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SYSTEMATIC REVIEWS

Cell-free DNA liquid biopsy for early detection of gastrointestinal cancers: A systematic review

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Abstract

BACKGROUND

Gastrointestinal tumors are among the most common cancer types, and early detection is paramount to improve their management. Cell-free DNA (cfDNA) liquid biopsy raises significant hopes for non-invasive early detection.

AIM

To describe current applications of this technology for gastrointestinal cancer detection and screening.

METHODS

A systematic review of the literature was performed across the PubMed database. Articles reporting the use of cfDNA liquid biopsy in the screening or diagnosis of gastrointestinal cancers were included in the analysis.

RESULTS

A total of 263 articles were screened for eligibility, of which 13 articles were included. Studies investigated colorectal cancer (5 studies), pancreatic cancer (2 studies), hepatocellular carcinoma (3 studies), and multi-cancer detection (3 studies), including gastric, oesophageal, or bile duct cancer, representing a total of 4824 patients. Test sensitivities ranged from 71% to 100%, and specificities ranged from 67.4% to 100%. Pre-cancerous lesions detection was less performant with a sensitivity of 16.9% and a 100% specificity in one study. Another study using a large biobank demonstrated a 94.9% sensitivity in detecting cancer up to 4 years before clinical symptoms, with a 61% accuracy in tissue-of-origin identification.

CONCLUSION

cfDNA liquid biopsy seems capable of detecting gastrointestinal cancers at an early stage of development in a non-invasive and repeatable manner and screening simultaneously for multiple cancer types in a single blood sample. Further trials in clinically relevant settings are required to determine the exact

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place of this technology in gastrointestinal cancer screening and diagnosis strategies.

Key Words: Cell-free DNA; Tumor DNA; Liquid biopsy; Next-generation sequencing; Cancer genomics; Pancreatic cancer; Colorectal cancer; Hepatocellular carcinoma; Multi-cancer detection; Cancer screening; Public health; Precision oncology

Core Tip: Liquid biopsy cell-free DNA represents a promising non-invasive method for detecting various gastrointestinal cancers at an early stage of development. The current literature suggests a high-performance profile for this technology and the potential to improve the global course of gastrointestinal cancers currently diagnosed at an advanced stage, such as pancreatic cancer. Prospective validation studies in relevant clinical settings are required to determine the applicability and added value of these new diagnostic and screening tests in global cancer care.

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INTRODUCTION

Tumors developing from the gastrointestinal tract are among the most common cancer types, colorectal and stomach cancer, counting for 19.5% and 11.1% respectively worldwide in 2020[1]. Risk factors notably include smoking, obesity, poor diet, genetic factors, and infections with hepatitis B virus or Helicobacter pylori bacteria[2]. Early detection and diagnosis represent a crucial component to allow effective treatment and improve survival. Nowadays, different screening strategies have been developed, such as colonoscopy for colorectal cancer or blood testing for alpha-fetoprotein (AFP) or magnetic resonance imaging in high-risk patients for liver cancer, but other types of tumors often lack screening strategies and non-invasive testing. For instance, so far, no efficient screening methods are available for pancreatic cancer; most patients experience their first symptoms at advanced and metastatic stages, explaining the 5-year survival rate of only 5% to 10%[3].

