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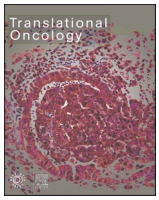

Potentialities and critical issues of liquid biopsy in clinical practice: An umbrella review

Nicola Veronese [a,*], Claudio Luchini [b], Stefano Ciriminna [a], Katia Spinelli [a], Santo Fruscione [a], Paola Mattiolo [b], Miriam Belluzzo [a], Veronica Messina [a], Lee Smith [c], Mario Barbagallo [a], Walter Mazzucco [a,d]

[a] Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy
[b] Department of Diagnostics and Public Health, Section of Pathology, University and Hospital Trust of Verona, Verona, Italy
[c] Centre for Health Performance and Wellbeing, Anglia Ruskin University, Cambridge, UK
[d] Division of Biostatistics & Epidemiology Research, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, United States

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ABSTRACT

Background: Liquid biopsy (LB) is a laboratory test performed on a fluid sample aiming at analyzing molecular data derived from circulating cells and related entities, or from nucleic acids. This umbrella review aims to map and evaluate the evidence supporting the use of LB in medicine across different medical specialities and conditions.

Methods: We searched three repositories from database inception up to October 1, 2023 and we included meta-analyses of observational studies reporting data on the use of LB, compared to gold standard, and its accuracy (area under the curve, AUC).

Results: Among 726 articles initially screened, 42 systematic reviews were included. Most of the outcomes explored (202/211) were related to cancer. We found that 75/211 had an excellent accuracy (AUC >0.90), with one comparison with an AUC equal to 1, i.e., Cell-Free Human Papillomavirus DNA (*cfHPV-DNA*) for HPV-positive oropharyngeal squamous cell carcinoma. However, considering published meta-analyses, all the outcomes were graded as very low on the GRADE criteria, and the heterogeneity was never reported.

Discussion: The literature about LB is rapidly increasing and some promising data about precision oncology are now available. However, this umbrella review on existing meta-analyses highlighted some critical issues for providing quantitative estimations on the different roles of LB.

Introduction

Liquid biopsy (LB) is a laboratory test performed on a fluid sample (e.g., blood, urine) aiming at analyzing molecular data derived from circulating cells and related entities or directly from nucleic acids [1–3]. Its use has been predominantly explored in oncology and related fields [4]. Indeed, LB represents a promising tool with potential diagnostic, prognostic, and predictive values [5–7].

Unlike traditional tissue biopsies, that necessitate invasive procedures, LB offers a minimally invasive approach for obtaining vital information about a patient's health status through the analysis of various biomarkers present in bodily fluids, such as blood, urine, saliva, and cerebrospinal fluid [8]. This transformative technique has garnered significant attention across medical disciplines due to its versatility, accessibility, and potential to revolutionize precision oncology and personalized medicine. Importantly, LB holds significant promise in the early detection and monitoring of various diseases, particularly cancer. By detecting circulating tumor cells (*CTCs*), cell-free DNA (*cfDNA*), microRNAs(*miRNAs*), and other molecular markers shed by tumors into the bloodstream or other bodily fluids, LB enables clinicians to detect cancer at its earliest stages, before the onset of clinical symptoms [8]. Early detection is crucial for improving patient outcomes, as it allows for timely intervention and treatment strategies tailored to the specific molecular profile of the tumour [9]. Furthermore, LB facilitates real-time monitoring of disease progression and response to treatment, offering valuable insights into treatment efficacy and the emergence of drug resistance [10].

Of interest, LB could bring promising innovations also beyond

* Corresponding author.
E-mail address: nicola.veronese@unipa.it (N. Veronese).

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oncology, extending its utility to various other medical specialties. In infectious diseases, for instance, it may enable the rapid detection of pathogens and monitoring of antimicrobial resistance, facilitating prompt and targeted therapeutic interventions [11]. In prenatal testing, the analysis of foetal DNA in maternal blood may offer a non-invasive alternative to traditional methods, reducing the risk of complications for both mother and foetus [12]. Additionally, LB holds promise in the field of organ transplantation, where it can aid in the early detection of graft rejection, emerging as a potential tool for guiding personalized immunosuppressive therapies [13].

The significance of LB in medicine is further underscored by its role in overcoming the limitations of conventional tissue biopsies. Unlike tissue biopsies, which may be hindered by sampling bias, accessibility issues, and tumour heterogeneity, LB offers a comprehensive and dynamic snapshot of the entire tumour landscape, allowing for more accurate molecular characterization and treatment selection [14]. However, LB may also suffer from potential issues, including lack of standardized approaches, propension to pre-analytical errors, and difficulties in the interpretation of data generated from multi-omics analysis [15].

In this complex scenario, this umbrella review (i.e., a systematic review of other systematic reviews on the same topic) aims to map and evaluate the evidence supporting the use of LB in medicine across different medical specialities and conditions, also taking into account the multiple issues potentially emerging in this setting.

Materials and methods

Protocol and registration

This umbrella review was conducted following the recommendations of the Cochrane handbook for systematic literature reviews to carry out the screening and selection of studies, and according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 updated guidelines [16,17]. The study protocol is freely available at https://osf.io/daz2t/.

PICO model and eligibility criteria

Following the PICOS (participants, intervention, control, outcomes, study design) model, we included as

- participants: any;
- intervention: liquid biopsy, defined as a simple venous blood sample in which molecular analyses can be performed such as the dosage of free tumoral cells, DNA, RNA, exosomes, or lysosomes [7];
- controls: none;
- outcomes: all health outcomes reported in terms of accuracy (area under the curve, AUC) with sensitivity and specificity or as precision (e.g., C-index). The data about accuracy were considered when reported against the gold standard approach for a condition;
- study design: systematic reviews of observational studies.

We excluded (i) meta-analyses of intervention studies and (ii) single studies.

Information sources and search strategies

Several relevant bibliographic databases were comprehensively searched, including Medline (via Ovid), Embase, and Web of Science from database inception up to the October 1, 2023 in Pubmed, Embase and Web of Science. The search strategies are fully available in **N** Table 1.

Table 1
Descriptive characteristics of the 42 systematic reviews included in the study.

AUTHOR	YEAR	TITLE	TOTAL SAMPLE SIZE	Number of studies
Bai S.	2023	Clinical diagnostic biomarker "circulating tumor cells" in breast cancer - a meta-analysis	1544	16
Borg M.	2023	Methylated Circulating Tumor DNA in Blood as a Tool for Diagnosing Lung Cancer: A Systematic Review and Meta-Analysis	4700	33
Chandrapalan S.	2022	A systematic review and meta-analysis: the diagnostic accuracy of methylated SEPTIN9 for the detection of hepatocellular carcinoma and the clinical evaluation of its use in combination with other surveillance modalities	1001	6
Cheng, J.	2017	Cell-Free Circulating DNA Integrity Based on Peripheral Blood as a Biomarker for Diagnosis of Cancer: A Systematic Review	2803	17
Duque G.	2022	Cancer Biomarkers in Liquid Biopsy for Early Detection of Breast Cancer: A Systematic Review	34,376	136
Elasifer H.	2023	The role of circulating viral and tumour DNA in the diagnosis and management of HPV associated anogenital cancers, a systematic review and meta-analysis	2247	31
Gally T.B.	2021	Circulating MicroRNAs as Novel Potential Diagnostic Biomarkers for Osteosarcoma: A Systematic Review	4970	35
He Y.	2020	Clinical performance of non-invasive prenatal testing for trisomies 21, 18 and 13 in twin pregnancies: A cohort study and a systematic meta-analysis	6618	21
Hong F.	2023	Exosomal microRNAs as novel diagnostic biomarkers in breast cancer: A systematic evaluation and meta-analysis	608	7
Hou F.	2023	Diagnostic value of cell-free DNA in thyroid cancer: A systematic review and meta-analysis	622	14
Jia S.	2021	Values of liquid biopsy in early detection of cancer: results from meta-analysis	108	17
Karkia R.	2022	Diagnostic Accuracy of Liquid Biomarkers for the Non-Invasive Diagnosis of Endometrial Cancer: A Systematic Review and Meta-Analysis	5527	59
Khetrapal P.	2018	The role of circulating tumour cells and nucleic acids in blood for the detection of bladder	58	15

(*continued on next page*)

Table 1 (*continued*)

