Cancer Biomarkers in Liquid Biopsy for Early Detection of Breast Cancer: A Systematic Review

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ABSTRACT

BACKGROUND: Breast cancer (BC) is the most common neoplasm in women worldwide. Liquid biopsy (LB) is a non-invasive diagnostic technique that allows the analysis of biomarkers in different body fluids, particularly in peripheral blood and also in urine, saliva, nipple discharge, volatile respiratory fluids, nasal secretions, breast milk, and tears. The objective was to analyze the available evidence related to the use of biomarkers obtained by LB for the early diagnosis of BC.

METHODS: Articles related to the use of biomarkers for the early diagnosis of BC due to LB, published between 2010 and 2022, from the databases (WoS, EMBASE, PubMed, and SCOPUS) were included. The MInCir diagnostic scale was applied in the articles to determine their methodological quality (MQ). Descriptive statistics were used, as well as determination of weighted averages of each variable, to analyze the extracted data. Sensitivity, specificity, and area under the curve values for specific biomarkers (individual or in panels) are described.

RESULTS: In this systematic review (SR), 136 articles met the selection criteria, representing 17 709 patients with BC. However, 95.6% were case-control studies. In 96.3% of cases, LB was performed in peripheral blood samples. Most of the articles were based on microRNA (miRNA) analysis. The mean MQ score was 25/45 points. Sensitivity, specificity, and area under the curve values for specific biomarkers (individual or in panels) have been found.

CONCLUSIONS: The determination of biomarkers through LB is a useful mechanism for the diagnosis of BC. The analysis of miRNA in peripheral blood is the most studied methodology. Our results indicate that LB has a high sensitivity and specificity for the diagnosis of BC, especially in early stages.

KEYWORDS: Breast neoplasms, liquid biopsy, early diagnosis, microRNAs, sensitivity and specificity, breast cancer

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Introduction

Breast cancer (BC) is the most frequently diagnosed malignancy and the leading cause of cancer-related deaths in women worldwide. In 2020, more than 2.2 million new cases were diagnosed and 684 996 deaths were reported globally.[1] This neoplasm originates in the epithelial cells that line the mammary ducts responsible for transporting milk to the nipple or in the lobules, which are the glandular structures that produce milk.

Despite recent developments for early detection of this disease, additional innovative and effective diagnostic methods in the early stages are needed to obtain the best possible outcomes during treatment. To date, progress in this area has been slow and continues to be an important challenge.[2,3]

Although ultrasound and mammography are the most widely used methods, both procedures depend on the radiologist's expertise, as well as the quality and technology of the equipment used during these procedures. Furthermore, as mammography applies ionizing radiation, the ability to use in patients younger than 30 years of age is limited.[3-5] During the last decade, nuclear magnetic resonance of the mammary glands has been used as a complementary method, with high sensitivity in the detection of small lesions. This approach, however, is an expensive procedure with a significant rate of false-positives.[6]

Nevertheless, to confirm the diagnosis of BC, all of the above methods require a tissue biopsy as an adjunct, which is an invasive procedure. The development of non-invasive techniques and methods that allow early diagnosis of BC is highly relevant, and several methods are being studied and researched worldwide. An example of the above would be the use of serum markers such as carcinoembryonic antigen (CEA) and Ca153, which may be interesting strategies, but show low sensitivity and specificity.[7]

Liquid biopsy (LB) is an approach that has also recently emerged. It identifies circulating biomarkers that can serve as a valuable and promising tool for early diagnosis of BC. This procedure, which is non-invasive, can be performed on blood and other body fluids such as urine, saliva, nipple discharge,

volatile respiratory fluids, nasal secretions, breast milk, and tears. Cellular components, such as circulating tumor nucleic acids (ctDNA), circulating tumor cells (CTC), vesicle-encapsulated extracellular RNA (EV-mRNA), and circulating microRNA (miRNA) molecules, are among the major components identified.[8]

The molecular classification of the disease based on the expression of estrogenic hormone receptors (ER), progesterone receptors (PR), human epidermal growth factor 2 (HER2), and Ki-67 proliferative index allows the following BC subtypes to be identified: luminal A (ER and/or PR+, Her2–, Ki-67 low), luminal B (ER and/or PR+, Her2–, Ki-67 high) or (ER and/or PR+ Her2+), Her2-enriched (ER and PR– Her2+), and triple-negative (ER– PR– Her2–), each of which is related to a specific gene expression and useful in the diagnosis of neoplasia.[9] In addition to molecular classification, the histological grade and stage of the disease are being investigated to determine their benefit in the early diagnosis of BC.

The aim of this study was to analyze the available evidence on the use of biomarkers obtained by LB in the early diagnosis of BC.

Materials and Methods

This study was written following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA 2020) statement[10] and is registered as a protocol in the PROSPERO database (ID: CRD42021255596).

Design

Systematic Review (SR).

Eligibility criteria

Articles related to LB and BC early detection in humans were included, without language restriction; the articles were published between January 2010 and June 2022. Review articles, letters to the editor, case reports, conference abstracts, and duplicate articles were excluded.

Information sources

A systematic search of related literature was conducted from the following sources: WoS, EMBASE, PubMed, SCOPUS. In addition, a manual cross-reference search was performed.

Search strategy

MeSH terms and free words were used: "circulating cell-free DNA," or "plasma cell-free DNA," or "serum cell-free DNA," or "liquid biopsy" or "biomarkers," or "circulating tumor cells," or circulating tumor DNA," or "detection of cancer DNA," or serum microRNA" and "breast cancer" or "early breast cancer detection" or "screening of breast cancer." In addition, Boolean operators "AND" and "OR" were used. The searches were adapted to each source of information and the corresponding language.