These past few years, researchers have focused their attention on a new promising diagnostic method, liquid biopsy, which uses biomarkers such as circulating cell tumor, RNA fragments, or cell-free DNA (cfDNA). Unlike tissue samples obtained by invasive methods like needle biopsies or endoscopies, biomarkers can be detected in body fluids, mostly blood[4], and address limitations of tissues biopsies not only in diagnosis and screening, but also in diagnosis and screening the treatment response and follow-up[5-7]. Among liquid biopsy options, cfDNA raises the most significant hopes in early cancer detection. Historically, it was first reported in 1948 by Mandel *et al* among healthy patients. In 1977, Leon *et al* described elevated levels of cfDNA in the serum of cancerous patients for the first time[4,8,9]. CfDNA is continuously released in the bloodstream through different mechanisms such as apoptosis, necrosis, and active secretion by the tumor cell. When originating from a cancer cell, cfDNA is called circulating tumor DNA (ctDNA)[4]. Concentration levels seem to correlate with the cancer stage and size; advanced-stage cancer patients show a higher concentration of cfDNA[8,9]. While cfDNA quantification in the bloodstream might indicate the presence or absence of cancer, sequencing and analyzing the mutation patterns of this cfDNA goes one step further: mutational profiling might give the researchers clues on the tumor's tissue of origin, providing information to target further specific investigations[9]. Recent progress in genomic technology also provides highly sensitive detection of low-prevalence mutations, even in high signal-to-noise configurations, thus theoretically enabling very early cancer diagnosis. The ability to run repeatable, non-invasive, multi-cancer early detection tests would bring significant advantages in

the global care of frequently hardly reachable cancer locations, such as gastrointestinal cancers.

The present systematic review of the literature aims to describe the current state of developing cfDNA liquid biopsies as a means of early gastrointestinal cancer detection and screening.

MATERIALS AND METHODS

A systematic review of the literature was performed following the PRISMA guidelines [10]. All articles written in English from January 2010 to January 2021 were searched on January 19th, 2021, through the PubMed database using the following research algorithm: (liquid biopsy OR cfdna) AND (multiple OR gastrointestinal OR colon OR colorectal OR gastric OR oesophag* OR liver OR hepatocellular OR pancreatic) AND (cancer OR tumor OR tumour) AND (screening OR diagnos* OR detect*) AND early AND (blood OR venous OR plasma) NOT review.

After a first selection based on titles for screening, eligible articles were selected based on abstract analysis. Then, full-text analysis of the eligible articles searched for criteria of the finally included articles. Two investigators (I Uhe, J Douissard) independently assessed the articles for eligibility and inclusion. Discordances in study inclusions were solved by re-evaluation between the two reviewers.

All relevant articles reporting human studies investigating cfDNA liquid biopsy as a screening method or diagnosis method for newly discovered untreated primary gastrointestinal cancers were included. Studies investigating multiple cancer screening, including gastrointestinal but not limited to them, were also included. Excluded articles were studies investigating cfDNA as a follow-up method after cancer treatment, minimal residual disease detection, studies investigating cfDNA as a prognosis method only, reviews, meta-analyses, theoretical papers, and biological studies not reporting clinical outcomes. Studies reporting cancer patients who were already treated, surgically or medically, have also been excluded. To improve the present review's clinical relevance, only the total number of participants in the papers' validations cohorts were considered. If available, test performances were reported in terms of sensitivity (Se), specificity (Sp), positive and negative predictive values, or area under the curve (AUC).

Literature search and studies characteristics

A total of 263 articles were identified through the PubMed search. Two articles were not written in English, 11 were not original publications, and 119 did not involve cfDNA. Thirty-five articles did not mention gastrointestinal cancer, and 44 did not investigate cfDNA as a screening or diagnosis method, leaving 52 articles. After full-text reading, thirteen studies were ultimately included for analysis, representing a total of 4824 patients (Table 1, Figure 1). The largest study included blood samples from 1194 participants[11], while the smallest study included samples of 130 participants[12]. Six studies took place in China[11,13-17], three in the United States[9,18,19], and four in Europe[12,20-22]. Five were multicentric[9,11,16,18,19], four monocentric[13,14,17,22] and four studies did not mention the information. Five studies focused on colorectal cancer (CRC)[9,12,17,20,22], three on various cancer types [14,19,21] of which two included gastric cancers[14,19], three on hepatocellular carcinoma (HCC)[11,15,16] and two on pancreatic ductal adenocarcinoma (PDAC)[13,18]. All studies compared cancer and non-cancer individuals. Five of them also included in their analysis a group of patients with pre-cancerous lesions, such as colorectal adenoma or hyperplasia, liver cirrhosis, or chronic hepatitis B virus infection [11,12,15,16,22] (Table 2).