AUTHOR	YEAR	TITLE	TOTAL SAMPLE SIZE	Number of studies
		cancer: A systematic review		
Leão R.	2021	Circulating MicroRNAs, the Next-Generation Serum Biomarkers in Testicular Germ Cell Tumours: A Systematic Review	45	31
Lyu M.	2019	The diagnostic value of circulating tumor cells and ctDNA for gene mutations in lung cancer	7244	47
McMahon J.T.	2022	Circulating Tumor DNA in Adults With Glioma: A Systematic Review and Meta-Analysis of Biomarker Performance	1651	7
Meng H.	2023	The diagnostic value of circulating abnormal cells in early lung cancer	53,728	7
Mlika M.	2018	Liquid biopsy as surrogate to tissue in lung cancer for molecular profiling: A meta-analysis	4782	33
Nakasu Y.	2023	Diagnostic accuracy of cerebrospinal fluid liquid biopsy and MRI for leptomeningeal metastases in solid cancers: A systematic review and meta-analysis	668	10
Öberg K.	2020	A meta-analysis of the accuracy of a neuroendocrine tumor mRNA genomic biomarker (NETest) in blood	549	18
Ohadi M.A.D.	2023	Micro RNAs as a Diagnostic Marker between Glioma and Primary CNS Lymphoma: A Systematic Review	918	8
Rapado-González O.	2019	miRNAs in liquid biopsy for oral squamous cell carcinoma diagnosis: Systematic review and meta-analysis	2562	16
Rapado-González O.	2020	Salivary biomarkers for cancer diagnosis: a meta-analysis	11,153	29
Rapado-González O.	2021	Salivary DNA Methylation as an Epigenetic Biomarker for Head and Neck Cancer. Part I: A Diagnostic Accuracy Meta-Analysis	8368	18
Song Z.	2019	The diagnostic accuracy of liquid exosomes for lung cancer detection: A meta-analysis	2413	13
Tonozzi T.R	2019	Liquid biopsies in endocrine neoplasia-a systematic review	not reported	65
Toraih E. A.	2021	Diagnostic and Prognostic Performance of Liquid Biopsy-Derived Exosomal MicroRNAs in Thyroid Cancer Patients: A Systematic Review and Meta-Analysis	1704	12
Uhe. I	2021	Cell-free DNA liquid biopsy for early detection of gastrointestinal cancers: A systematic review	4824	13
Van Westrhenen A.	2018	Diagnostic markers for CNS lymphoma in blood and cerebrospinal fluid: a systematic review	268	25
Wuerdemann N.	2020	Cell-Free HPV-DNA as a Biomarker for Oropharyngeal Squamous Cell Carcinoma-A Step Towards Personalized Medicine?	1284	11
Xu Y.	2021	Meta-Analysis of the Diagnostic Value of Cell-free DNA for Renal Cancer	1109	8
Xu Y.	2021	Urinary Exosomes Diagnosis of Urological Tumors: A Systematic Review and Meta-Analysis	3224	22
Ye J.	2023	Glutathione-S-Transferase p1 Gene Promoter Methylation in Cell-Free DNA as a Diagnostic and Prognostic Tool for Prostate Cancer: A Systematic Review and Meta-Analysis	2610	14
Yinzhong W	2023	Diagnostic accuracy of circulating-free DNA for the determination of hepatocellular carcinoma: a systematic review and meta-analysis	3686	15
Yu W.	2022	The diagnostic significance of blood-derived circRNAs in NSCLC: Systematic review and meta-analysis	2052	12
Zhang C.	2022	Cell-free DNA as a Promising Diagnostic Biomarker in Prostate Cancer: A Systematic Review and Meta-Analysis	2022	14
Zhang J.	2022	Evaluating the Diagnostic Potentials of Circulating Tumor DNA against Melanoma: A Systematic Review and Meta-Analysis	1430	10
Zhao Q.	2021	Role of circulating tumor cells in diagnosis of lung cancer: a systematic review and meta-analysis	3997	21
Zhou S.	2020	Detection of epidermal growth factor receptor mutations in peripheral blood circulating tumor DNA in patients with advanced non-small cell lung cancer A PRISMA-compliant meta-analysis and systematic review	4527	32
Zhou Z.	2020	A Meta-Analytic Review of the Value of miRNA for Multiple Sclerosis Diagnosis	989	11
Zhu Y.	2020	Diagnostic performance of various liquid biopsy methods in detecting colorectal cancer: A meta-analysis	18,739	62
Zhu Y.	2020	Diagnostic value of various liquid biopsy methods for pancreatic cancer: A systematic review and meta-analysis	1872	19

Study selection

The selections were independently carried out by four review authors (KS, SF, MB, VM), with consensus meetings to discuss the studies for which divergent selection decisions were made by two review authors. A third senior member of the review team (NV) was involved, if necessary. The studies selection process involved, first, a selection based on title and/or abstracts, then a selection of studies retrieved from this first step based on the full-text manuscripts. The freely accessible software Rayyan was used for the title/abstract screening [18].

Data collection and data items

From the eligible full-text articles the following data has been extracted: first author name and affiliation, year of publication, journal name, title of the manuscript; data on the characteristics of the population considered, for individual observational studies (e.g., sample size, mean age, gender), the name of the marker used in the LB, and the outcome. Data regarding estimates were extracted as reported in the original systematic review and reported as AUC, since no one reported data using C-indexes. These data were collected using a RedCap [19]. Data extraction was carried out by four authors (KS, SF, MB, VM) and systematically double-checked by another author (SC).

Assessment of risk of bias

Four authors (KS, SF, MB, VM) rated the methodological quality of the included systematic reviews using "A MeaSurement Tool to Assess systematic Reviews 2 (AMSTAR 2)" [20,21], which ranks the quality of a meta-analysis in one of 4 categories, ranging from "critically low" to "high", according to 16 predefined items [20]. Another author (SC) double checked this evaluation.

Statistical analysis and synthesis of the data

The analyses are proposed by estimates of accuracy (AUC), in agreement with the NICE guidelines [22]. While no definitive threshold exists, values of AUC/C-index of 0.50 was considered to indicate accuracy or precision no better than chance, while values between 0.50 and 0.60 reflecting a very poor accuracy, between 0.60 and 0.70 a poor, between 0.70 and 0.80 a good, between 0.80 and 0.90 a very good, and more than 0.90 an excellent accuracy [23,24].

We used the GRADE (Grading of Recommendations, Assessment, Development, and Evaluations) assessment, adapted for prognostic and diagnostic studies [25]. Briefly, we considered the risk of bias as reported in the systematic reviews; statistical heterogeneity (inconsistency) between different studies was assessed using the I^2 score, with a low heterogeneity that was based on an I^2 ranging from 30 to 49%, a moderate heterogeneity from 50 to 74%, and a high heterogeneity from 75% and above [26]; indirectness was based on the assumption that the analyses should reflect the PICO question and, in particular, if only a specific population was included; imprecision: the evidence was downgraded by 1 increment if the individual studies varied across 2 areas (for example, 0.5–0.8 and 0.8–1) and by 2 increments if the individual studies varied across 3 areas (for example, 0–0.5, 0.5–0.8 and 0.8–1) [27]; publication bias was assessed using the Egger bias test [28]. When one domain of data was missing, we downgraded the evidence by two levels for that domain.

Lastly, the GRADE gave four different degrees of certainty of evidence from very low (there is a very little confidence in the estimate, i.e., the true diagnosis is likely to be substantially different from the estimate) to high (we are very confident that the true diagnosis lies close to that of the estimate) [25].

RESULTS

Summary of the literature search

As shown in Fig. 1, we initially screened 726 articles at title/abstract level. Of them, 94 full-texts were examined, finally including 42 systematic reviews after eligibility assessment [27,29–69]. The main reasons of exclusion were the lack of information about accuracy ($n = 17$) and the wrong study design ($n = 15$).

Descriptive findings

Table 1 shows the main descriptive findings of the systematic reviews included in the study. All the systematic reviews were published after the year 2017. Overall, the 42 systematic reviews included an approximate total of 213,600 individuals and a total of 1010 studies, with a mean of 24 studies and 5209 participants for each systematic review, respectively. Altogether, 211 meta-analytic outcomes were evaluated.

