comes to early-stage CRC detection, it could be that sDNA assays or other types of blood-based biomarker assays is way forward in a screening setting.

4.2 | AA Detection

Unfortunately, the detection rate of AAs fell below 25% for 15 out of 17 included studies with data on AAs. It is a general issue in the literature that ctDNA assays cannot find AAs, despite their importance for CRC prevention strategies [17]. According to a systematic review and meta-analysis by Goyal et al. the sensitivity of a ctDNA assay should be $\geq 50\%$ with the specificity being $\geq 90\%$ for AAs in order to be equivalent to FIT and clinically acceptable [50, 54]. Only the ColoScape test by Scimia et al. managed to fulfill these parameters with a sensitivity of 58.8% and specificity of 92.3% [39]. However, as noted by Goyal et al. a sample size of 67 to 100 AA cases would be required in order to observe a significant improvement of a ctDNA assay in comparison to FIT [55, 56]. Of note, the study by Scimia et al. only analyzed 13 cases with AA, meaning that this study heavily lacks the statistical power needed. The ColoScape assay has since been developed to include additional ctDNA targets, and in a larger cohort of 50 AAs and 27 early-stage CRC cases has shown sensitivities of 44% for AAs and 63% and 78% for Stage I and II CRC, respectively. These results are, however, only presented in an abstract [57].

The relatively low performance related to AA and early-stage CRC detection could also simply be explained by the fact that

early-stage CRC and AA do not produce and shed sufficient amounts of ctDNA into the bloodstream for it to be detected in a small blood sample [58]. With an estimated half-life of ctDNA in the bloodstream being 114 min [59], it is essentially a balance between the amount of ctDNA released (being proportional to tumor size) and the speed of ctDNA clearance in the blood [48]. Evidence also shows that symptomatic AAs are easier to detect with ctDNA than asymptomatic AAs (sensitivity of 50% vs. 25%, respectively) indicating that symptoms may affect the presence of ctDNA in the blood or vice versa [60]. This begs the question of whether ctDNA sheds in measurable amounts from smaller, asymptomatic tumors and whether the solution is simply more blood, larger tumors or other biomarkers (e.g., miRNA or protein).

4.3 | Cost-Efficency Considerations

Finally, before implementing any screening tool, the cost needs to be considered. A single target assay such as ColoHealth (earlier known as Epi proColon) is much cheaper compared to complex assays such as Shield (Table 3). Generally, the best performing blood-based ctDNA tests include DNA methylations of several targets (149 methylated targets in the ColonSecure assay described by Zhao et al. [31]) or use several molecular methods for ctDNA detection (DNA mutation, DNA methylation, fragmentomics, and machine learning algorithms used in both Shield and FMBT-CRC [21, 22]). A study by van den Puttelaar et al. found that ColoHealth was more cost-efficient compared to Shield, although FIT was more cost-efficient than both Shield and ColoHealth [61]. Another study by Hutchinson et al. found that screening with a hypothetical cell-free (cf) DNA assay matching the results of Shield would result in more CRC deaths and higher cost compared to FIT if adherence and participation rates matched that of FIT [62]. However, they noted that at 100% adherence and 90% participation (based on real-world data from a withdrawn study by Guardant Health Inc. [63]) Shield had increased CRC detection and reduced mortality [63]. In this scenario, the cost would still be higher than FIT due to the price of Shield. These studies have not been done on the Galleri or the Freenome assay as the research is still ongoing.

A large consideration when designing blood-based ctDNA assays is whether individual cancer screening or pan-cancer screening is more cost-efficient. Due to the higher cost of comprehensive ctDNA assays (Table 3), several studies have looked into pan-cancer approaches such as fragmentomics or methylation signatures with promising results [19, 64–66]. However, very few have screening-relevant cohorts for pan-cancer screening with cohorts/trials such as the PLCO from West-Szymanksi et al. and the HUNT from Brenne et al. being rare exceptions [40, 42].

5 | Limitations

This systematic review is limited by the small number of included studies, many of them based on small cohorts, as it proved difficult to find studies that were based on screening populations.

TABLE 3 | Commercially available blood-based ctDNA tests.