Risk of bias of included studies

The risk of bias of included studies was determined using the ROBINS-I tool (2016) [23]. Except for one study with an overall low risk of bias[16], all included studies were at moderate risk (Table 3).

Extraction and sequencing methods

All studies collected cfDNA from plasma samples. Kits used for cfDNA extraction from plasma samples can be found in Table 4. The QIAamp circulating nucleic acid kit was the most employed, a spin column-based kit (n = 7/13). A large majority of studies used next-generation sequencing (NGS) (n = 9/13), two used real-time polymerase chain reaction (RT-PCR), one digital droplet PCR, and one multiplex

Table 1 Characteristics of included studies

Ref.	Year	Country	Mono/multicentric	Type of cancer	Total number of patients in validation cohort	Type of groups analyzed
Li *et al*[13]	2020	China	Monocentric	PDAC	208	Cancer *vs* healthy
Chen *et al*[14]	2020	China	Monocentric	Gastric, esophagus, colorectal, lung or liver	418	Cancer diagnosed *vs* healthy; Pre-diagnosed patients *vs* healthy
Guler *et al*[18]	2020	United States	Multicentric	PDAC	228	Cancer *vs* healthy
Junca *et al*[12]	2020	France	NA	Colorectal	130	Cancer *vs* healthy *vs* advanced-adenoma *vs* non-advanced adenoma and/or hyperplastic polyp(s)
Tao *et al*[15]	2020	China	NA	HCC	175	HBV-related HCC *vs* cancer-free HBV patients
Cristiano *et al*[19]	2019	United States	Multicentric	Breast, colorectal, lung, ovarian, pancreatic, gastric, bile duct	423	Cancer *vs* healthy
Li *et al*[17]	2019	China	Monocentric	Colorectal	140	Cancer *vs* healthy
Qu *et al*[16]	2019	China	Multicentric	HCC	331	HBsAg1 positive without cancer based on screening with serum AFP and ultrasonography
Cai *et al*[11]	2019	China	Multicentric	HCC	1194	Cancer *vs* healthy *vs* 392 LC/HB *vs* BLL
Wan *et al*[9]	2019	United States	Multicentric	Colorectal	817	Cancer *vs* healthy
Jensen *et al*[20]	2019	Denmark	NA	Colorectal	234	Cancer *vs* healthy
Nunes *et al*[21]	2018	Portugal	NA	Breast, colorectal, lung	356	Cancer *vs* healthy
Perrone *et al*[22]	2014	Italy	Monocentric	Colorectal	170	Cancer *vs* healthy *vs* premalignant lesion (adenoma/hyperplasia)

PDAC: Pancreatic ductal adenocarcinoma; HCC: Hepatocellular carcinoma; LC/HB: Liver cirrhosis/hepatitis B; BLL: Benign liver lesions; HBV: Hepatitis B virus; AFP: Alpha-fetoprotein.

methylation-specific PCR. Various mutational patterns and genomic profiling strategies were investigated (Table 4). Most studies focused on methylation variations (n = 7/13), while others investigated specific mutation locations such as *KRAS* and *BRAF* or more complex mutational patterns.

Tests performance

Overall test performances for each cancer subgroup are described in Table 5.

RESULTS

CRC

Clinically relevant sensitivities and specificities to detect colorectal adenocarcinoma were achieved in three studies[9,20,21], Li *et al*[17] and Jensen *et al*[20] focusing on tumor-specific methylations. In contrast, Wan *et al*[9] investigated complex cfDNA mutational patterns using a machine-learning-based model. Sensitivities ranged from 74% to 85%, while specificities ranged from 85% to 99%. In a fourth study, Perrone *et al*[22] reported an AUC of 0.709 when discriminating CRC from healthy patients. However, for premalignant lesions, the performance was lower, with an AUC of 0.535 [22]. Similarly, investigating adenomas and adenocarcinomas through cfDNA *KRAS* and *BRAF* mutations, Junca *et al*[12] found a mean sensitivity of 16.9% for a 100% specificity reflecting a still lower sensitivity in premalignant lesions detection but allowing a high level of precision.