As shown in Table 2, among the markers investigated, 121 comparisons over 211 used RNA and, in particular, microRNA (*miRNA*), followed by DNA markers (53/211) and genetic biomarkers (40/211). Furthermore, eight comparisons used free tumoral cells, two exosomes, one nucleosomes, and one salivary DNA biomarkers. The large majority of the meta-analytic comparisons included cancer, at any stage, as outcome (202/211), with only nine comparisons about prenatal diagnosis and one about multiple sclerosis.

Main findings

Table 2 presents the main findings of the umbrella review, whilst **Supplementary Table 2** includes the GRADE assessment for each outcome. When considering the AUC as estimation of the accuracy of the LB markers, we found that 75/211 (45.4%) had an excellent accuracy (AUC >0.90), with one comparison having an AUC equal to 1, indicating a perfect correspondence between LB and the gold standard, i.e., *cfHPV-DNA* for HPV-positive oropharyngeal squamous cell carcinoma. Moreover, 81/211 outcomes showed an AUC between 0.80 and 0.90, thus being classified with a very good accuracy, while 39 had an AUC between 0.70 and 0.80, and 16 an AUC 0.60–0.70.

However, when the level of evidence using the GRADE was evaluated, all the outcomes were graded as very low, as further detailed in **Supplementary Table 2**. Briefly, only 95 out of 211 outcomes (44.8%) reported the 95% CI for AUCs, thus increasing the risk of imprecision. Moreover, the risk of bias was graded as very serious in almost all outcomes included (197/211, 92.9%) and the heterogeneity was never reported, making inconsistency very serious in all the comparisons. Lastly, the presence of publication bias was present in almost a half of outcomes (102/211, 48.1%) (**Supplementary Table 2**).

Risk of bias evaluation

Supplementary Table 3 reports the results on the possible risk of bias evaluated on the 42 systematic reviews included in the study. Of these, 30 were ascertained as having a critically low quality, 11 a low quality and only one of high quality. The main reasons of this possible bias were also identified as the lack of sufficient information about statistical methods used for meta-analysis and the poor consideration of the risk of bias in affecting results in original systematic reviews.

Discussion

This study represents a quantitative summary of evidence related to LB, highlighting its potentialities and limitations. In this umbrella review, evaluating the accuracy of several LB markers, we highlighted that in 42 systematic reviews published after 2017, including a total of

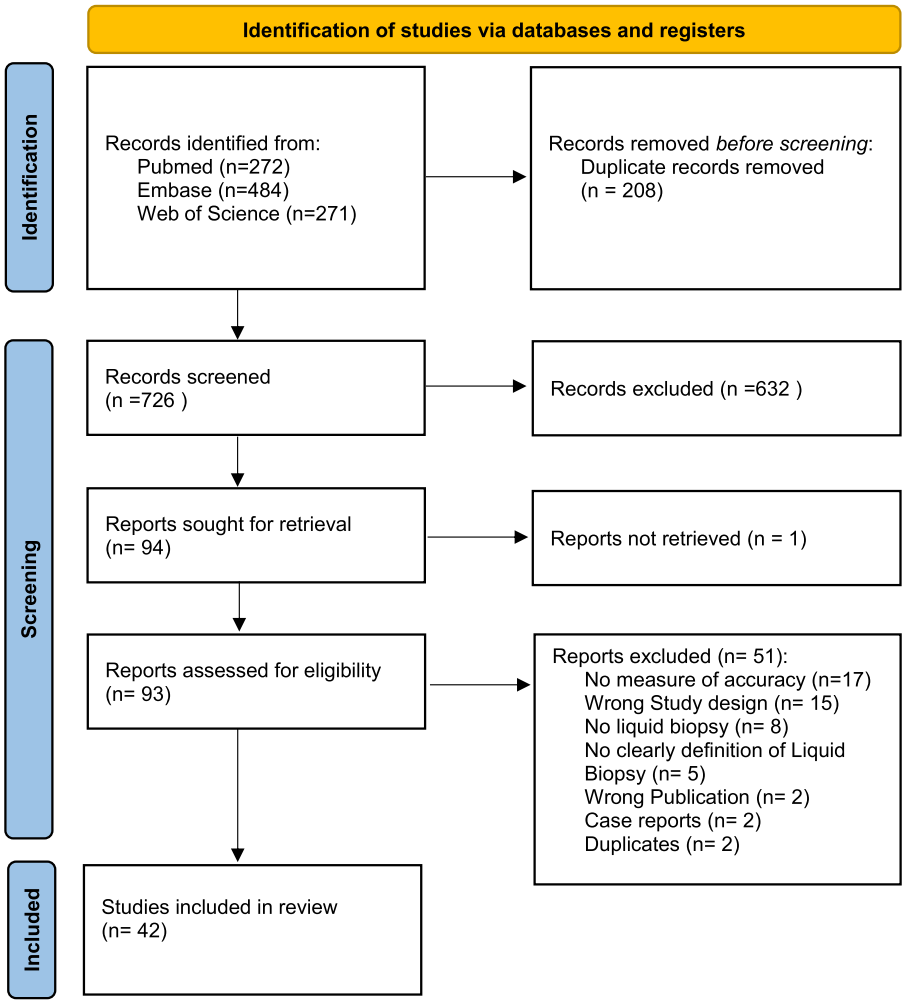

Fig. 1. PRISMA flow-chart.

213,600 individuals from 1010 studies, a significant fraction of the outcomes considered had an excellent or very good accuracy, respectively. Based on this accuracy, this study may corroborate recent evidence of the need for implementing LB into clinical practice. However, as expected, most systematic reviews and meta-analysis in the literature regards oncology and related fields (95.7%).

Although LB has been mainly explored in the context of precision oncology, it should be acknowledged that it may bring crucial advantages into different clinical levels, including: i) avoiding invasive procedures for the molecular characterization of a disease, even for patients with multiple comorbidities and/or unfit for surgery, ii) allowing molecular investigation for the molecular characterization of tumours, even for stage IV / metastatic patients, where surgical resections cannot be considered and tissue biopsies are not always practicable, iii) giving the possibility of overcoming intratumor heterogeneity, representing a possibility of showing a comprehensive molecular landscape of a given neoplasm, independent from tissue's related biases. These advantages are unique for LB and related methodologies and call for further research along those lines for implementing this promising tool into clinical practice.

The use of integrated biomarkers allows for a real-time, minimally invasive assessment of the tumour's genetic and epigenetic alterations. For instance, ctDNA can reveal mutations in key oncogenes, such as *EGFR*, providing valuable information for therapy selection, particularly for targeted treatments [70]. The inclusion of microRNAs and exosomal content further enhances the diagnostic accuracy, as these components can reflect various biological processes involved in cancer progression and metastasis. In lung cancer management, the integration of these biomarkers supports longitudinal monitoring [71]. By analysing changes in ctDNA levels or the presence of specific mutations over time, clinicians can adjust treatment strategies more effectively, optimizing outcomes for patients. This approach enables personalized medicine, where treatment is tailored based on the evolving molecular profile of the tumour, improving both the prognosis and quality of life for cancer patients.

It is important to remark that more recent artificial intelligence (AI) systems could significantly help to implement the use of LB in the daily clinical practice (e.g., by helping to manage big data derived from multi-omics analyzes on LB) [72,73]. AI can improve the sensitivity and specificity of liquid biopsies for early cancer detection; more in depth, by integrating data from multiple biomarkers and employing sophisticated algorithms, AI can distinguish between benign and malignant conditions more effectively than traditional methods, therefore potentially leading to earlier diagnosis and better prognostic outcomes [74]. Finally, the use of AI in the LB field may track changes in biomarker levels over time, providing valuable insights into disease progression and patient response to treatments, allowing for personalized treatment adjustments [75].

The role of LB in the central nervous system (CNS), specifically for detecting cell-free DNA (cfDNA) in patients with meningioma and glioblastoma, is critical in improving non-invasive diagnostic and monitoring approaches. LB may provide a minimally invasive method to detect tumor-derived DNA in bodily fluids, offering a real-time reflection of tumor dynamics [76]. This approach has shown promise in CNS

Table 2
Main findings of the umbrella review.