Selection process. The eligibility assessment of the primary articles was performed by 2 groups of 2 reviewers each (G.D.-C.A. and B.G.-J.P.H.), who worked independently and blinded. Disagreements between review groups were resolved by consensus. Item recruitment closed on May 30, 2022.

Data collection process. For data extraction, an Excel sheet (PC Excel, version 15.24; 2016 Microsoft Corporation) was created. Five authors extracted data from the included studies (G.D., C.A., D.P., J.P.H., and B.G.) and 2 additional authors checked the extracted data (M.M. and L.A.). Disagreements between the reviewers were resolved by consensus.

Variables studied

The variables considered were year of publication, country, number of cases, type of design, body fluid used for LB (peripheral blood, saliva, fluid aspirated from the nipple, sweat, urine, tears, and volatile compounds in the breath), type of biomarkers in the blood (CTC, ctDNA, circulating free DNA [cfDNA], circulating miRNA, circulating extracellular RNA vesicles [EV-RNA], and others), type of biomarkers in other body fluids (CTC, EV-RNA, miRNA, ctDNA, cfDNA, and others), determined biomarkers, sensitivity, specificity, and methodological quality (MQ) of the primary studies.

Study risk of bias assessment. The internal validity (MQ) of the primary studies was assessed using the MInCir-Dg scale[11] (MQ_assessment scale for diagnostic studies), composed of 9 items grouped into 3 domains, with a minimum and maximum score of 9 and 45 points, and a cut-off point of 20 points, which defines the dichotomy of the MQ_construct for diagnostic studies.

Effect measures

Descriptive statistics (percentages, frequencies) and determination of variable weighted means (weighting of the result of each variable by the MQ_of the primary study from which it originated) were used to analyze the extracted data.

Synthesis methods

The identified documents were filtered by duplication between databases. Titles and abstracts were screened using selection criteria. Finally, an in-depth analysis of each of the selected primary articles was performed; critical reading guides were applied, thus organizing the synthesis of the information.

Table 1. Search strategy: databases used and primary articles found.

DATABASES	NO. OF ARTICLES FOUND	NO. OF SELECTED ARTICLES
EMBASE	3036	32 (23.5%)
WoS	8819	74 (54.4%)
PubMed	3286	3 (2.20%)
SCOPUS	1001	27 (19.9%)
TOTAL	16142	136

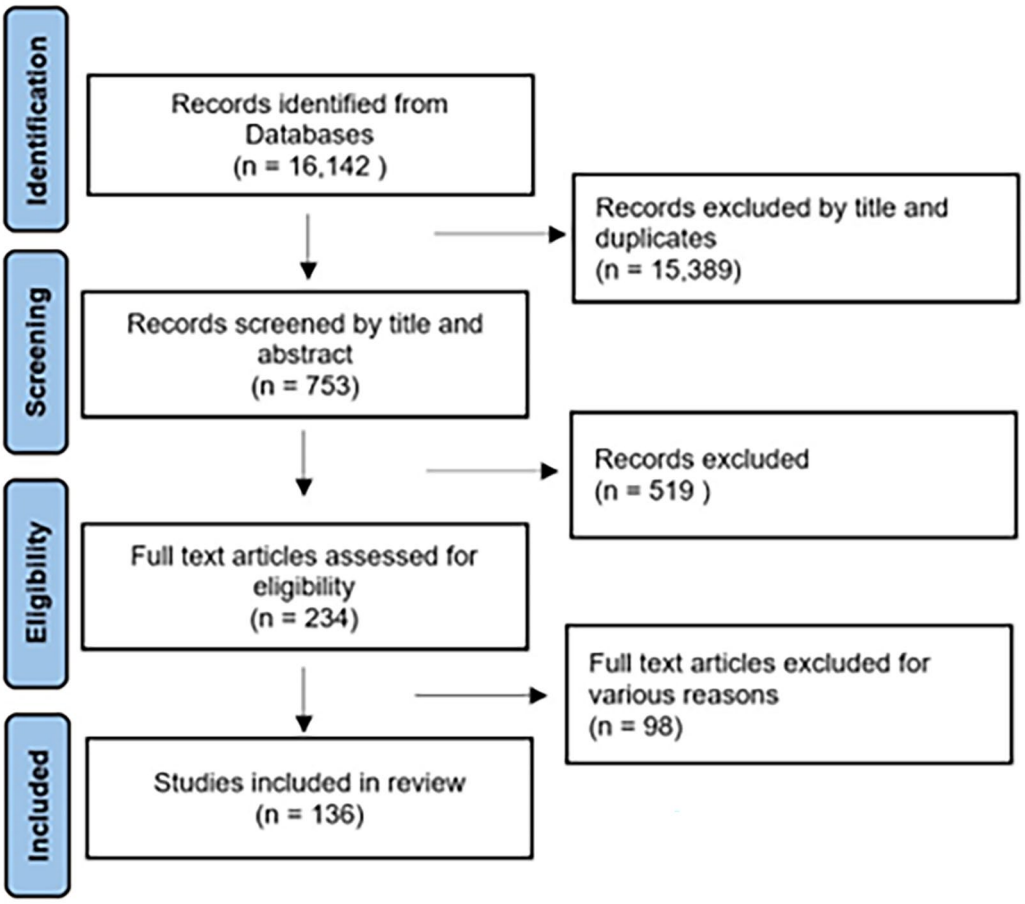

Figure 1. Flow chart of primary articles used in this SR.

Assessment of reporting bias

Potentially missing studies were identified by cross-reference searches.

Certainty assessment

Not considered.

Ethics

The authors and centers of the primary studies used were masked.

Results

Study selection

In total, 16142 articles were identified in the aforementioned databases (Table 1). However, 234 articles were retained for full reading, following the elimination of duplicates and articles whose title and abstract did not meet the eligibility criteria; of these, only 136[12-147] met the inclusion criteria and are the basis of the qualitative and quantitative analysis of this SR (Figure 1).