Table 2 Number of patients in each group

Ref.	Total patients in validation cohort	Nbr patient cancer group	Nbr patient healthy group	Nbr patient additional group 1	Nbr patient in aditionnal group 2
Li *et al*[13]	208	101	107	-	-
Chen *et al*[14]	418	113	2071	98 pre-diagnosed patients	-
Guler *et al*[18]	228	23	205	-	-
Junca *et al*[12]	130	20	40	39 advance adenoma	31 non-advance adenoma
Tao *et al*[15]	175	89	86	-	-
Cristiano *et al*[19]	423	208	215	-	-
Li *et al*[17]	140	74	66	-	-
Qu *et al*[16]	331	-	-	HBsAg (+)	-
Cai *et al*[11]	1194	809	256	129 LC/CHB	-
Wan *et al*[9]	817	546	271	-	-
Jensen *et al*[20]	234	143	91	-	-
Nunes *et al*[21]	356	253	103	-	-
Perrone *et al*[22]	170	34	63	73 adenoma/hyperplasia	-

LC: Liver cirrhosis, CHB: Chronic hepatitis B virus infection. 90 GC patients without surgery and 110 who had undergone surgery.

Pancreatic cancer

Examining methylation patterns in cfDNA, Li *et al*[13] described eight methylation markers in patients suffering from PDAC; SIX3, TRIM73, MAPT, FAM150A, EPB41L3, MIR663, LOC100130148, and LOC100128977. These markers identified PDAC patients efficiently, with a sensitivity of 93.2% and a specificity of 95.2% (AUC = 0.943). By investigating 5-hydroxymethylcytosine (5hmC) changes in circulating cfDNA, Guler *et al*[18] achieved similar performance with an AUC of 0.921.

Hepatocellular carcinoma

Cai *et al*[11] found promising results using a mutational pattern of 32 gene markers to discriminate HCC patients from healthy individuals, with a sensitivity and specificity of 82.7% and 76.4%, respectively. Furthermore, when comparing HCC patients with cancer-free high-risk patients (chronic hepatitis B or liver cirrhosis), the model performed similarly with an 82.7% sensitivity and 67.4% specificity[11].

Comparing HCC patients with cancer-free asymptomatic HBV patients based on cfDNA mutational pattern of specific locations, Qu *et al*[16] achieved a sensitivity and specificity of 100% and 94%, respectively. Further, using somatic copy number aberration in cfDNA as an alternative to methylation or specific mutations analysis, Tao *et al*[15] investigated the possibility of discriminating HBV-related HCC from cancer-free chronic HBV patients. Their predictive model performed appropriately, showing a high level of precision in two validation cohorts, with an AUC of 0.92 and 0.81.

Multi-cancer detection

Nunes *et al*[21] investigated the possibility to diagnose lung, breast, and colorectal cancer patients simultaneously from healthy individuals by detecting aberrant methylations on specific locations. They achieved an overall specificity of 73.5% and a sensitivity of 74.2%. For colorectal cancer, specificity was 69.9%, and sensitivity was 78.4%[21].

With a comparable strategy targeting five cancers (gastric, oesophageal, lung, liver, and colorectal), Chen *et al*[14] demonstrated the potential ability of cfDNA liquid biopsy to achieve multicancer detection several years before the actual diagnosis. Based on blood samples from a large biobank, they analyzed samples from 3 groups. The post-diagnosis group included patients with a newly discovered and untreated

Table 3 Risk of bias of included studies, determined using the ROBINS-I tool (2016)

Ref.		Entry	Judgement	Support for judgement
Li *et al*[13]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	No information	No information about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	No information	No information about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Chen *et al* [14]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	Low risk	Information provided about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	Low risk	Information provided about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Guler *et al* [18]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	No information	No information about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	No information	No information about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Junca *et al* [12]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	No information	No information about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	No information	No information about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention

	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analysesconsistent with a priori plan
Tao *et al*[15]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	Low risk	Information provided about the start of follow up and intervention for the participants in the supplementary materials
	C	Bias in classification of interventions	Low risk	Information provided about the start of follow up and intervention for the participants in the supplementary materials
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Cristiano *et al*[19]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	No information	No information about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	No information	No information about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Li *et al*[17]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	No information	No information about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	No information	No information about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Qu *et al*[16]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	Low risk	Information provided about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	Low risk	Information provided about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Low risk	Pre-registered protocol available (NCC201709011)

Cai *et al*[11]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	Low risk	Information provided about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	Low risk	Information provided about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Wan *et al*[9]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	No information	No information about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	No information	No information about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Jensen *et al*[20]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	Low risk	Information provided about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	Low risk	Information provided about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Nunes *et al*[21]	A	Bias due to confounding	Low risk	No confounding factors
	B	Bias in selection of participants into the study	No information	No information about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	No information	No information about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan
Perrone *et al*[22]	A	Bias due to confounding	Low risk	No confounding factors

	B	Bias in selection of participants into the study	Low risk	Information provided about the start of follow up and intervention for the participants
	C	Bias in classification of interventions	Low risk	Information provided about the start of follow up and intervention for the participants
	D	Bias due to deviationsfrom intended interventions	Low risk	No deviations from the planned interventions
	E	Bias due to missing data	Low risk	All data were reported
	F	Bias in measurement of outcomes	Low risk	Comparable methods of outcome assessment in the groups, intervention received in each group unlikely to influence the outcome measure, any error in measuring the outcome is unrelated to intervention
	G	Bias in selection of the reported result	Moderate risk	No pre-registered protocol available; outcome measurements and analyses consistent with a priori plan

Table 4 Details of extraction and sequencing methods used in each of the included studies

Ref.	Source of cfDNA	Focus in cfDNA	Extraction method (used kit)	Sequencing method	Sequencing method details
Li *et al*[13]	Plasma	Methylated markers	QIAamp Circulating Nucleic Acid Kit (Qiagen, 55114)	NGS	Illumina HiSeq 2000 platform
Chen *et al*[14]	Plasma	Cancer-specific methylation signatures	QIAamp Circulating Nucleic Acid kit (Qiagen, 55114)	NGS	APA Library Quantification Kit for Illumina (KK4844) and sequenced on an Illumina NextSeq 500
Guler *et al*[18]	Plasma	5hmC modifications	QIAamp Circulating Nucleic Acid Kit (QIAGEN, Germantown, MD)	NGS	NextSeq550 instrument with version 2 reagent chemistry (Illumina, San Diego, CA).
Junca *et al*[12]	Plasma	*KRAS* and *BRAF* mutational status	QIAamp Circulating Nucleic Acid kit (Qiagen, Hilden, Germany)	RT-PCR	Q24 PyroMark system (Qiagen, Hilden, Germany)
Tao *et al*[15]	Plasma	Somatic copy number aberration	QIAamp CirculatingNucleic Acid Kit (Qiagen)	NGS	Next generation sequencing (Illumina)
Cristiano *et al*[19]	Plasma	Fragmentation size	Qiagen Circulating Nucleic Acids Kit (Qiagen GmbH)	NGS	NEBNext DNA Library Prep Kit for Illumina
Li *et al*[17]	Plasma	Aberrant DNA hypermethylation of CpGislands	DNeasy Blood & TissueKit (Qiagen)	NGS	Methylated CpG tandem ampli-fication and sequencing
Qu *et al*[16]	Plasma	Specific mutations	ARCHITECT i2000SR Chemical luminescence immunity analyzer	NGS	Next generation sequencing
Cai *et al*[11]	Plasma	5hmC modifications	NA	NGS	5hmC-Seal
Wan *et al*[9]	Plasma	cfDNA mutations patterns	MagMAX cfDNA Isolation Kit	NGS	Illumina NovaSeq 6000 Sequencing System
Jensen *et al*[20]	Plasma	Tumour-specific DNA methylation	Gentra Puregene Tissue Kit (Qiagen)	DD-PCR	Bisulfite sequencing and methylation-specific droplet digital PCR
Nunes *et al*[21]	Plasma	Aberrant DNA methylation	QIAamp MinElute ccfDNA (Qiagen, Hilden, Germany)	qMSP	qMSP
Perrone *et al*[22]	Plasma	*KRAS* mutated cfDNA	Qiamp DNA Blood Extraction Kit (Qiagen)	RT-PCR	RT-PCR