Name Of The Marker	Outcome	AUC	95% CI Low For AUC	95% Ci High For AUC	Sensitivity	Specificity
cfHPV-DNA	Oropharyngeal Squamous Cell Carcinoma	1			73	100
cHPV DNA	HPV Associated anal cancer	0.9955	0.9886	1	95	100
ALK CtDNA	Lung Cancer	0.994	0.953	1		
NIPT screening for trisomy 21	prenatal testing for trisomies 21 in twin pregnancies	0.9916	0	0	0.99	1
cfHPV-DNA (First Diagnosis)	Oropharyngeal Squamous Cell Carcinoma	0.99			81	98
miR10b	Breast cancer	0.99			97.1	100
miR-148a	Breast cancer	0.99			94.7	90.9
miR-155	Breast cancer	0.99			97.4	94.4
miR-21	CNS lymphoma in blood and cerebrospinal fluid:	0.99			91.70	95. 7
miR-30c	Breast cancer	0.99			97.3	96.4
PTEN	Breast cancer	0.99			100	94
miR-19b	CNS lymphoma in blood and cerebrospinal fluid:	0.98	0.91	1	95.70	0.9
miRNA-373	Breast cancer	0.98			93.4	99
miRNA-373	Breast cancer	0.98			90.8	98.4
S100A4 gene	Bladder cancer	0.978			90	92
hsa-miR-548ar-5p	Breast cancer	0.97			100	77
miR21	Breast cancer	0.97			95.7	98.5
miR-92a	CNS lymphoma in blood and cerebrospinal fluid:	0.97	0.93	1	95.70	80
NIPT screening for trisomy 13	prenatal testing for trisomies 13 in twin pregnancies	0.9655	0	0	0.85	1
hsa-miR-21–5p	Breast cancer	0.96			86.7	93.3
miR-27a	Breast cancer	0.96			92	92
MiR-152	Osteosarcoma	0.956	0	0	96.2	92.5
Panel of 6 miRNAsc	Bladder cancer	0.956	0.922	0.978	90	90
APC	Breast cancer	0.95			93.4	95.4
miR-373	Breast cancer	0.95			85	100
NIPT screening for trisomy 18	prenatal testing for trisomies 18 in twin pregnancies	0.948	0	0	0.88	1
CTC (DAPI+/CD45-, and CK+ or CEA, CD45-/DAPI+/CEP8, chromosome 8, CD45,CK19,Pdx-1, CEP8, CK, CD45 and DAP, KRAS), ctDNA (GNAS, KRAS, ADAMTS1, BNC1), Exosomes (miR-191,miR-21,miR-451a, miR-10b, miR-21, miR-30c, miR-181a,miR-let7a, CD44v6, Tspan8, EpCAM, MET and CD104, GPC1, miR-17–5p,miR-21, miR-1246, miR-4644)	Pancreatic cancer	0.9478			80	89
ctDNA(CSF)	Glioma	0.947	0.808	0.957		
S100A9	Bladder cancer	0.944			81.7	92
cfDNA	Pancreatic Cancer (ductal adenocarcinoma)	0.943			93.2	95.2
cfDNA	Various cancer types	0.94			80	95
miR-155	Breast cancer	0.94			86	90
miR-21	CNS lymphoma in blood and cerebrospinal fluid:	0.94	0.87	1	95.70	83.30
miR-598–3p	Breast cancer	0.94			95	85
RARB2	Breast cancer	0.94			95.5	92.4
S100A8	Bladder cancer	0.935			85	92
S100A11	Bladder cancer	0.934			83.3	91
CTC	leptomeningeal metastases in solid cancers	0.931			0.9	0.9
miR-371a-3p for diagnosis	Serum Biomarkers in Testicular Germ Cell Tumours	0.93			70.8–100	61–100
cf-DNA	Hepatocellular carcinoma	0.93	0. 90	0.95	83	90
cfDNA (*EGFR -TP53-NF1- MET -BRAF -KRAS -ALK*)	Lung Cancer	0.93			0.6	1
ctDNA	Any Cancer	0.93			83.6	91.9
miR-21 Mao et al. (2014)	CNS lymphoma in blood and cerebrospinal fluid:	0.93	0.88	0.98		
miR-99a	Breast cancer	0.93			76.7	95
ctDNA (BRAF)	Evaluating the Diagnostic Potentials of Circulating Tumor DNA against Melanoma	0.9287			73	94
DNA del cHPV	HPV Associated with cervical cancer	0.9277	0.8863	0.9691	0.36	0.96
S100A6 gene	Bladder cancer	0.924			86.7	84
cfDNA	HCC	0.92				
hsa-miR-25–3p	Breast cancer	0.92			92	83
methylated *SEPTIN9*	hepatocellular carcinoma	0.92			80	90
miR-21	Breast cancer	0.92			92.3	81.2
Urinary Exosomes [Panel of lncRNAs (MALAT1+PCAT-1+SPRY4-IT1), Panel of lncRNAs (UCA1–201+UCA1–203+ MALAT1+LINC00355), Panel of miRNAs (miR-19b1–5p+miR-136–3p+miR139–5p), CD9 protein, miR-21–5p, miR-30c-5p, Panel of miRNAs (miR-126–3p+miR-449a, the best combination), Panel of sncRNAs (Selected miRNAs+ selected snoRNAs), miR-196a, miR-19b, Panel of mRNAs (PCA3 and ERG), Panel of mRNAs and miRNAs(ANXA3, CD24, TMPRSS2-ERG, SLC45A3,FOLH1, HPN, ITSN1, miR-375–3p, miR-	Urological tumors	0.92	0.89	0.94	83	88

(*continued on next page*)

Table 2 (*continued*)

Name Of The Marker	Outcome	AUC	95% CI Low For AUC	95% Ci High For AUC	Sensitivity	Specificity
574–3p), Panel of miRNA isoforms(isomiRs of miR−21, miR−204 and miR−375), Urinary vesicle-associated PSA extraction ratio						
miR-320a	Osteosarcoma	0.9188	0	0		
miR-374a-5p	Osteosarcoma	0.9173	0	0		
CTC	Lung Cancer	0.91	0.88	0.93	72	96
ctDNA (EGFR)	Growth factor receptor mutations in peripheral blood circulating tumor DNA in patients with advanced non-small cell lung cancer	0.91	0.88	0.93	70	98
KRAS in CtDNA group	Lung Cancer	0.91	0.804	1	65.1	95.5
LncRNA-ATB	Breast cancer	0.91			80	90
miRNAs (miR-24; miR-10b; miR-31; miR-338–3p; miR-29a; miR-223; miR-16 let-7b; miR-146a; miR-21; miR-27b; miR-136; miR-125b; miR-4677; miR-483–5p; miR-196a; miR-196b; miR-196a e miR-196b; miR-139–5p; miR-223; miR-200b-3p; miR-99a; miR-150–5p; miR-423–5p; miR-150–5p e miR-423–5p	oral squamous cell carcinoma	0.91	0.88	0.93	78	82
Panel of 40 miRNAs	Bladder cancer	0.91			90	89
RNU2–1f (Baraniskin et al. 2016)	CNS lymphoma in blood and cerebrospinal fluid:	0.91			68. 1	91. 4
miR-195–5p	Osteosarcoma	0.9029				
miR-199a-3p	Osteosarcoma	0.9025				
Panel of 7 gene mRNA (IGFBP7, SNX16, CSPG6, CTSD, CHD2, NELL2, TNFRSF7)	Bladder cancer	0.901	0.803	0.96	83	90
CTC, Exosomes, cfDNA	Colorectal cancer	0.9004			77	89
CCN1	Breast cancer	0.9			80	99
cfDNA	Any Cancer	0.9			67.3	99.3
CTCs	Any Cancer	0.9			90	94.1
liquid exosomes:miRNA; Proteins; lipids	Lung cancer	0.9	0.87	0.92	82	84
miR-1246	Breast cancer	0.9			93	75
miR-195–5p-5p	Breast cancer	0.9			77.8	100
miR-301a-3p	Breast cancer	0.9			85	78
miR-495	Breast cancer	0.9			100	66.7
Plasma cfDNA concentrations	Any Cancer	0.9			90.5	80.5
miR-210	Bladder cancer	0.898	0.855	0.931	97.6	69.2
miR-371a-3p post chemo	Serum Biomarkers in Testicular Germ Cell Tumours	0.8975			82.6–100	58–100
MiR-326	Osteosarcoma	0.897			83.7	94.5
mRNA	Neuroendocrine tumor	0.897	0.877	0.917		
S100A7 gene	Bladder cancer	0.895			73.3	93.3
MiR-195	Osteosarcoma	0.892	0	0	88	83.3
cf-DNA	Thyroid cancer	0.89	0.86	0.91	0.76	0.87
HERV-K (HML-2) type levels.	Breast cancer	0.89			80	84.6
microRNA	Endocrine Neoplasia	0.89			90	76.50
miR-34a	Breast cancer	0.89			91	75
MiR-375	Osteosarcoma	0.89			82.1	74.7
−31G/C polymorphism in the survival promoter gene	Breast cancer	0.89			92.7	86.9
Community Verified icon	Colorectal cancer	0.887				
EGFR in CTC group	Lung Cancer	0.885	0.778	0.993	75.4	85.2
miR21	Glioma and Primary CNS Lymphoma	0.883	0.813	0.954		
Hypomethylation and copy number aberrations	Any Cancer	0.88			81	94
MiR-574–3p	Osteosarcoma	0.88	0	0		
BRAF IN CTDNA	Lung Cancer	0.877	0	1	31.3	99.5
circulatory abnormal cells (CAC)	Lung Cancer	0.87	0.84	0.9	0.8	0.9
cfDNA (APP gene integrity)	Endocrine Neoplasia	0.87				
miR-17–5p	Breast cancer	0.87			100	75.5
Telomeric sequences in cfDNA	Breast cancer	0.87			91.5	76.2
MiR-25–3p	Osteosarcoma	0.868	0	0	71.4	92.3
APC, GSTP1 or TIG1	Bladder cancer	0.867	0.785	0.948	80	93.3
MiR-27a	Osteosarcoma	0.867	0	0	70	98.3
TIG1, GSP1, APC or PTGS2	Bladder cancer	0.867	0.785	0.948	80	93.3
microRNA (miR-130a-3p, miR-129–2, miR-889, miR-29a, miR-148a-3p, miR-25–3p, miR-296–5p, miR-92a-3p, miR-5189–3p, miR-5010–3p, miR-598–5p, miR-3161, miR-6516–5p, miR-4644, miR-1283, miR-1227–3p, miR-149–3p, miR-210–5p, miR-3662, miR-187–5p, miR-16–2–3p, miR-223–5p, miR-34c-5p, miR-182–5p, miR-223–3p, miR-146b-5p, miR-16–2–3p, miR-223–5p, miR-146b-5p, miR-221–3p, miR-222–3p, miR-21–5p, miR-204–5p, miR-485–3p, miR-4433a-5p, miR4306, miR-376a-3p, miR-204–3p, miRNA423–5p, miR-346, miR-10a-5p, miR-34a-5p, miR-181a,	Thiroid Cancer	0.866			82	76
miR-371a-3p for early stage disease	Serum Biomarkers in Testicular Germ Cell Tumours	0.8625			83.4–100	60.1–100
MiR-199a-5p	Osteosarcoma	0.8606	0	0	88.3	76.67