Study characteristics

Of the 136 primary articles, 130 (95.6%) were case-control studies and 6 (4.4%) were cohort studies. The population

Table 2. Characterization of the primary articles on the diagnosis of BC by means of LB (n = 136).

TYPE OF BC	N	%
Several	73	53.7
Not specified	59	43.4
Triple-negative	2	1.5
HER2-enriched	2	1.5
PUBLICATION YEAR	**NO. OF ARTICLES**	**%**
2022	11[12-22]	8.1
2021	35[23-57]	25.7
2020	17[58-74]	12.5
2019	14[75-88]	10.3
2018	10[89-98]	7.4
2017	4[99-102]	2.9
2016	12[103-114]	8.8
2015	8[115-122]	5.9
2014	8[122-130]	5.9
2013	2[131,132]	1.5
2012	9[133-141]	6.6
2011	2[142,143]	1.5
2010	4[144-147]	2.9

Abbreviations: BC, breast cancer; HER2, human epidermal growth factor 2; LB, liquid biopsy.

represented in these articles comprised 34 376 patients, of which 17 709 are BC carriers and 16 667 correspond to controls (defined as healthy subjects with benign breast disease or other types of cancer). In all, 75.7% of the articles were published between 2016 and 2022 (Table 2).

Results of individual studies

Evidence on the early diagnosis of BC through LB came from 31 countries. China (n = 45) and Egypt (n = 18) were the countries that contributed the most articles (Figure 2).

The molecular classification of BC (Figure 3) was not described in 59 articles, while 73 articles included patients with various types, based on the molecular classification (luminal A, luminal B, HER2-enriched, and triple-negative). In addition, 2 articles studied only patients with triple-negative BC,[101,116] and 2 included patients with HER2-enriched BC.[76,95]

In reference to the disease stage, 57.4% of the articles detailed the stages that the patients were in at the time. In 43 articles, patients in all stages (I, II, III, and IV) were included, representing a population of 5382 patients with BC (stage II was the most frequent, representing 3230 patients). The details are available in Table 3.

The histological grade was identified in 40.4% of the articles, which represents 5102 patients. Patients with all grades (1-3) were included in 51 articles. In 3 articles, grades 2 and 3 were included,[68,96,140] and 1 article included only grade 3 patients[120] (Table 4).

Diagnostic role of LB in BC

The body fluids in which LB was analyzed are detailed in Table 5 emphasizing that in 96.3% of the studies, it was performed in peripheral blood. Additional body fluids analyzed included urine[69,74,86] and saliva.[71,120]

Regarding the different LB methods, miRNA analysis (56.8%) predominated, followed by cfDNA (20.5%), in the studies that used blood samples. In those that analyzed saliva or urine, the diagnosis was performed by miRNA analysis.

The validity of the different tools for early diagnosis of BC described by the primary studies (markers obtained in DNA or RNA) is noted in Tables 6 to 8. It is noteworthy that the sensitivity of most is higher than 70% (Table 6).

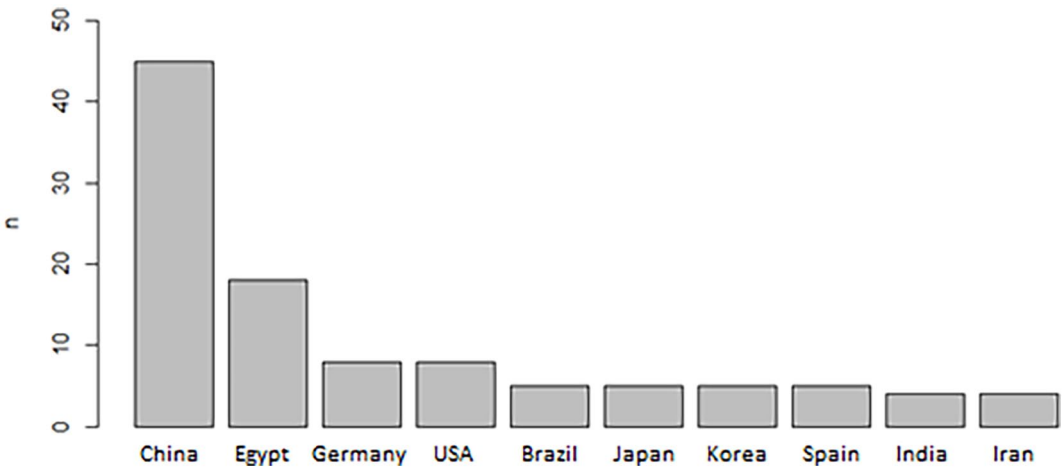

Figure 2. Main countries of origin of articles on BC diagnosis through LB.
BC indicates breast cancer; LB, liquid biopsy.

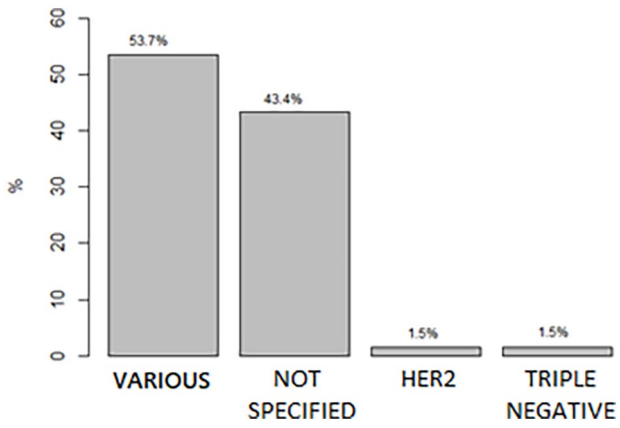

Figure 3. Molecular classification of BC reported in 136 primary articles. BC indicates breast cancer.