NGS: Next-generation sequencing; RT-PCR: Real-time polymerase chain reaction; qMSP: Multiplex methylation-specific polymerase chain reaction.

malignancy at the time of sampling. The pre-diagnosis group included patients with no known malignancy at the sampling time but who developed cancer within four years after sampling (pre-diagnosis). Finally, the control group included healthy individuals who were still free of malignant disease four years after sampling. Their model achieved an overall detection specificity of 96% when comparing healthy individuals to pre-diagnosis and post-diagnosis groups. Overall sensitivity was 87.5% for the post-diagnosis group, ranging from 75% in colorectal cancer to 96% in lung cancer. It reached 94.9% in the pre-diagnosis group, ranging from 91% in oesophageal cancer to 100% in liver cancer[14].

Table 5 Sensibility and sensitivity of included studies

	Ref.	Group of validation cohorts	Sensitivity	Specificity	Positive predictive value	Negative predictive value	AUC
PDCA	Li *et al*[13]	Cancer *vs* healthy	93.2	95.2	NA	NA	0.943
	Chen *et al*[14]	Cancer *vs* healthy	NA	NA	NA	NA	0.921
HCC	Guler *et al*[18]	HBV-related HCC *vs* cancer-free HBV group 1	18	97.4	NA	NA	0.92
		HBV-related HCC *vs* cancer-free HBV group 2	29	95.6	NA	NA	0.81
	Junca *et al*[12]	HCC *vs* cancer-free HBV	100	94	17	100	NA
	Tao *et al*[15]	HCC *vs* healthy	82.7	76.4	NA	NA	0.884
		HCC *vs* high risk (HBV and cirrhosis)	82.7	67.4	NA	NA	0.846
Various cancer types	Cristiano *et al*[19]	Pre-diagnosis *vs* healthy	84.9	96.1	NA	NA	NA
		Post-diagnosis *vs* healthy	87.5	96.1			
	Li *et al*[17]	All cancer *vs* healthy	80	95	NA	NA	0.94
			73	98			
		Gastric cancer *vs* healthy	81	95			
			81	98			
		Colorectal cancer *vs* healthy	81	95			
			70	98			
		Bile duct cancer *vs* healthy	88	95			
			81	98			
		Pancreatic cancer *vs* healthy	71	95			
			65	98			
	Qu *et al*[16]	All cancer *vs* healthy	74.2	73.5	87.1	52.1	NA
		Colorectal cancer *vs* healthy	78.4	69.9	48.3	90	
Colorectal	Cai *et al*[11]	Cancer/adenoma *vs* healthy	16.9	100	100	59.2	NA
	Wan *et al*[9]	Cancer *vs* healthy	74	90	NA	NA	0.887
	Jensen *et al*[20]	Cancer *vs* healthy	85	85	NA	Na	0.92
	Nunes *et al*[21]	Cancer *vs* healthy	85	99	NA	NA	NA
	Perrone *et al*[22]	Cancer *vs* healthy	NA	NA	NA	NA	0.709
		Adenomas *vs* healthy	NA	NA	NA	NA	0.535

HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; PDAC: Pancreatic ductal adenocarcinoma.