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Table 2 (*continued*)

Name Of The Marker	Outcome	AUC	95% CI Low For AUC	95% Ci High For AUC	Sensitivity	Specificity
ccfDNA	Breast cancer	0.86			67	90
Delta181CTmir155	Breast cancer	0.86			83.3	82.4
hsa-miR-888–5p	Breast cancer	0.86			83	75
miR-195	Breast cancer	0.86			69	89.2
miR-222	Breast cancer	0.86			91.2	78.6
MiR-663a	Osteosarcoma	0.86	0	0	67.4	89.8
miRNA-21	Breast cancer	0.86			70.8	91.8
MiR-191	Osteosarcoma	0.858	0	0	74	100
MiR-194	Osteosarcoma	0.855	0	0	84.2	79.1
miR21	Glioma and Primary CNS Lymphoma	0.851	0.755	0.947		
Salivary biomarkers (CA-125; c-erbB-2; VEGF, EGF, CEA; 1472.78 Da, 2936.49 Da, 6556.81 Da, 7081.17 Da KRAS, MBD3L2, ACRV1, CDKL3; CSTA, TPT1, IGF2BP1, GRM1, GRIK1, H6PD, MDM4, S100A8, CA6; N elongate, S mitis, G adiacens; miR-21 BRAF, CCNI, EGFR, FGF19, FRS2, GREB1, LZTS1; calprotectin, AZGP1, HP H3F3A, SRGN, B2 M, BASP1, AGPAT1, IL1B, IER3 miR-10b, miR-144, miR-451, miR-21; miR-144; miR-21, miR-23a, miR-23b, miR-29c, miR-216, miR-210, let-7c; miR-3679–5p, miR-940 miR-21 SFAA: Phe, Trp, Met, Pro, Thr, Asp, Ser, Cit, Orn; His, Gln, Leu, Val, Glu, Lys LRP; Capnocitophaga, Veillonela, Neisseria; miR-4644, miR-1246 miR-21 Polyamines: SPM, N1-Ac-SPD, N8-Ac-SPD, Ac-PUT, CAD,; Ac-SPM, DAc-SPM, DAc-SPD, PUT; SPD, ORN, DAP lncRNAs: HOTAIR, PVT1 CSTB, TPl1, DMBT1 LysoPC (18:2), Palmitic amide, Phytosphingosine,; LysoPC(18:1), PS(14:1/16:1), LysoPC(16:0), Acetylphenylalanine, Propionylcholine, LysoPC(22:6), MG(0:0/14:0/0:0), LysoPE(18:2/0:0), PC(18:1/16:0), Phenylalanine, Citrulline, Histidine, N-Acetylneuraminic acid, PE(22:0/20:4), 4-Hydroxyphenylpyruvic acid; Lectins: model GC (VVA and SBA), VVA; PPP2CA, PTGS2, ROCK1, SKP1, SLK I; EX-1; Lectins: model BC (BS-I, NPA, PNA, PTL-II and MAL-I)	Salivary biomarkers for cancer diagnosis	0.85	0.84	0.87	76	76
Delta181CTmir125a	Breast cancer	0.85			83.3	64.7
hsa-miR-548a-5p	Breast cancer	0.85			83	83
MiR-101	Osteosarcoma	0.85	0	0	78.95	82.86
miRNA (miR-15b-5p,miR-451a, miR-30b-5p,miR-342–3p, miR-127–3p, miR-370–3p, miR-409–3p, miR-432–5p, miR-145 and miR-223, miR-122–5p, miR-196b-5p, miR-301a-3p,miR-532–5p, miR-484, miR-140–5p, miR-320a, miR-486–5p, miR-320c, miR-7–1–3p,miR-7–1–3p, miR-191–5p, mi-RNA-145, miR-572, miR-30e, miR-150, miRNA-181c)	Multiple Sclerosis Diagnosis	0.85	0.82	0.88	81	75
SMAD4	Breast cancer	0.85			100	100
miR-20a-5p	Osteosarcoma	0.8471	0	0		
MiR-124	Osteosarcoma	0.846	0	0	79.8	86
MiR-139–5p	Osteosarcoma	0.846	0	0	76.5	80
GSTP1 or APC	Bladder cancer	0.844	0.757	0.931	75.6	93.3
MiR-221	Osteosarcoma	0.844	0	0	65.7	100
TIG1 or APC	Bladder cancer	0.844	0.757	0.931	68.9	100
MiR-542–3p	Osteosarcoma	0.841	0	0	77.8	93.6
circRNAs	non-small cell lung cancer (NSCLC)	0.84	0.8	0.87	78	76
miR-185–3p	Breast cancer	0.84			95	66
EGFR in CtDNA group	Lung Cancer	0.8391	0.759	0.919	67.1	96.1
PTGS2 gene	Bladder cancer	0.836	0.753	0.918	95.6	62.2
microRNAs	Breast cancer	0.8325			67	81
APC, GSTP1, PTGS2, p14(ARF), p16(INK) and RASSF1A	Any Cancer	0.83			67	97
cfDNA integrity	Breast cancer	0.83			77.5	90
miRNA-222	Breast cancer	0.83			97.8	75.5
Opz	Breast cancer	0.83			80	76
Micro RNA-21	Non-Invasive Diagnosis of Endometrial Cancer	0.825	0.735	0.915		
Panel of 9 hypermethylated segments	Bladder cancer	0.825	0.761	0.89	62.1	88.7
hsa-miR-144 5p	Bladder cancer	0.824	0.633	0.929	70	82.4
hsa-miR-374–5p	Bladder cancer	0.824	0.633	0.929	60	94
miR-26b-5p hsa-miR-144 5p hsa-miR-374–5p	Bladder cancer	0.824	0.633	0.929	65	94.1
APC, RASSF1A, CDH1, RUNX3, TFPI2, SFRP5, OPCML	Any Cancer	0.82			85.3	90.5
Cf-DNA	renal cancer	0.82	0.79	0.85	71	79
mtDNA	Breast cancer	0.82			77	83
Lower cfDI	Different types of Cancer (Breast cancer, Prostate cancer, HCC, CRC,Glioma,Melanoma, Bladder cancer, Renal cell cancer,Acute leukemia,TGCC,HNC,PAC,Mixed)	0.814			0.74	0.84
Micro RNA-223	Non-Invasive Diagnosis of Endometrial Cancer	0.813	0.735	0.89		