Commercial name, price, and company	Characteristics
ColoHealth New Day Diagnostics $ 199	Target: methylated *SEPT9* FDA approved Covered by MediCare Previously known as Epi proColon from Epigenomics Inc., now rebranded by New Day Diagnostics
Colvera Clinical Genomics Price unknown	Target: methylated *BCAT1*, and *IKZF1* FDA approved Was previously covered by MediCare Company out of business, patent inactive
ColoScape DiaCarta Inc. $299	Target: 8 hotspot mutations in *APC*, *CTNNB1*, *KRAS*, and *BRAF* Not FDA approved Not covered by MediCare
Shield Guardent Health $ 895	Target: Specific targets unknown, but alleged somatic mutations, methylations, and an AI-scoring algoritm FDA approved Covered by MediCare
FMBT-CRC Freenome Holdings Inc. Price unknown	Target: Specific targets unknown, but alleged methylation patterns, and an AI-scoring algorithm (possibly also investigating several types of omics) Not FDA approved Not covered by MediCare Ongoing Research Not yet available for purchase

Abbreviations: AI, artificial intelligence; CRC, colorectal cancer; FDA, U.S. food and drug administration.

Most studies categorized as "Cohort Type A" consisted of screening participants from national FIT-based CRC screening programs where only FIT-positive individuals undergoing colonoscopies were analyzed for ctDNA [8, 23, 38, 39, 41]. This would result in an over-representation of CRC cases in the study cohort—all with blood in the stool—while inhibiting detection of CRC cases otherwise missed by FIT. Studies categorized "Cohort Type B" included population-based cohorts counteracting the previous limitation but introducing a lack of AA data (due to registers and/or patient records used for follow-up) [42]. Study cohorts of "Type C" included symptomatic CRC cases which may lead to overestimated sensitivities. Only the Shield and FMBT-CRC assays were tested in prospectively and consecutively included unselected population-based cohorts with

all participants undergoing colonoscopies [4, 21]. Shield and FMBT-CRC are both comparable to FIT in regard to CRC detection, but since participants did not take a FIT test in these studies, it is impossible to know if Shield and FMBT-CRC could replace the FIT in parts of the world where it is implemented. To fully assess the clinical potential of a CRC screening tool, it should be benchmarked against the closest best standard which is currently FIT.

A key clinical limitation for many of these studies is that UICC Stage II CRC is often found to have a higher detection rate than UICC Stage III as was the case with FMBT-CRC (100% for Stage II and 82% for Stage III) [22], a Colvera study (90% for Stage II and 74% for Stage III) [23], an Epi proColon study (80% for Stage II and 65% for Stage III) [44], and a KRAS/BRAF study (30% for Stage II and 20% for Stage III) [46] included in this review. This is likely caused by the larger T3 and T4 tumors categorized under UICC Stage II, whereas UICC Stage III also includes smaller T1 and T2 tumors with lymph nodes [67]. T-stage has been shown to impact the level of ctDNA shedding in CRC, meaning that reporting ctDNA sensitivities for CRC based on UICC stages is somewhat problematic [48]. UICC Stage II ends up drastically increasing the early-stage (UICC Stage I–II) detection based on large T3 and T4 tumors with worse prognosis [68]. Thus, more studies should report CRC detection rates for ctDNA assays stratified by T-stage in the future.

6 | Conclusion

In summary, there is a considerable lack of solid studies investigating ctDNA screening tools in asymptomatic cohorts of population-based average-risk individuals. None of the ctDNA assays reviewed in here showed sufficient sensitivities towards both AA and early-stage CRC detection to be considered as future screening tools. ctDNA research in solid tumors is an evolving field, and while the goal for many is cancer prevention and early detection, the technology and limits of detection are not yet developed. Hopefully, future innovative techniques and validation in asymptomatic target populations can remove some of the challenges of finding the ctDNA target in the haystack.

Author Contributions

Caroline Ledertoug Kahn: conceptualization, methodology, formal analysis, investigation, data curation, visualization, writing – original draft, writing – review and editing. **Maria Wissing Rasmussen:** conceptualization, methodology, formal analysis, investigation, data curation, writing – review and editing. **Jakob Kleif:** investigation, supervision, writing – review and editing. **Inge Søkilde Pedersen:** investigation, supervision, writing – review and editing. **Christina Therkildsen:** conceptualization, methodology, formal analysis, investigation, data curation, visualization, supervision, project administration, funding acquisition, writing – review and editing.

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Conflicts of Interest

C.L.K., J.K., and C.T. are authors of a study included in the review [23]. I.S.P. is an author of a study included in the review [42]. M.W.R. declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Quality assessment for all included articles.