In contrast to these two studies focused on cfDNA methylations, Cristiano *et al*[19] explored a multi-cancer detection model analyzing cfDNA fragmentation patterns, including gastric, bile duct, colorectal and pancreatic cancers. Their model reached an overall detection sensitivity of 80% for a specificity of 95%, or a sensitivity of 73% for a specificity of 98%, and a global AUC of 0.94. Furthermore, enhanced by a machine-learning algorithm, they were able to identify the tissue of origin of cancer samples with a 61% accuracy[19]. Detailed performances per cancer type of this model can be found in Table 3.

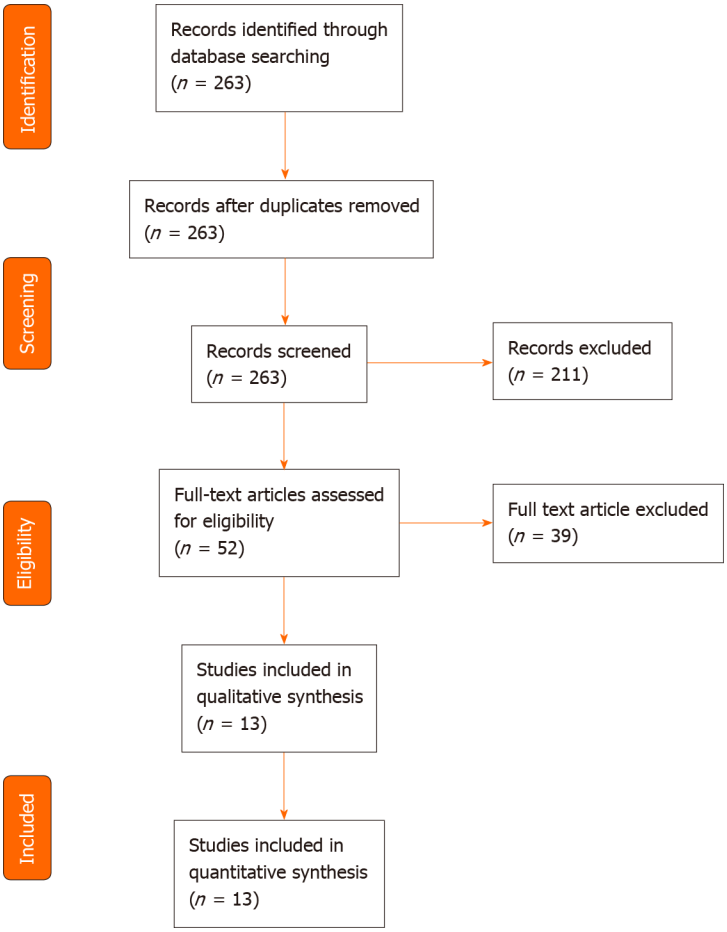

Figure 1 PRISMA flow diagram summarizing the search strategy.

DISCUSSION

Liquid biopsy appears as a promising non-invasive method for the initial screening and diagnosis of various gastrointestinal cancers. High levels of sensitivity and specificity described in the included studies seem within acceptable ranges for eventual clinical use. In the case of HCC, cfDNA tests demonstrated better detection performances when compared to the standard surveillance of high-risk patients combining AFP dosage and ultra-sound monitoring. It also appears to be a viable solution regarding the challenge of pancreatic cancer screening; due to the paucity of symptoms in the early phases and the absence of acceptable screening strategies even for high-risk groups, this type of cancer remains frequently detected at metastatic or locally advanced and unresectable stages. Conversely, colorectal cancer is one of the few cancers with a standardized and efficient large-scale screening strategy based on the colonoscopy and the fecal occult blood test. Still, there is room for improved and more cost-effective strategies. Of note, cfDNA liquid biopsy's ability to detect several cancer types simultaneously appears as a potential paradigm shift in global cancer care, and studies investigating such application achieved a high level of performance. Further, as demonstrated by Chen *et al*[13], this technology bears the potential to predict cancer several years before the onset of clinical symptoms and identify or direct investigations towards specific tissues of origin.