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Table 2 (*continued*)

Name Of The Marker	Outcome	AUC	95% CI Low For AUC	95% Ci High For AUC	Sensitivity	Specificity
Circulating tumor cells(CTC)	Breast cancer	0.8129			50	93
cfDNA methylation score	Breast cancer	0.81			93	73.5
Salivary DNA	Salivary DNA Methylation as an Epigenetic Biomarker for Head and Neck Cancer	0.81	0.77	0.84	39	87
LncRNA H19	Breast cancer	0.81			56.7	86.7
methylated circulating tumor DNA (ctDNA)	Lung Cancer	0.81	0.77	0.84	46.9	92.9
ALU-247	Breast cancer	0.8			70	100
cfDNA, GADD45, CDH13promoter, Hypermethylation APC, Hypermethylation GSTP1, Hypermethylation MDR1, Hypermethylation RASSF1, Hypermethylation PTGS2, RARβ2	Prostate Cance	0.8	0.76	0.83	56	89
GSTP1 or TIG1	Bladder cancer	0.8	0.704	0.895	66.7	93.3
miR-155	Breast cancer	0.8			65	81.8
MiR-214	Osteosarcoma	0.8	0	0		
APC	Bladder cancer	0.798	0.698	0.897	59.5	100
miR-25–3p	Osteosarcoma	0.7961	0	0		
miR-451a	Osteosarcoma	0.7961	0	0		
ALU-115	Breast cancer	0.78			67.5	100
GSTP1	Prostate Cancer	0.78	0.75	0.82	37	97
MiR-335–5p	Osteosarcoma	0.78	0	0		
GSTP1	Bladder cancer	0.777	0.676	0.877	59.1	93.2
miR-425–5p	Osteosarcoma	0.7765	0	0		
cfDNA	Breast cancer	0.77			70	76
Delta192CTmir155	Breast cancer	0.77			77.8	6.7
Hotair	Breast cancer	0.77			76	76
hsa_circ_0005046	Breast cancer	0.77			85	51
miR-10b	Breast cancer	0.77			60	93
miR-16–5p	Osteosarcoma	0.7686	0	0		
miR-371a-3p for chemotherapy	Serum Biomarkers in Testicular Germ Cell Tumours	0.759			83.4–92.9	60.1–100
miR-639	Bladder cancer	0.752	0.571	0.934		
Serum DNA	Any Cancer	0.75			62.9	87
Higher cfDI	Different types of Cancer (Breast cancer, Prostate cancer, HCC, CRC,Glioma,Melanoma, Bladder cancer, Renal cell cancer,Acute leukemia,TGCC,HNC,PAC,Mixed)	0.742			0.58	0.78
ctDNA(Plasma)	Glioma	0.741	0.332	0.927		
KRAS in CTC group	Lung Cancer	0.741	0.472	1	38.7	92.1
FAM83H-AS1	Breast cancer	0.74			70	76.7
miR-184	Breast cancer	0.74			87.5	71
miR-382–3p	Breast cancer	0.74			52	92.5
miR 30a 3p	Glioma and Primary CNS Lymphoma	0.737	0.66	0.81		
AFP, CEA, CA19–9, CYFRA21–1, SCC, PSA	Any Cancer	0.73			76	76
DNA integrity index	Breast cancer	0.73			51	90
Neat1	Breast cancer	0.73			80	80
miR-106a-5p	Osteosarcoma	0.7255				
Circulating nucleosomes	Any Cancer	0.72			91	90
HER2 mRNA	Breast cancer	0.72			90	50
MiR-17–3p	Osteosarcoma	0.72			64.3	84.6
miR-505–5p	Breast cancer	0.72			75	60
miR-96–5p	Breast cancer	0.72			73	66
miR 6803 3p	Glioma and Primary CNS Lymphoma	0.716	0.646	0.786		
miR-141	Bladder cancer	0.714	0.519	0.91		
Pai-1	Breast cancer	0.71			64	68
miR-139–5p	Osteosarcoma	0.7098				
Delta181CTmir21	Breast cancer	0.7			72.2	64.7
MiR- 205–5p	Osteosarcoma	0.7				
cfDNA (APP gene integrity)	Endocrine Neoplasia	0.699				
miR 4751	Glioma and Primary CNS Lymphoma	0.693	0.602	0.778		
ACTB-106	Bladder cancer	0.686	0.617	0.755	91.6	43.3
miR 3918	Glioma and Primary CNS Lymphoma	0.682	0.586	0.766		
miR 487a 3p	Glioma and Primary CNS Lymphoma	0.681	0.593	0.762		
miR 6820 3p	Glioma and Primary CNS Lymphoma	0.681	0.579	0.774		
hsa-miR-423–5p	Breast cancer	0.68			66	68
9 miRNAs (miR-15a, miR-18a, miR-107, miR- 133a, miR-139–5p, miR-143, miR-145, miR- 365, miR-425)	Any Cancer	0.67			83.3	41.2
miR-181a	Breast cancer	0.67			70.7	59.9
miR 371a 3p	Glioma and Primary CNS Lymphoma	0.669	0.578	0.757		
miR 4756 5p	Glioma and Primary CNS Lymphoma	0.669	0.578	0.755		
TIG1	Bladder cancer	0.659	0.536	0.782	31.8	100
miR 146a 3p	Glioma and Primary CNS Lymphoma	0.656	0.551	0.754		
miR548am 3p	Glioma and Primary CNS Lymphoma	0.639	0.531	0.743		
4-miRNAs	Osteosarcoma	0.608			91.1	94.4
CTCs	Any Cancer	0.6			30.6	88

(*continued on next page*)

Table 2 (*continued*)

Name Of The Marker	Outcome	AUC	95% CI Low For AUC	95% Ci High For AUC	Sensitivity	Specificity
PTGS2	Bladder cancer	0.3622	0.506	0.739	24.4	100
DAPK	Bladder cancer	0.3512	0.385	0.638	24	100