As shown in Table 6, highlighted in gray, 28 biomarkers presenting a Sensitivity greater than 90% were identified: miR-17-5p, miR-155, miR-222,[89] miR-202,[60] PTEN, SMAD4,[24] APC, RARB2,[115] miRNA-222, miRNA-373,[92] miR-27a,[85] cfDNA methylation score,[25] HER2 mRNA,[95] miR-21,[28] polymorphism -31G/C in survivin promoter gene,[79] hsa-miR25-3p, hsa-miR-548ar-5p,[80] miR-598-3p, miR-1246,[105] miR-495,[117] telomeric sequences in cfDNA,[121] miR-30c, miR-148a,[39] miR-185-3p,[49] miR-34a,[17] miR10b, miR21,[12] and miRNA-373.[34]

As shown in Table 7, highlighted in gray, 16 biomarker panels in peripheral blood can be observed[19,22,27,29,30,42,44,54,61,81,93,98,106,111,112,123] and 2 biomarker panels in saliva[74,86] with a sensitivity greater than 90%.

As shown in Table 8, highlighted in gray, 8 individual biomarkers and 7 panel biomarkers taken from peripheral blood had statistically significant area under the curve (AUC) values (greater than 0.80). However, 21 studies (20 in peripheral blood and 1 in urine) presented statistically significant *P* values (less than .05).

Sensitivity, specificity, AUC, or *P* value of the test analyzed was not described in 17 of the articles studied. These articles, however, did address frequencies and associations with patients' clinical pathological characteristics.

Methodological quality. The average MQ of the articles was 24.7 points (Table 9). Most of the articles were cases and control studies. None of the studies validate the sample size used, and from a methodological standpoint, there is a lack of homogeneity throughout all of the articles reviewed. Furthermore, not all studies describe the inclusion and exclusion criteria; and in some, the study population involves less than 100 patients. However, the study test is described in sufficient detail in all of these studies, and regardless of the results, the same reference standard was applied to all study subjects. Furthermore, in most of the articles, the objectives of the study were clear and precise.

Table 3. Characterization of primary articles based on the stage of the disease.

STAGES	NO. OF ARTICLES	NO. OF PATIENTS BY STAGES							
		TOTAL	I	II	III	IV	I AND II	I, II, AND III	III AND IV
I and II	15	1291	428	637	–	–	226	–	–
I, II, and III	20	1842	544	793	365	–	114	26	–
I, II, III, and IV	43	5382	1259	1800	846	406	625	–	446
Unknown	–	91	–	–	–	–	–	–	–
Total	78	8606	2231	3230	1211	406	965	26	446

Table 4. Characterization of primary articles based on the histological grade.

HISTOLOGICAL GRADE	NO. OF ARTICLES	NO. OF PATIENTS BY GRADE					
		TOTAL	1	2	3	1 AND 2	2 AND 3
1, 2, and 3	51	4695	646	2049	1676	274	50
2 and 3	3	153	–	110	43	–	–
3	1	5	–	–	5	–	–
Unknown	–	249	–	–	–	–	–
Total	55	5102	646	2159	1724	274	50

Table 5. Characterization of LB analyzed in primary articles.

BODY FLUIDS	NO. OF ARTICLES (N = 136)
Peripheral blood	131 (96.3%)
Urine	3 (2.2%)
Saliva	1 (0.7%)
Saliva and peripheral blood	1 (0.7%)
BIOMARKERS USED IN PERIPHERAL BLOOD	**NO. OF ARTICLES (N = 132)**
miRNA	75 (56.8%)
cfDNA	27 (20.5%)
ctDNA	8 (6.1%)
RNA	8 (6.1%)
DNA	5 (3.8%)
cfRNA	4 (3.0%)
Vesicles	4 (3.0%)
Others	1 (0.8%)
BIOMARKERS IN OTHER BODY FLUIDS	**NO. OF ARTICLES (N = 4)**
miRNA	4 (100%)

Abbreviation: LB, liquid biopsy.

Discussion

There are 3 SRs related to this issue. One of them studied circulating tumor ctDNA with disease-free survival in patients with BC,[148] another described the clinical uses of LB in BC,[149] and the last one reported the validity of HER2/ERBB2 copy number variation in LB from BC patients.[150] This is the first SR aimed at establishing the main biomarkers obtained by LB, useful for the early diagnosis of BC. This evidence is highly relevant because the identification of biomarkers in the early diagnosis of BC would undoubtedly be valuable in reducing mortality rates resulting from this neoplasm.

The LB approach shows promise, given that the standard BC screening technology is limited. For instance, the sensitivity of mammography depends on age, ethnic origin, personal history, the experience of the radiologist, and the quality of the technique applied.[151] In addition, ultrasound imaging of the breast also depends on the radiologist's expertise.[148]

The primary use of serum markers CA-153, CA27-29, CA-125, and CEA is applied to monitor response to treatment. However, these markers are not recommended as screening methods in light of their low diagnostic sensitivity in early disease, and their lack of specificity.[148] Despite scientific technological advancements, LB has not yet been standardized as a routine diagnostic method in the clinical setting.

It is expected that the sequencing of the genetic material obtained through LB and the significant amount of research being conducted in this area will prompt the implementation of this diagnostic tool for diagnosis, early detection, and follow-up of BC patients.

Our study found that only 4 primary articles researched the determination of biomarkers in urine and saliva, in such a way that although the use of LB in different bodily fluids has been described in BC, peripheral blood is still the most frequently used.