The central role of early cancer detection in improving oncologic and public-health outcomes is well established. However, it is a challenge for liquid biopsy since smaller and earlier-stage tumors tend to release lower levels of ctDNA[24]. The signal-to-noise ratio of ctDNA is thus meager compared to non-cancer-derived cfDNA, with a detection percentage ranging from 0 to 11.7%[25,26]. The extraction method plays a critical role in improving detection performance. Different procedures have been developed, the more widespread being column-based, polymer-based, phenol-chloroform, or magnet-based[9,27]. These methods are efficient and allow to reach a high DNA concentration but remain expensive and time-consuming[9,27]. In this context, some authors proposed plasma processing methods without the need for

DNA extraction. Breitbach *et al*[28] notably used quantitative RT-PCR to measure cfDNA concentration in plasma. Not only did the method showed great feasibility with higher levels of cfDNA found among cancer patients, but it also proved to be more time effective and more efficient than the eluate of the QIAamp DNA Blood Mini Kit, for example, with levels of cfDNA in unpurified plasma 2.79 fold higher[28].

Regarding the sequencing method, some authors focused their attention on specific mutations while others analyzed the whole genome searching for non-specific mutational patterns, most of them using NGS methods. Different factors can explain the apparent predominance of NGS over other PCR methods such as RT-PCR in the published studies. Although more technically demanding and expensive, NGS is a hypothesis-free approach that carries a higher discovery power of new mutational patterns, in addition to a higher sensitivity to rare variants[29,30]. Further, its superior multiplex capabilities tend to improve the workflow when studying a large number of locations and samples. These high throughput and detection sensitivity capabilities might be valuable in a screening configuration for early cancer detection, which deals with lower levels of mutation than advanced stage cancers and aims at testing a high volume of patients.

As the field is at an early stage of clinical exploration, there is still a high variability in trial designs and reporting methods, thus undermining the global quality of tests' performance analysis. Of note, biocomputational trials based on biobank samples often report higher levels of sensitivity and specificity but are less likely to translate into clinically relevant performances as prospective trials would. Applicability to real-life clinical applications is thus the most awaited step to achieve for the scientific validation of this technology, and upcoming clinical trials will need to address many questions, such as the appropriate balance between sensitivity and specificity in a screening purpose, the timing of screening tests, patient selection, socio-economic parameters and dealing with the uncertainty around tissues of origin in positive tests.

CONCLUSION

Liquid biopsy cfDNA represents an efficient, non-invasive, and promising method for detecting various gastrointestinal cancers at an early stage of development. These tools could improve the global prognosis of cancers currently diagnosed at an advanced stage due to the lack of effective screening and diagnostic methods, such as pancreatic cancer. Allowing early detection of several types of cancers and reducing the burden of multiple screening tests, cfDNA liquid biopsies could change the course of gastrointestinal cancers care for a significant number of patients and induce a paradigm shift in cancer-related public health policies, provided that they can demonstrate their clinical relevance in future studies.

ARTICLE HIGHLIGHTS

Research background

Liquid biopsy cell-free DNA (cfDNA) represents a promising non-invasive method for detecting various gastrointestinal cancers at an early stage of development.

Research motivation

Various and recent literature is available on this topic, with exponentially growing interest.

Research objectives

To review the current state of development of cfDNA liquid biopsy in the field of gastrointestinal cancer early detection.

Research methods

A systematic review of the literature according to the PRISMA guidelines.

Research results

The current literature suggests a high-performance profile for this technology and the potential to improve the global course of gastrointestinal cancers currently diagnosed at an advanced stage, such as pancreatic cancer.

Research conclusions

cfDNA liquid biopsy showed high potential in the diagnosis of early gastrointestinal cancers and simultaneous screening of multiple cancer types.

Research perspectives

Further trials in clinically relevant settings are required to determine the exact place of this technology in future diagnosis strategies.

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