Data are reported ranked by area under the curve values, from higher to lower values. Abbreviations: *OSCC*: Oropharyngeal Squamous Cell Carcinoma, *HPV*: Human Papillomavirus; *LC*: Lung Cancer; *NIPT*: Non-Invasive Prenatal Testing; *CNS*: Central Nervous System; *BC*: Breast Cancer; *BLC*: Bladder Cancer; *OLC*: Oral Squamous Cell Carcinoma; *OC*: Ovarian Cancer; *HCC*: Hepatocellular Carcinoma; *G*: Glioma; *PC*: Pancreatic Cancer, *O*: Osteosarcoma; *LEP*: Leptomeningeal Metastases; *TGC*: Testicular Germ Cell Tumors; *NE*: Neuroendocrine Tumor, *ThyC*: Thyroid Cancer; *EN*: Endocrine Neoplasia; *CRC*: Colorectal Cancer; *MS*: Multiple Sclerosis; *NSCLC*: Non-Small Cell Lung Cancer; *RCC*: Renal Cell Cancer; *AC*: Acute Leukemia; *TGCC*: Testicular Germ Cell Cancer; *HNC*: Head and Neck Cancer; *PAC*: Pancreatic Adenocarcinoma; *SBC*: Salivary Biomarkers for Cancer; *DNA*: Deoxyribonucleic Acid; *cfHPV-DNA*: Circulating cell-free Human Papillomavirus DNA; *cHPV DNA*: Circulating Human Papillomavirus DNA; *ALK ctDNA*: Anaplastic Lymphoma Kinase circulating tumor DNA; *NIPT*: Non-Invasive Prenatal Testing; *miR10b*: MicroRNA-10b; *miR-148a*: MicroRNA-148a; *miR-155*: MicroRNA-155; *miR-21*: MicroRNA-21; *miR-30c*: MicroRNA-30c; *PTEN*: Phosphatase and Tensin Homolog gene; *miR-19b*: MicroRNA-19b; *miRNA-373*: MicroRNA-373; *S100A4*: S100 Calcium Binding Protein A4; *hsa-miR-548ar-5p*: Homo sapiens microRNA-548ar-5p; *miR-92a*: MicroRNA-92a; *hsa-miR-21–5p*: Homo sapiens microRNA-21–5p; *miR-27a*: MicroRNA-27a; *miR-152*: MicroRNA-152; *APC*: Adenomatous Polyposis Coli gene; *CTC*: Circulating Tumor Cells; *ctDNA*: Circulating tumor DNA; *S100A9*: S100 Calcium Binding Protein A9; *cfDNA*: Cell-Free DNA; *miR-598–3p*: MicroRNA-598–3p; *RARB2*: Retinoic Acid Receptor Beta 2 gene; *S100A8*: S100 Calcium Binding Protein A8; *S100A11*: S100 Calcium Binding Protein A11; *miR-371a-3p*: MicroRNA-371a-3p; *cf-DNA*: Cell-Free DNA; *EGFR*: Epidermal Growth Factor Receptor gene; *TP53*: Tumor Protein P53 gene; *NF1*: Neurofibromin 1 gene; *MET*: MET Proto-Oncogene; *BRAF*: B-Raf Proto-Oncogene; *KRAS*: KRAS Proto-Oncogene; *ADAMTS1*: A Disintegrin And Metalloproteinase with Thrombospondin Motifs 1; *BNC1*: Basonuclin 1; *Exosomes*: Small extracellular vesicles; *CD44v6*: Isoform of CD44 protein; *Tspan8*: Tetraspanin 8; *EpCAM*: Epithelial Cell Adhesion Molecule; *GPC1*: Glypican-1; *miR-1246*: MicroRNA-1246; *GNAS*: GNAS Complex Locus; *miR-4644*: MicroRNA-4644; *S100A6*: S100 Calcium Binding Protein A6; *SEPTIN9*: Septin 9 gene; *lncRNA*: Long Non-Coding RNA; *MALAT1*: Metastasis Associated Lung Adenocarcinoma Transcript 1; *PCAT-1*: Prostate Cancer Associated Transcript 1; *SPRY4-IT1*: Sprouty RTK Signaling Antagonist 4 Intron Transcript 1; *UCA1–201*: Urothelial Cancer Associated 1 Transcript 201; *LINC00355*: Long Intergenic Non-Protein Coding RNA 355; *CD9*: CD9 Protein; *miR-19b1–5p*: MicroRNA-19b1–5p; *miR-136–3p*: MicroRNA-136–3p; *miR-139–5p*: MicroRNA-139–5p; *PCA3*: Prostate Cancer Antigen 3; *ERG*: ETS-Related Gene; *TMPRSS2-ERG*: Fusion gene between TMPRSS2 and ERG; *FOLH1*: Folate Hydrolase 1; *HPN*: Hepatocyte Growth Factor Activator; *ITSN1*: Intersectin 1; *miR-375–3p*: MicroRNA-375–3p; *miR-574–3p*: MicroRNA-574–3p; *ANXA3*: Annexin A3 gene; *CCN1*: Cellular Communication Network Factor 1; *HERV-K*: Human Endogenous Retrovirus K; *miR-99a*: MicroRNA-99a; *GSTP1*: Glutathione S-Transferase Pi 1 gene; *TIG1*: Tazarotene-Induced Gene 1; *PTGS2*: Prostaglandin-Endoperoxide Synthase 2 gene; *miR-25–3p*: MicroRNA-25–3p; *Pdx-1*: Pancreatic and Duodenal Homeobox 1 gene; *KRAS in CTC group*: KRAS mutations detected in circulating tumor cells group; *cfDNA integrity*: Integrity of cell-free DNA; *miR-191*: MicroRNA-191; *miR-125b*: MicroRNA-125b; *miR-223*: MicroRNA-223; *miR-196a*: MicroRNA-196a; *miR-146a*: MicroRNA-146a; *miR-4677*: MicroRNA-4677; *miR-483–5p*: MicroRNA-483–5p; *miR-141*: MicroRNA-141; *miR-222*: MicroRNA-222; *miR-663a*: MicroRNA-663a; *miR-195*: MicroRNA-195; *Delta181CTmir21*: A specific mutation (Delta181) in microRNA-21; *miR-205–5p*: MicroRNA-205–5p; *miR-495*: MicroRNA-495; *miR-301a-3p*: MicroRNA-301a-3p; *ALU-247*: ALU repeat region of 247 base pairs; *miR-20a-5p*: MicroRNA-20a-5p; *ctDNA (Plasma)*: Circulating tumor DNA in plasma; *Neat1*: Nuclear Enriched Abundant Transcript 1; *Pai-1*: Plasminogen Activator Inhibitor 1; *AFP*: Alpha-fetoprotein; *CEA*: Carcinoembryonic Antigen; *CA19–9*: Cancer Antigen 19–9; *SCC*: Squamous Cell Carcinoma Antigen; *PSA*: Prostate-Specific Antigen; *ALU-115*: ALU repeat region of 115 base pairs; *HER2*: Human Epidermal Growth Factor Receptor 2 gene; *cf-DNA*: Cell-Free DNA; *mtDNA*: Mitochondrial DNA; *hsa-miR-423–5p*: Homo sapiens microRNA-423–5p; *miR-146b-5p*: MicroRNA-146b-5p; *ALU*: ALU sequences (repetitive elements in the genome); *miR-486–5p*: MicroRNA-486–5p; *miR-34a*: MicroRNA-34a; *miR-425–5p*: MicroRNA-425–5p; *SMAD4*: SMAD Family Member 4 gene; *miR-181a*: MicroRNA-181a; *miR-106a-5p*: MicroRNA-106a-5p; *miR-193b*: MicroRNA-193b; *PTEN*: Phosphatase and Tensin Homolog gene; *PTGS2*: Prostaglandin-Endoperoxide Synthase 2 gene; *PTEN*: Phosphatase and Tensin Homolog *SMAD4*: SMAD Family Member 4 gene; *ADAMTS1*: A Disintegrin And Metalloproteinase with Thrombospondin Motifs 1; *miR-92b*: MicroRNA-92b; *CFAP69*: Cilia and Flagella Associated Protein 69 gene; *LOXL2*: Lysyl Oxidase Like 2 gene; *MLH1*: MutL Homolog 1 gene; *PAX8*: Paired Box 8 gene; *miR-29a*: MicroRNA-29a; *miR-143*: MicroRNA-143; *NDRG2*: N-Myc Downstream Regulated Gene 2; *hTERT*: Human Telomerase Reverse Transcriptase gene; *PIK3CA*: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha gene; *TFF1*: Trefoil Factor 1 gene; *TFF3*: Trefoil Factor 3 gene; *NID1*: Nidogen 1 gene; *TM4SF1*: Transmembrane 4 L6 Family Member 1 gene; *miR-122*: MicroRNA-122; *cfmiRNA*: Circulating free microRNA; *CCAT1*: Colon Cancer Associated Transcript 1 gene; *miR-18a*: MicroRNA-18a; *miR-146b*: MicroRNA-146b; *MEG3*: Maternally Expressed 3 gene; *MMP9*: Matrix Metallopeptidase 9 gene; *PIK3CA ctDNA*: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha circulating tumor DNA; *miR-214*: MicroRNA-214; *miR-34b*: MicroRNA-34b; *miR-210*: MicroRNA-210; *miR-224*: MicroRNA-224; *lncRNA CCAT1*: Long Non-Coding RNA Colon Cancer Associated Transcript 1; *cfmiR-101*: Circulating free MicroRNA-101; *hsa-miR-30e-5p*: Homo sapiens MicroRNA-30e-5p; *miR-125a-5p*: MicroRNA-125a-5p; *SEPT9 cfDNA*: Septin 9 Cell-Free DNA; *miR-376a*: MicroRNA-376a; *miR-150–5p*: MicroRNA-150–5p; *TM4SF1 cfDNA*: Transmembrane 4 L6 Family Member 1 circulating tumor DNA; *MALAT1 cfmiRNA*: Metastasis Associated Lung Adenocarcinoma Transcript 1 circulating free microRNA; *hsa-miR-144–3p*: Homo sapiens MicroRNA-144–3p; *cf-DNA integrity*: Integrity of circulating free DNA; *TCF21*: Transcription Factor 21 gene; *miR-451*: MicroRNA-451; *miR-125a*: MicroRNA-125a; *miR-29c*: MicroRNA-29c; *mtDNA cfDNA*: Mitochondrial DNA in circulating free DNA; *BRCA1*: Breast Cancer Type 1 susceptibility gene; *BRCA2*: Breast Cancer Type 2 susceptibility gene; *miR-663b*: MicroRNA-663b; *ITGA3*: Integrin Subunit Alpha 3 gene; *HDAC4*: Histone Deacetylase 4 gene; *hsa-miR-548ar-5p cfDNA*: Homo sapiens MicroRNA-548ar-5p circulating tumor DNA; *hsa-miR-151a-5p*: Homo sapiens MicroRNA-151a-5p; *FGF19*: Fibroblast Growth Factor 19 gene; *miR-506*: MicroRNA-506; *HLA-G*: Human Leukocyte Antigen G gene; *hsa-miR-23b-3p*: Homo sapiens MicroRNA-23b-3p; *hsa-miR-320a-3p*: Homo sapiens MicroRNA-320a-3p; *miR-508–5p*: MicroRNA-508–5p; *hsa-miR-153–5p*: Homo sapiens MicroRNA-153–5p; *TUSC2*: Tumor Suppressor Candidate 2 gene; *cMet ctDNA*: Circulating MET Proto-Oncogene tumor DNA; *cf-DNA plasma integrity*: Integrity of cell-free DNA in plasma; *CD9 cfmiRNA*: CD9 circulating free microRNA; *cfDNA fragments*: Fragments of cell-free DNA; *STK11*: Serine/Threonine Kinase 11 gene; *cfRNA*: Circulating Free RNA; *miR-214–5p*: MicroRNA-214–5p; *cfDNA epigenetic markers*: Epigenetic markers in cell-free DNA; *HOTTIP*: HOXA Transcript at the Distal Tip gene; *cfRNA expression*: Expression of circulating free RNA; *cfDNA methylation markers*: Methylation markers in cell-free DNA; *hsa-miR-335–5p*: Homo sapiens MicroRNA-335–5p; *miR-7*: MicroRNA-7; *AKT1*: AKT Serine/Threonine Kinase 1 gene; *miR-92a cfmiRNA*: MicroRNA-92a circulating free microRNA; *cfDNA fragment size*: Size of circulating free DNA fragments; *hsa-miR-146b-5p cfDNA*: Homo sapiens MicroRNA-146b-5p circulating tumor DNA; *cfRNA markers*: Markers of circulating free RNA; *cfmiR-30b*: Circulating free MicroRNA-30b; *cfmiR-200c*: Circulating free MicroRNA-200c; *cfmiR-375*: Circulating free MicroRNA-375; *cfDNA fragment ratio*: Ratio of circulating free DNA fragments; *cfmiR-196a*: Circulating free MicroRNA-196a; *cfmiR-141*: Circulating free MicroRNA-141; *hsa-miR-181a-5p cfDNA*: Homo sapiens MicroRNA-181a-5p circulating tumor DNA; *cfmiR-486–5p*: Circulating free MicroRNA-486–5p; *cfmiR-34a*: Circulating free MicroRNA-34a; *cfDNA epigenetics*: Epigenetic modifications in circulating free DNA; *cfmiR-424–5p*: Circulating free MicroRNA-424–5p; *cfmiR-145*: Circulating free MicroRNA-145; *cfmiR-99b*: Circulating free MicroRNA-99b; *cfmiR-221*: Circulating free MicroRNA-221; *cfmiR-26b*: Circulating free MicroRNA-26b.