Contrary to what we reported in our previous review, in which most of the primary articles applied the determination of biomarkers using ctDNA,[149] in this SR, miRNA expressions were researched in 56 studies, with the aim of identifying biomarkers that differentiated between tumor tissue, healthy tissue, benign tumor breast tissue, and BC. This could be explained because the levels of cfDNA and ctDNA are significantly low in the preclinical stages, which reduces the sensitivity for screening.[152] Thus, the Yong Tay study determined that although ctDNA had a specificity greater than 99% for detecting BC, its sensitivity was only 33%.[153]

The explanation may be related to the miRNA biomarker normal signals derived from active metabolic processes occurring in all living, growing cells, increasing the pool of cellular biomarkers in earlier stages. The expressions obtained from cfDNA originate from tumor cells that detach from a tumor at an advanced stage of its development.[154]

In 29 articles,[23-25,28,59,60,68,73,79,80,85,89,92,95,96,99,103,105,108,109,115,117,119,121,126,128,129,136,141] sensitivity and specificity were reported individually for a single biomarker. In 21 of these, sensitivity was greater than 70%. However, in 10 of the studies in which sensitivity was less than 70%, and in one, 80% greater specificity was reported. In contrast, 26 articles reported sensitivity and specificity figures greater than 70% for combined biomarkers in the form of panels[27,29,30,61,76,77,81,82,87,90,93,94,97,98,101,104,106,111-114,118,123,124,132,147] leaving only 6 panels with figures lower than 70%.[61,76,93,114,124,132] Finally, 27 articles reported AUC and *P* values,[26,62,63,67,71,72,78,83,84,88,102,110,116,120,122,125,127,130,133,135,137,139,140,142,143,145,146] and of these, AUC was lower than 0.7[67,133,137] only in 3 articles. In contrast, 2 articles reported AUC values above 0.9[26,88]

A study worth noting is by Hua Zhao, in which 31 miRNA biomarkers were found in White patients, and 18 in African Americans, all with adequate sensitivity and specificity to discriminate between BC and healthy subjects.[145] Despite the above, to be considered useful, a biomarker must meet a set of analyses and clinical criteria. The benefit provided by the biomarker is underscored in the clinical setting to reduce mortality from BC and clinical validity (the ability to accurately identify a patient with BC).[155,156]

Consequently, even though research results are increasingly promising, the use of biomarkers for the early diagnosis of BC requires time to better understand the mechanisms related to circulating tumor material and to achieve adequate reproducibility.[157,158]

Table 6. Individually tested biomarkers for early diagnosis of BC in peripheral blood.

AUTHOR	BIOMARKERS	SENSITIVITY (%)	SPECIFICITY (%)	AUC
Guo et al[103]	miR-155	84.2	88.1	NR
Garrido-Cano et al[59]	miR-99a-5p	68.8	65.3	NR
Swellam et al[89]	miR-17-5p	100	75.5	0.87
	miR-155	97.4	94.4	0.99
	miR-222	91.2	78.6	0.86
Kim et al[60]	miR-202	90	93	NR
Adam-Artigues et al[23]	miR-30b-5p	78.3	72.3	NR
Swellam et al[24]	PTEN	100	94	0.99
	SMAD4	100	100	0.85
Swellam et al[115]	APC	93.4	95.4	0.95
	RARB2	95.5	92.4	0.94
Zhao et al[126]	miR-195	69	89.2	0.86
El-Ashmawy et al[68]	LncRNA-ATB	80	90	0.91
	FAM83H-AS1	70	76.7	0.74
Swellam et al[92]	miRNA-21	70.8	91.8	0.86
	miRNA-222	97.8	75.5	0.83
	miRNA-373	93.4	99	0.99
Guo and Zhang[136]	miR-181a	70.7	59.9	0.67
Swellam et al[85]	miR-27a	92	92	0.96
Bozhenko et al[108]	Mammaglobin	60.6	92.3	NR
Zhang et al[109]	LncRNA H19	56.7	86.7	0.81
Xia et al[128]	mtDNA	77	83	0.82
Zhang et al[99]	miR-30b-5p	80	100	NR
	miR-96-5p	53.3	100	NR
	miR-182-5p	53.3	92.3	NR
	miR-374b-5p	86.7	69.2	NR
	miR-942-5p	66.7	100	NR
Yousif et al[73]	miR-99a	76.7	95	0.93
Liu et al[25]	cfDNA methylation score	93	73.5	0.81
Wu et al[95]	HER2 mRNA	90	50	0.72
Hussein et al[96]	ALU-247	70	100	0.80
	ALU-115	67.5	100	0.78
	cfDNA integrity	77.5	90	0.83
Diansyah et al[28]	miR-21	92.3	81.2	0.92
Motaw et al[79]	Polymorphism -31G/C in survivin promoter gene	92.7	86.9	0.89
Souza et al[80]	hsa-miR-25-3p	92	83	0.92

(Continued)

Table 6. (Continued)

AUTHOR	BIOMARKERS	SENSITIVITY (%)	SPECIFICITY (%)	AUC
	hsa-miR-548a-5p	83	83	0.85
	hsa-miR-888-5p	83	75	0.86
	hsa-miR-548ar-5p	100	77	0.97
Fu et al[105]	miR-382-3p	52	92.5	0.74
	miR-598-3p	95	85	0.94
	miR-1246	93	75	0.90
	miR-184	87.5	71	0.74
Mishra et al[117]	miR-195-5p-5p	77.8	100	0.90
	miR-495	100	66.7	0.90
Matamala et al[119]	miR-505-5p	75	60	0.72
	miR-96-5p	73	66	0.72
Wu and Tanaka[121]	Telomeric sequences in cfDNA	91.5	76.2	0.87
Wang-Johanning et al[129]	HERV-K type (HML-2) levels	80	84.6	0.89
Sun et al[141]	miR-155	65	81.8	0.80
Bartkowiak et al[13]	CCN1	80	99	0.90
Canatan et al[37]	Delta181CTmir155	83.3	82.4	0.86
	Delta181CTmir125a	83.3	64.7	0.85
	Delta192CTmir155	77.8	64.7	0.77
	Delta181CTmir21	72.2	64.7	0.70
El-Fattah et al[38]	Hotair	76	76	0.77
	Neat1	80	80	0.73
	Pai-1	64	68	0.71
	Opn	80	76	83.00
Elhelaly et al[14]	ccfDNA	67	90	0.86
	DNA integrity index	51	90	0.73
	VEGF	74	34	0.55
Elhelbawy et al[39]	miR-30c	97.3	96.4	0.99
	miR-148a	94.7	90.9	0.99
Mahmoud et al[49]	miR-185-3p	95	66	0.84
	miR-301a-3p	85	78	0.90
Majumder et al[51]	pri-miR526b	86	71.8	NR
Mohamed et al[17]	miR-155	86	90	0.94
	miR-373	85	100	0.95
	miR-10b	60	93	0.77
	miR-34a	91	75	0.89
Ali et al[12]	miR10b	97.1	100	0.99
	miR21	95.7	98.5	0.97

(Continued)

Table 6. (Continued)

AUTHOR	BIOMARKERS	SENSITIVITY (%)	SPECIFICITY (%)	AUC
Ameli-Mojarad et al[33]	hsa_circ_0005046	85	51	0.77
	hsa_circ_0001791	10	87	100
Bakr et al[34]	miRNA-373	90.8	98.4	0.98
Han et al[41]	cfDNA	70	76	0.77
Liu et al[46]	hsa-miR-423-5p	66	68	68
Liu et al[47]	hsa-miR-21-5p	86.7	93.3	0.96

Abbreviations: AUC, area under curve; BC, breast cancer; cfDNA, circulating cell-free DNA; HER2, human epidermal growth factor 2; miRNA, microRNA; NR, not reported.

Table 7. Biomarker panels tested in LB for early diagnosis of BC.

FLUID	AUTHOR	BIOMARKER	SENSITIVITY (%)	SPECIFICITY (%)	AUC
Peripheral blood	Shan et al[104]	HOXD13, SFN, RASSF1A, P16, PCDHGB7, Hmlh1	79.6	72.4	NR
	Fan et al[90]	c-miR-16, c-miR21, c-miR155, c-miR195	88.9	86.7	0.936
	Luo et al[123]	miR-451, miR-148a, miR-27a, miR-30b	94.7	82.8	0.953
	Li et al[76]	miR-23a-3p,	86.5	45.9	0.699
		miR-130a-5p, miR-144-3p, miR-148a-3p, miR-152-3p			
	Li et al[81]	miR let-7b-5p, miR-122-5p, miR-146b-5p, miR-210-3p, miR-215-5p	94.4	88.9	0.978
	Kodahl et al[124]	miR-15a, miR-18a, miR-107, miR-133a, miR-139-5p, miR-143, miR-145, miR-365, miR-425	83.3	41.2	0.665
	Fang et al[82]	hsa-miR-324-3p/hsa-miR-382-5p, hsa-miR21-3p/hsa-miR-324-3p, hsa-miR-30a-5p/has-miR-30e-5p, hsa-miR-221-3p/hsa-miR-324-3p	89.0	92.5	0.901
	Liu et al[93]	PD-1 + IL-10 + IL-2Rα + CA15-3	93.3	61.4	0.811
	Salta et al[94]	APC, FOXA1, RASSF1A	81.8	76.9	NR
	Ozawa et al[61]	EV-miR-142-5p, miR320a, miR-4433b-5p	93.3	68.8	0.8387
	Liu et al[101]	ANRIL, HIF1A-AS2, UCA1	76.0	97.1	0.934
	Murillo Carrasco et al[27]	PUM1 y RNasa P	100	93.8	0.989
	Raheem et al[87]	miR-34a y CA15-3	77.7	83.3	0.842
	Shimomura et al[111]	miR1246, miR1307-3p, miR4634, miR6861-5p, miR6875-5p	97.3	82.9	0.971
Peripheral blood	Thakur et al[112]	miR21, miR-221, miR-210	100	100	1
	Nunes et al[97]	Methylation cfDNA APC, FOXA1, RASSF1A (PanCancer)	72.4	73.5	NR
	Wang et al[29]	Methylation GCM2, ITPRIPL1 and CCDC181	92.9	87.5	0.961
	Jang et al[30]	miR-1246, miR6, miR-24, miR-373	98.0	96.0	0.992
	Mijnes et al[77]	SPAG6 - PER1 - ITIH5 - NKX2-6	70.0	79.0	0.842
	Yu et al[98]	miR-21-3p, miR-21-5p, miR-99a-5p	97.9	73.5	0.895
	Uehiro et al[113]	Methylation RASGRF1, CPXM1, HOXA10 and DACH1	86.2	82.7	0.876

(Continued)

Table 7. (Continued)