tumors, which are typically challenging to access surgically due to the blood-brain barrier. Corticosteroid treatment, commonly used to manage edema in CNS tumors, may affect the levels of cfDNA detected in liquid biopsies [77]. The administration of corticosteroids can reduce inflammation and vascular permeability, potentially lowering the amount of cfDNA released into circulation [77]. This can influence the sensitivity of liquid biopsies in detecting tumor-derived cfDNA, thereby impacting the accuracy of real-time tumor monitoring during

corticosteroid therapy.

At the same time, this umbrella review serves also as a critical tool for analyzing the limitations associated to LB, and in particular to the published attempts for proving quantitative summaries on this group of methodology through the use of meta-analysis. Indeed, according to the GRADE evaluation, all the outcomes were graded as very low, thus highlighting important limitations. The measured outcomes suffered from the lack of essential parameters for their evaluation in meta-analysis, including the 95% confidence intervals in the majority of studies, the total lack of reporting/analyzing heterogeneity, and the significant proportion of publication bias risk. All these observations clearly showed that to date there are critical issues in the available literature for enabling a comprehensive, impartial, and objective evaluation of such limitations on the use of LB. For example, we don't know if we can apply LB in all individuals independently from the demographic characteristics (e.g., age and gender). Moreover, the available literature seems to be affected by a publication bias. For the mentioned reasons, the methodological considerations emerging from our umbrella review represents a warning when considering the current literature available on this topic. At the same time, our findings warmly support for a more rigid and transparent assessment of the findings, while suggesting where to better direct future research efforts. Overall considered, the most essential areas where LB should be better addressed are: i) improving LB sensitivity, starting from more specific analyses on the role of LB in the early detection of cancer, which now is very challenging, since LB is still depending on the bulk of circulating tumor cells / nuclei acids from a given neoplasm [1,78]; ii) improving LB specificity, also considering the discrepancies of the molecular profiling between LB and tissue samples [14,45]; iii) standardizing sample extraction procedures, starting from pre-analytical platforms to arrive to the several and different methodologies of isolation of circulating tumor cells and related material. This task should aim to extrapolate the use of the different circulating molecules into the "real world" of the clinic. On top of that, the "only one analyte" barrier should be overcome, trying to focus more on multi-omics approaches to finally unleash the full clinical utility of LB [79]; iv) lastly, as this study clearly shows, high-quality meta-research is urgently needed to better evaluate the real impact of LB into the clinic, along with its intrinsic potentialities and limitations. Systematic reviews with meta-analyses represent indeed one of the most important tools for providing evidence-based quantitative summary on a topic. Based on the results of our umbrella review, however, we clearly show that current literature based on meta-analyses on LB suffers from critical methodological issues. Thus, upcoming meta-research efforts along this line should consider our findings, trying to address such crucial limitations.

Our study does have some limitations. First, all the meta-analytic outcomes were practically about cancer, thus limiting possible quantitative explorations on the role of LB in different areas of medicine. Second, according to the AMSTAR 2 evaluation, the systematic reviews and meta-analysis included were rated as critically low or low quality, possibly introducing critical biases. Finally, umbrella reviews were limited to outcomes studied in the respective meta-analyses and do not provide in-depth data on disease severity, dose–response effects, or specific subgroups such as by sex, environmental conditions, or age.

In conclusion, the literature about LB is exponentially increasing and some promising data about cancer are available to date. However, no definitive statements can yet be claimed in relation to LB since the meta-analyses published to date are affected by several methodological limitations such as missing data about heterogeneity or publication bias, overall indicating that the true diagnostic value of LB could be substantially different from gold standard techniques, therefore indicating that high-quality meta-research is urgently needed.

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Ethics approval and consent to participate

Being a systematic review with meta-analysis of previously published literature, no informed consent was obtained and we did not ask for the approval to our local Ethical Committee.

Data available statement

The data underlying this article will be shared on reasonable request to the corresponding author. All authors declare manuscript is an honest, accurate, and transparent account of the study being reported; no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned (and, if relevant, registered) have been explained in published protocol.

CRediT authorship contribution statement

Nicola Veronese: Writing – original draft, Formal analysis. **Claudio Luchini:** Writing – review & editing. **Stefano Ciriminna:** Formal analysis, Data curation. **Katia Spinelli:** Data curation. **Santo Fruscione:** Data curation. **Paola Mattiolo:** Data curation. **Miriam Belluzzo:** Data curation. **Veronica Messina:** Data curation. **Lee Smith:** Writing – review & editing. **Mario Barbagallo:** Writing – review & editing. **Walter Mazzucco:** Writing – review & editing.

Declaration of competing interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2024.102172.

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