FLUID	AUTHOR	BIOMARKER	SENSITIVITY (%)	SPECIFICITY (%)	AUC
	Li et al[114]	EGFR + PPM1E	77.9	50.7	0.734
	Wang et al[106]	Survivin + VEGF	95.4	84.0	0.898
	Zhang et al[118]	miR-199a, miR-29c y miR-424	77.2	88.9	0.905
	Kloten et al[132]	RASSF1A, ITIH5 y DKK3	67.0	69.0	0.697
	Aaroe et al[147]	738 gene expression profile	80.6	78.3	0.88
	Adam-Artigues et al[31]	miR-30b-5p, miR-99a-5p	82.3	87.5	0.92
	Itani et al[42]	miR-145, miR-425-5p, miR-139-5p, miR-130a	97.0	91.0	0.97
	Jang et al[43]	miR-1246, miR-202, miR-21, and miR-219B	85.3	93.3	0.96
	Kim et al[44]	miR-9, miR-16, miR-21, and miR-429	96.8	80.0	0.88
	Lopes et al[48]	miR-210, miR-152	83.3	68.0	0.75
	Rajkumar et al[19]	Panel 6 (Adipsin, Leptin, Syndecan-1, Basic fibroblast growth factor, Interleukin 17B and Dickopff-3)	65.0	80.0	NR
		Panel 3 (SOSTDC1, DACT2, WIFI)	100	90.0	NR
	Sadeghi et al[52]	hsa-miR-106b-5, –126-3p, –140-3p, –193a-5p, –10b-5p	67.0	80.0	74
	Yu et al[22]	hsa_circ_0000091, hsa_circ_0067772, and hsa_circ_0000512	97.0	90.0	0.97
	Zhang et al[54]	miR-185-5p, miR-362-5p	92.7	92.3	0.96
	Zhang et al[55]	cg00594560 cg01348584 cg04541368 cg07458308 cg08279008 cg08402365 cg08599259 cg09760908 cg13973436 cg14140881 cg14868703 cg15321298 cg15634980 cg16304215 cg17632299 cg18087672 cg18786873 cg20072171 cg20631750 cg21501525 cg22778178 cg23035715 cg25566568 cg25756435 cg25924096 cg26371731	89.0	100	0.97
	Zhang et al[56]	tRF-Gly-CCC-046, tRF-Tyr-GTA-010 and tRF-Pro-TGG-001	84.0	67.0	0.73
	Zou et al[57]	let-7b-5p, miR-106a-5p, miR-19a-3p, miR-19b543 3p, miR-20a-5p, miR-223-3p, miR-25-3p, miR-425-544 5p, miR-451a, miR-92a-3p, miR-93-5p, and miR-16- 545 5p	87.2	89.3	0.94
Saliva	Ando et al[86]	miR.21 y MMP1/CD63	95.0	79.0	NR
	Hirschfeld et al[74]	miR-424, miR-423, miR-660, let7-i	98.6	100	0.995

Abbreviations: AUC, area under curve; BC, breast cancer; cfDNA, circulating cell-free DNA; LB, liquid biopsy; NR, not reported.

Table 8. Analysis through AUC values or *P* values of different individual biomarkers and panel biomarkers in peripheral blood and urine. (Sensitivity and specificity were not reported in these primary articles.).

FLUID	AUTHOR	BIOMARKERS	AUC	
Peripheral blood	Cuk et al[133]	miR-148b, miR-376c, miR-409-3p, miR-801	0.69	
	Guo et al[67]	miR-21-5p, miR-1273g-3p	0.51	
	Madhavan et al[125]	cfDNA integrity	0.75	
	Yan et al[83]	Vesicles mR-375, mRNA-655-3p, mR-548b-5p	0.81	
	Shin et al[116]	miR-16	0.79	
		miR-21	0.87	
		miR-199a-5p	0.88	
	Zhao et al[145]	hsa-miRNA-595	0.75	
		hsa-miRNA-493	0.70	
		hsa-miRNA-155	0.72	
	Huang et al[84]	tDR-7816, tDR-5334, tDR-5236, tDR-6954 y tDR-4733	0.86	
	Schrauder et al[137]	miR375, miR655-3p, miR548b-5p, miR24-2-5p	0.68	
	Bao et al[26]	genomic instability MIR421, MIR128-1 y MIR128-2	0.92	
	Tahmouresi et al[62]	LncRNAs DSCAM-AS1 y MANCR	0.76	
	Loke et al[88]	miR-3162-5p, miR-6869-5p, miR-6781-5p, miR-1249, miR-7108-5p, miR 6804-3p, let-7e-3p y miR-1306-5p	0.95	
	Farina et al[102]	hsa-miR-3124-5p, hsa-miR-1184, hsa-miR-4423-3p, hsa-miR-4529-3p, hsa-miR-7855, hsa-miR-766-3p	0.89	
	Cappetta et al[58]	CYFIP1	0.73	
	Giussani et al[40]	hsa-miR-423-5p-002340; hsa-miR-181c-000482; hsa-miR-625-002431; hsa-miR-301b-002392	0.71	
		hsa-miR-423-5p-002340; hsa-miR-181c-000482; hsa-miR-301b-002392; hsa-miR-370-002275	0.68	
		hsa-miR-181c-000482; hsa-miR-625-002431; hsa-miR-301b-002392	0.70	
		hsa-miR-423-5p-002340; hsa-miR-625-002431; hsa-miR-370-002275	0.68	
		hsa-miR-423-5p-002340; hsa-miR-625-002431; hsa-miR-301b-002392	0.66	
		hsa-miR-181c-000482; hsa-miR-301b-002392; hsa-miR-370-002275	0.66	
		hsa-miR-181c-000482; hsa-miR-301b-002392	0.63	
	Lin et al[45]	circRNAs in plasma EVs	0.83	
FLUID	AUTHOR	BIOMARKERS	AUC	*P*
Peripheral blood	Mahmoudian et al[50]	miR 25-3p	0.83	
		miR29a-5p	0.84	
		miR105-3p	0.82	
		miR181b1-5p	0.88	
		miR 335-5p	0.81	
		miR 339-5p	0.77	

(Continued)

Table 8. (Continued)

FLUID	AUTHOR	BIOMARKERS	AUC	
	Wang et al[21]	MIAT, LINC0096, LINC01140	0.87	
	Wang et al[53]	circ_0000745, circ_0001531 and circ_0001640	0.91	
	Su-Ying et al[135]	miRNA-155		<.05
	Liu et al[122]	methylation FHIT		.002
	Delmonico et al[120]	ATM		.999
		p14		.582
		p16		.003
	Ahmed et al[146]	RASS + DAPK1		<.001
	Habeeb et al[72]	B-actin DNA integrity index		<.001
	Zhou et al[142]	polymorphism CD44 exon2		<.001
	Hamam et al[110]	hsa-miRNA-4270		.001
	Chen et al[78]	Let-7a-5p		<.001
		miR-21-5p		<.001
	Kandula et al[139]	KRAS mRNA		.001
		PTEN mRNA		.006
	Sochor et al[127]	miRNA-155		.026
		miRNA-19a		.026
		miRNA-181b		.025
		miRNA-24		.009
	Kim et al[143]	Slit2 factor hypermethylation		<.001
	Radwan et al[140]	Mammaglobin		.017
	Holubekova et al[63]	miRNA-99a, miRNA-130a, miRNA-484 y miRNA-1260a		<.005
	Ramadan et al[130]	polymorphism Arg399Gln del gen XRCC1		.017
		polymorphism Arg194Trp del gen XRCC1		<.001
Urine	Bentata et al[71]	* RNA splicing factors: HNRNPA1, HNRNPA2BQ, SRSF6, HNRNPA3, HNRNPK, HNRNPK exon 8 inclusion, PTBP1		.005

AUC, area under curve; cfDNA, circulating cell-free DNA; EV, extracellular vesicles; miRNA, microRNA.

The studies by Ming et al[70] and Yoshinami et al[64] also evaluated gene profiles and the presence of mutations, coinciding with Jimenez et al[75] and Duque et al,[149] in which the most frequently found mutations affected these loci: PIK3CA, TP53, and AKT1.

MiRNA-34a expression was low and miRNA-155 expression was elevated in BC vs controls with a significant *P* value. In addition, a correlation was demonstrated between the expression of miRNA-155 or miRNA-34a and TNM, presence of nodes, and histological grade.[107] Similarly, the Nadeem study agrees with this result by showing that low miRNA-195 expression was correlated with clinical stage, nodes, and histological grade.[100]

However, the studies of Delmonico et al[120] and Ritter et al,[69] which analyzed methylation promoters in DNA in saliva and blood, as well as miRNA in urine and blood, did not find significant associations.

As is noted in Figure 2, 45 studies from China (33%), 18 from Egypt, 8 from the United States, and 8 from Germany representing more than half of the primary articles were found. The figures clearly indicate considerable interest in ongoing research by these countries, regarding this area.

Table 9. MQ scores of the primary articles studied by year (n = 136).

YEAR	NO. OF CASES	MQ SCORE	
		M (SD)	MEDIAN (MAX-MIN)
2022	11[12-22]	25.6 (3.17)	26.0 (20-30)
2021	35[23-57]	26.5 (3.11)	27.0 (18-34)
2020	17[58-74]	22.4 (3.94)	22.0 (17-30)
2019	14[75-88]	24.5 (3.20)	24.5 (19-29)
2018	10[89-98]	24.2 (2.57)	24.5 (20-28)
2017	4[99-102]	22.8 (2.99)	22.0 (20-27)
2016	12[103-114]	25.0 (3.52)	25.5 (19-31)
2015	8[115-122]	23.1 (2.42)	23.0 (20-26)
2014	8[122-130]	23.6 (3.07)	24.5 (18-27)
2013	2[131,132]	27.5 (2.12)	27.5 (26-29)
2012	9[133-141]	25.4 (4.64)	25.0 (19-34)
2011	2[142,143]	23.5 (0.71)	23.5 (23-24)
2010	4[144-147]	23.3 (5.12)	23.0 (18-29)

Abbreviations: MQ, methodological quality; SD, standard deviation.

Finally, and in reference to the MQ analysis of the primary studies (applying the MInCir-Dg scale),[11] it is important to emphasize that the median score was 25 points (17-34 points) and the average was 24.7 points, which represents a regular MQ. It should be highlighted that the lowest scores were associated with the type of design (most of the studies correspond to cases and controls) and not having estimated the sample size, which determines that the level of evidence of the primary articles is 2b and 3b for diagnostic studies, with a grade B recommendation.[159]

Regarding the limitations of this study, the heterogeneity of the primary studies should be highlighted, as various methods are used, both for the identification of different biomarkers through LB (CTCs, ctDNA, cfDNA, miRNA, and EV-RNA), as well as the fact that some studies evaluate biomarkers individually, while others do so through combinations, establishing biomarker panels under evaluation. Another important limitation of the study was that none of the primary studies performed a sample size calculation, and in the articles, the number of participants varied and was inconsistent. In addition, some primary studies established sensitivity and specificity, while others only reported AUC values, and some only reported *P* values. These variables are made for a difficult analysis and comparison. Despite these limitations, the strengths of the primary studies are that a significant number maintained a methodological strategy to perform the analyses in test cohorts, and then in validation cohorts, maintaining groups of cases and control groups in each of the studies.

Conclusions

Integrating LB in clinical practice as part of the process for early diagnosis of BC is a promising alternative. The biomarkers, obtained from samples obtained through LB, consisting of miRNA molecules, were the most frequently investigated biomarkers in the early diagnosis of BC. MiR-21, miR-155, and miR-195 have the greatest potential to discriminate between healthy individuals, BC, and benign breast tumors. There are panels of combined biomarkers, with the potential to increase diagnostic sensitivity. Our results reflect that LB has a high sensitivity and specificity for the diagnosis of BC, especially in early stages.

Author Contributions

GD and CM contributed to the concept and design of the research. TO reviewed and approved the study design. GD, CA, JPH, and BG performed the selection process for article recruitment. GD, CA, DP, JPH, and BG extracted the data from the studies included, and MM and LA verified the extracted data. GD, CM, CA, and BG collaborated in the analysis of the results and presentation of data. GD and BG contributed to final revisions. Data sharing is not applicable to this article as no new data were created or analyzed in this study. All authors contributed in the drafting of the article.

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